



Organic Seed Alliance

*Advancing the ethical development and stewardship
of the genetic resources of agricultural seed*

PO Box 772, Port Townsend, WA 98368

How to Breed Tomatoes for Organic Agriculture



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I. Introduction

Tomatoes (*Solanum lycopersicum*) are an excellent crop to do plant breeding work with and can be used to clearly demonstrate the steps of breeding with a self-pollinated crop species. Not only do tomatoes readily self-pollinate, performing hand pollinations for controlled crosses is relatively easy. Tomatoes are also capable of producing prolific amounts of seed as each individual fruit can yield several hundred. Tomato varieties also span an abundant array of colors, shapes, sizes, and flavors, creating an exciting palette for the aspiring breeder to work with.

Although tomatoes are not impressively high in nutrients, they make up a significant amount of the U.S. diet. While North Americans consume tomatoes mainly in processed form, most often in sauces or pastes, the consumption and popularity of fresh market tomatoes is on the rise. This may be due to improved varieties and an increasing interest in the wide range of tomatoes available, such as grape, specialty, heirloom, and greenhouse types.

Modern tomatoes span a number of distinct market classes. These classes include cherry types with round, grape, and pear shapes; saladette types that are similar to cherry types but larger and round; large, round slicer types; paste or plum types that have thick walls with high total solids, mainly used for processing; and heirloom types. There are also wild species, such as *Solanum pimpinellifolium*, that will readily cross with domesticated types.

Breeding projects can be done within or between any of these market classes. For example, if improving flavor in a slicing type is the goal, then one approach is to cross a modern slicer and an heirloom type known for superior flavor, such as 'Brandywine.' If the goal is to create something novel, making a cross between two distinctly different tomato types from two different classes will provide abundant genetic variation from which to select. In general, if you are seeking to improve a variety for only one or a few traits, making crosses within a class will be most effective and efficient. On the other hand, if you want to create something

novel or improve a number of traits, you may want to consider making crosses between two different tomato classes.

Several decades ago, it was common for a single tomato variety to serve multiple purposes and markets. Today, tomatoes are bred for specific markets, such as processing, fresh market, greenhouse, or home garden use. This change occurred largely because the combination of quality and cultural requirements for each of these four markets has become quite distinct, driving a relatively new and unprecedented specialization within the tomato breeding industry. To develop new varieties that thrive under the pressures and challenges of specific conditions, and to incorporate traits most important to each market, it is vital that breeding work use the same cultural practices and environmental conditions in which the finished variety will be produced.

II. Introduction to Breeding

All good breeding projects need a foundation of intimate familiarity with the crop. And good breeders start with good material. Take time to investigate which varieties and other **germplasm** resources are available to you before beginning a breeding project. A legacy of breeding precedes you, and it will benefit you to take advantage of the products of this experience and expertise. Finding good breeding material will save you time and potential frustration. In brief, the steps involved in breeding will be to:

- Develop and define your breeding goals
- Identify parent material or varieties you'd like to work with or cross
- Make crosses
- Grow out and select over multiple generations, potentially making more crosses

On-farm plant breeding involves making the best use of limited resources. The breeding plan for any crop will be based on a few key factors:

- Determining how much time, space, and re-

*Definitions for technical words that are italicized and bolded can be found in the glossary

- sources you have to devote to breeding work
- What your breeding goals are and how likely they are to be achieved
- How much genetic diversity is available or created at the beginning of the project

The combination of these factors will determine how many plants you will need to evaluate, particularly in the early stages of your work. For example, a cross between two genetically similar varieties with the goal to enhance eating quality may require relatively small populations. On the other hand, larger populations will be needed for crosses between two genetically distinct varieties with the goal to create a novel variety with good agronomic and culinary qualities.

Below, in the **Breeding Methods** section, we offer examples of Organic Seed Alliance's (OSA) approach to breeding self-pollinated crops in addition to a commercial pedigree breeding scheme. You can perform an interesting and successful breeding project without following an established commercial breeding procedure. OSA's approach allows you to incorporate a greater degree of genetic diversity into your breeding work while still achieving stability and an acceptable level of uniformity in a new variety. Incorporating and retaining diversity in your breeding work is good insurance against failure and environmental challenges. Retaining diversity is an especially good idea if you are new to plant breeding, because it keeps you from narrowing genetic material too much and too quickly, and buffers against making poor choices due to inexperience.

III. Breeding Goals

It is important to set clear and attainable goals for your tomato breeding work. Do you want to create a tomato variety that combines your favorite qualities from two varieties? Do you want to enhance or create disease, pest, or insect resistance in your favorite variety? Do you want to improve eating quality in an existing variety? Or do you want to create something entirely new? Are you breeding for your own use or do you intend to share, sell, or even license your creation? The goals and intended outcomes of your work will help you identify where to

start and how to develop, create, and implement a successful breeding plan. While it is not necessary to be a trained geneticist to do good plant breeding, it is extremely valuable to understand basic genetic concepts and how they affect your breeding work. For an overview and in-depth discussion of the genetic basis of plant breeding, please refer to OSA's *Introduction to On-Farm Organic Plant Breeding*.

Below is a list of traits commonly included in tomato breeding work. A chart of how these traits are inherited and when to make selections is included at the end of the **Breeding Methods** section.

Agronomic traits

Growth habit: Indeterminate varieties grow continuously with three leaf nodes occurring between each inflorescence. Determinate varieties express a finite amount of growth with only one leaf node between inflorescences. Semi-determinate varieties express intermediate growth with two leaf nodes between each inflorescence.

Plant stature: Tall plants are most common, dwarf varieties are less common.

Leaf shape: Heirloom types often express potato leaf shape whereas most modern standard varieties have regular shaped leaves. Potato leaf types commonly have exerted stigmas.

Canopy cover: Minimal leaf coverage can contribute to sunscald. However, minimal leaf coverage can also enhance color in purple types that have light responsive compounds in the skin.

Maturity: Maturity is related to the intended production environment. For example, early maturing varieties are needed in colder climates with short growing seasons.

Pedicel attachment: Fruit breaks off at the calyx in jointless pedicel types. In plants with jointed *pedicels*, fruit breaks off with a bit of stem. This is of particular significance for processing tomatoes where attached stems can puncture fruit and reduce quality.

Defensive traits

Disease resistance: Some varieties have single gene resistance, also known as vertical resistance, for certain races of the following diseases: leaf mold, Septoria, late blight, Alternaria, Stemphylium, and verticillium and fusarium wilts, Dominant resistance to tobacco mosaic virus and nematode resistance is also available. Other forms of complex, **polygenic** (multi-gene) resistance, also known as horizontal resistance, exist and are extremely beneficial to robust breeding work. Wild tomato types are often used in breeding work as sources of disease resistance.

Drought tolerance: Complex and polygenic, little is known about the specific components of drought tolerance. Selection for this complex trait needs to be done under drought conditions.

Fruit quality

Fruit color: This trait involves the combination of skin and flesh color. Skin is either yellow or colorless and flesh is red, yellow, orange, green, or purple/brown. Color is an indication of the dominant compounds in the fruit, with lycopene dominant in red types, beta-carotene in orange types, delta-carotene in yellow types, and phytoene in green types. There are a number of genes that control and contribute to flesh color in tomatoes. Modifying genes may cause gradations in expression and production of color compounds. The particular combination of skin and flesh color result in the following colors of tomato fruits:

- Red types have red flesh and yellow skin
- Pink types have red flesh and colorless skin
- Yellow types have yellow flesh and skin (colorless skin and yellow flesh results in cream to pale yellow fruits)
- Orange types have orange flesh and either yellow or colorless skin
- Green types have green flesh and either yellow or colorless skin
- Black and brown types have red/green flesh with yellow skin
- Purple types have red/green flesh with colorless skin

Fruit stripe: This trait refers to dark green radial stripes that occur opposite the **locules**. Stripes tend

to fade as the fruit matures.

Green striping: This trait refers to irregular longitudinal green striping on the fruit. Stripes will either stay green or turn yellow at full maturity.

Skin texture: Most tomato varieties have smooth skin, but peachy, fuzzy textured skin also exists.

Flavor: Flavor is a complex and polygenic trait. Locule size and number, as well as texture, are significant contributors to a tomato's flavor profile.

Texture: Heirloom types are known for their "meaty" flesh texture. This is often a desirable trait in tomato breeding work.

Shape and size: There are numerous possibilities for fruit shape and size as a large diversity exists in tomato germplasm. Your market will likely be the largest determining factor in what size and shape is most desirable.

Fruit ripening: Green shoulder types have dark green shoulders when immature whereas fruit of uniform ripening types are entirely light green when immature. Green shoulder types have recently been associated with superior flavor to uniform ripening types. Uniform ripening types are associated to some extent with crack resistance and are desirable for paste tomatoes.

Parthenocarpy: This trait refers to a plant's ability to set seed under limited pollination, which is important for setting fruit under hot or cold conditions when pollen cannot function normally.

Obtaining a specific combination of traits can be challenging in self-pollinating crops. Each generation of self-pollination increases homozygosity within the plant (i.e., the state in which the two alleles at a gene location are the same). Once the two **alleles** become the same at a given gene location, the trait it controls or contributes to is considered to be fixed and will no longer segregate in successive generations. Refer to figure 1 for an explanation of this phenomenon. As the gene combinations sort out and the degree of homozygosity increases, specific combinations of traits become more settled. If the desired combination of

traits is not achieved after five or more generations of self-pollination, then additional crossing and genetic recombination may be necessary. Because of this phenomenon, growing and retaining as large a population as is practical in the first few years is critical to optimizing your success.

Obtaining Traits

Repeated generations of self-pollination in tomatoes and other self-pollinating crops leads to increased genetic uniformity. With each generation of self-pollination, the genes in a tomato become 50% more **homozygous**; meaning, half of the allele combinations at each gene location become the same. As an example, let's look at a controlled cross between variety A and variety B. The first generation following the cross will be 100% heterozygous, with every gene location having an allele from parent A and one from parent B (AB). There are four ways for these alleles to recombine after self-pollination, two of which retain a heterozygous pairing (AB and BA) and two of which are homozygous (AA, BB).

Allele	A	B
A	AA	AB
B	BA	BB

This 50% chance of **homozygosity** occurs at every locus (gene location) in every generation of self-pollination. Once an allele pair becomes homozygous (AA or BB), it becomes "fixed" and will remain that way through future generations (until a mutation or an outcross occurs).

Allele	A	A
A	AA	AA
A	AA	AA

Therefore, the genetic makeup of material selected from a cross becomes very genetically stable after five or six generations.

Generation	% Heterozygosity	% Homozygosity
F1	100	0
F2/S1	50	50
F3/S2	25	75
F4/S3	12.5	87.5
F5/S4	6.25	93.75
F6/S5	3.125	96.875

F = filial-a, the general term for a generation following the parent generation (F1 is the first generation following a cross, F2 is the second, F3 the third, and so on). S = selfed-a, the generation following the parent generation that has been created by an act of self-pollination.

This phenomenon is why it is important to retain as much diversity as possible in early generations, and that you also make stringent evaluations and selections several generations after the cross is made. This process ensures you will be selecting on a stable arrangement of genetic material.

A short discussion on yield: Although it is tempting to make selections for higher yield, selecting directly for yield is seldom very effective. Yield is strongly influenced by environmental factors and cultural practice. Instead, it is advised to base your selections on component qualities and characteristics that contribute to robust yields. This may mean placing emphasis on other traits, such as disease resistance, maturity, growth habit (determinate, semi-determinate, or indeterminate), concentration of fruit set, resistance to cracking, and other factors that contribute to a high percentage of quality, marketable fruit rather than focusing solely on the relative abundance of fruit.

It is important to recognize and make selections based on favorable genetic combinations that are heritable, and thus can be passed on from one generation to the next. Just because a plant looks excellent in the field does not mean that it has excellent genetics. The plant may look good because it was placed in a superior location that had added fertility, or was subjected to very few stressors, such as disease, pest pressure, or lack of water. Incorporating family-based selection, also known as progeny selection, is the best way to minimize the chance

of selecting plants that look good but may in fact be harboring unfavorable or weak genetic combinations. Refer to OSA's Introduction to *On-farm Organic Plant Breeding* for details and suggestions on incorporating family-based selection into your breeding work.

Fortunately, there is no right or wrong way to do plant breeding. There exists boundless genetic variation and potential both in nature and in our work to intentionally cross one tomato to another. There is more genetic variability and potential than you could possibly capture in a single lifetime. You will certainly miss or discard excellent genetic combinations, but you will capture others. Plant breeding requires a certain amount of ruthlessness and a lot of decisiveness. Combined with an artistic eye and an openness to unexpected results, plant breeding can become a dynamic and engaging lifelong passion.

III. Sourcing Germplasm

There are essentially two ways to begin a tomato breeding project: either create genetic variability or begin with genetically diverse material. To create genetic variability, you will need to make a controlled cross, create the opportunity for a natural cross to occur, or wait for an off-type or mutant variant to arise. What type of material you start with and where you source it from will depend on your objectives and how much time and energy you are willing to invest.

One place to begin is by acquiring a population of material that is known to be genetically diverse. This material is sometimes referred to as “breeders’ mixes” or collections. If you start with a diverse population, be sure to acquire the material from a reputable breeder, seed producer, or seed company. Some genetically diverse material is available in an unimproved or wild state. There may be some good material in these collections but it will likely take many years to tease it out.

You can also start by saving seed of a variety you know and love. This can be done with either a standard (non-hybrid) or a **hybrid** variety. Older standard varieties and heirlooms often contain surpris-

ing amounts of genetic variation. Standard varieties will give you less genetic variability and diversity to work with than a hybrid variety. Hybrid varieties of tomatoes are highly heterozygous (having different alleles at a number of gene locations across the genome), whereas standard varieties are much more homozygous (alleles are the same at most gene locations). However, nature is dynamic, and new genetic variation is always arising. By paying attention to this genetic variation, you can identify variation that may be useful in a breeding project.

A common way to initiate a breeding project is to create genetic variability by making a cross. Controlled crosses are made by hand pollination, transferring the pollen from one desirable plant to the flower of another (described in the **Pollination Techniques** section below). Planting two varieties in very close proximity to each other can generate natural crosses. Natural crossing will most readily occur with older standard and heirloom varieties or cherry types with **exserted** stigmas that facilitate and encourage cross-pollination (see the **Reproductive Biology** section for more information). Nearly all commercial tomato breeders base their programs on controlled crosses followed by pedigree selection. Pedigree breeding follows a single line derived from a single cross to create a new variety. An example of breeding by the pedigree method is outlined in the **Breeding Schemes and Methods** section below.

You can also begin a breeding project by making a controlled cross between one or two quality F1 hybrids, which will create a plethora of genetic variability to select from. However, it will be difficult at best to predict what kind of variability you will get and if any of it will be useful or valuable. If you choose to start by crossing hybrids, we recommend that you grow out and observe the progeny of the hybrids you want to use as parents for at least one or two generations before making the crosses. This will allow you to see the variability and the genetic potential encompassed in each hybrid. It is not uncommon that the phenotypes of the parents of a hybrid are quite different from the **phenotype** of the hybrid itself. Knowing the genetic makeup and potential of your breeding material is important to any successful breeding project.

There are many sources for obtaining varieties or other germplasm to begin your breeding work. The USDA Germplasm Resources Information Network (GRIN) system is a great resource for sourcing both wild and cultivated tomato material. It is a searchable database housed within the USDA Agriculture Research Service. Tomato collections are housed at the Geneva, New York, and UC Davis, California, locations and material from them is available for free by request. The Seed Savers Exchange houses a large collection of heirloom and older standard varieties that have a wealth of potential for breeding work. Public universities, particularly land grant institutions, often have advanced breeding material that can be licensed or shared for breeding work. North Carolina State University, Cornell University, and Oregon State University have excellent tomato breeding programs. Local, regional, and national seed companies and seed libraries are also good places to source varieties for breeding work.

Once you have sourced potentially useful germplasm, conducting variety trials can assist you in identifying promising breeding material. Variety trials are a way of observing and evaluating a number of different varieties, or sources of a single variety, in a side-by-side comparison. Spending at least one or more seasons conducting trials is a great way to get to know a crop and to see the variation available for breeding work. A variety trial can be set up as either an unreplicated observation trial or as a replicated trial that will allow you to determine if the differences you see between varieties are due to chance or to the varieties themselves. For more information on variety trials and how to conduct them, refer to OSA's *On-Farm Variety Trials: A Guide for Organic Vegetable, Herb, and Flower Producers*, which is available for free download at www.seedalliance.org.

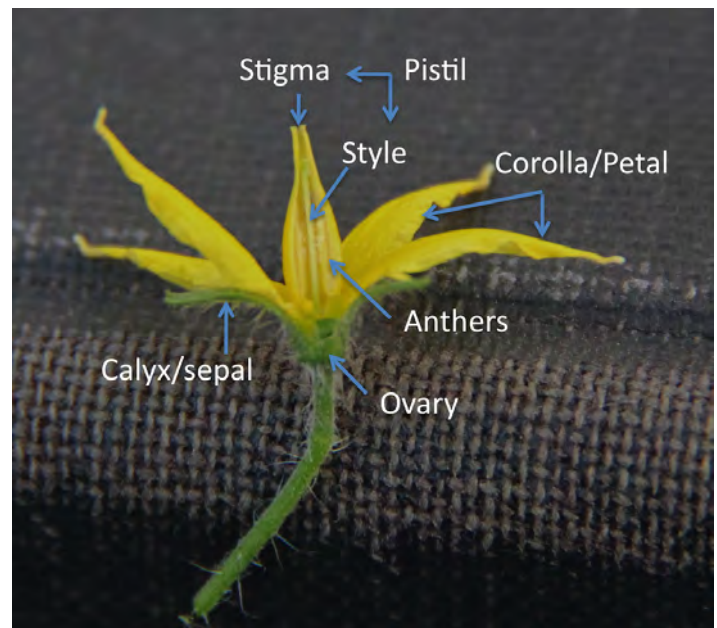
A word of caution on intellectual property protections: Unfortunately, there are some very restrictive intellectual property protections on agricultural seed. It is critical that you know what, if any, restrictions exist for the material you want to use. You need to be aware of whether a variety is protected through the use of a certificate granted through under the Plant Variety Protection Act (PVPA), a Material Transfer Agreement (MTA), or a utility patent. These forms of legal protection all

have varying degrees of restrictions that may affect your ability to use certain varieties or other forms of crop germplasm, so be sure to research any possible legal restrictions on your breeding material before you begin.

IV. Reproductive Biology

Grown under optimal conditions, tomatoes have a 95 to 115 day lifecycle. The first flowers will appear and open 7 to 8 weeks after seeding and mature fruits will follow 6 to 8 weeks later.

Tomatoes have perfect flowers that contain both functional male and female parts. The male parts are known as the stamens and are each made up of an anther borne on a filament. The female parts are known as the pistils and are made up of a style and stigma positioned above an ovary.



Flowering can occur for weeks, a feature of tomatoes that facilitates the ability to make crosses between varieties with distinctly different maturities. Normally at least 4 to 8 flowers are borne on each inflorescence, and a single tomato plant may produce as many as 20 or more inflorescences over a season, giving you ample opportunities for making crosses.

Most modern tomato varieties are almost entirely self-pollinating. This is facilitated by the floral

structure in which a tight, protective anther cone forms around the stigma, greatly reducing the incidence of natural cross-pollination. In older standard and heirloom varieties, and often in cherry types, the degree of natural cross-pollination can be much higher than the occurrence in modern standard varieties. Older standard and heirloom varieties often have longer styles, pushing the stigma either flush with or sometimes beyond the tip of the anther cone. This arrangement facilitates higher rates of natural cross-pollination. The photo below illustrates this floral structure difference.



Bee and insect activity can also increase natural cross-pollination, which tends to be higher on organically managed land due to diverse cropping systems and a lack of pesticides. If you are working with two older standard or heirloom varieties, planting them right next to each other may result in a natural cross.

Domesticated tomatoes, including both modern and heirloom types, are biologically compatible with many of their wild relatives. Unlike the modern, domesticated tomato, many wild relatives have self-incompatibility systems that enforce cross-pollination. Wild species have played an important role in tomato improvement as they have been, and continue to be used as, critical sources of disease resistance in most modern breeding programs.

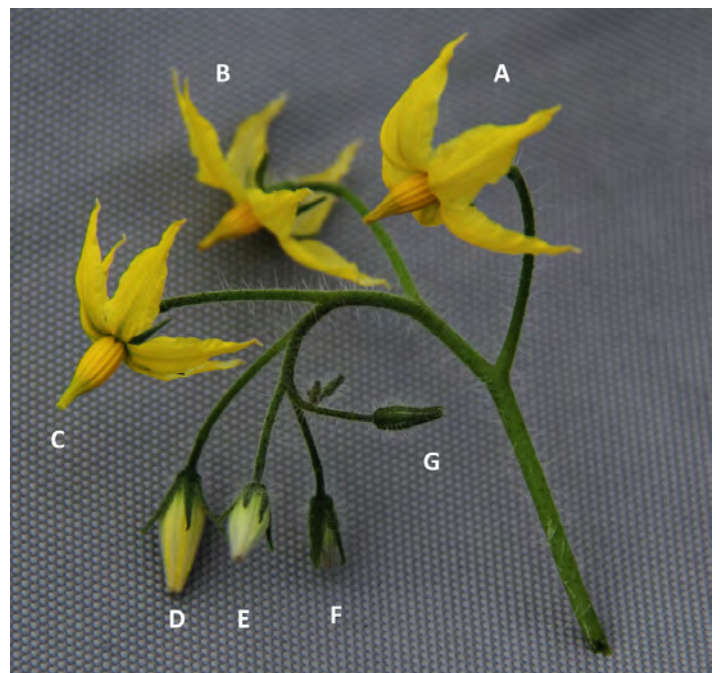
V. Pollination Techniques

Following is a description of how to perform controlled crosses using simple hand tools and techniques. Making a controlled cross entails

emasculating immature flowers by removing the androecium, or male structures, of the chosen female parent (pollen acceptor) and collecting and transferring pollen from mature flowers of the chosen male parent (pollen donor).

The first step in making a hand pollination cross with tomatoes is to identify which flowers are at the correct maturity to serve as the male and female. Since tomato flowers are perfect, the age and maturity of the flower will dictate whether it can be used as a male or female in the intended cross. Flowers open progressively along the inflorescence, with the oldest and most mature flower on the basal end and the youngest on the terminal end. You can expect 1 to 2 flowers to open each day.

Flowers in the late bud stage are most appropriate for emasculation. At this stage the corolla (petals) and anthers remain tightly closed around the pistil, the sepals will have begun to open, and the petals will be changing from pale to bright yellow. If the petals have already opened, or have begun to open, it is too late. The pollen will have already *dehiscid* (been released from the anthers) and likely pollinated the stigma. There will often be more than one bud on an inflorescence that is appropriate for emasculation. The highest success rates for hand pollination are achieved when using buds near the basal end of the inflorescence. In the photo below, flowers A, B, and C are too old for *emasculation* but are appropriate for pollen collection. Flowers D



and E are appropriate for emasculating and flowers F and G are too young.

Emasculating can be accomplished with the use of forceps (recommended) or a pair of needle nosed tweezers. The corolla and stamen cone are tightly folded up around the pistil at the late bud stage, forming a two-layered cone that can be removed in a single step. Insert one point of your forceps into the top of the cone, between the pistil and the anthers, and work it in all the way to the bottom of the cone. Place the other point between the calyx (sepals) and the corolla. Grasp and firmly, but gently, pull straight up, removing the entire cone and leaving the pistil exposed and unharmed. If the cone does not detach in one piece, carefully remove any remaining anthers and pieces of corolla. It is also possible to pull off each layer of the flower, starting with the corolla and then removing the anthers individually. This method will work, though it may be more difficult to avoid damaging the style and stigma. Successful emasculating takes practice. Don't be discouraged if it takes several attempts.

For pollen collection, choose a fully mature flower from the plant you have designated to be the male parent. Removing the petals can be helpful, but is not necessary. Look for the series of longitudinal slits that run along the anther cone, work your tweezers into one of the slits, and pry the cone open.



There are three ways to transfer pollen: 1) remove a set of anthers and rub them against the stigma on your emasculated flower; 2) pull the style out of the emasculated flower and rub the remaining anther cone over your emasculated stigma; or 3) collect pollen using a bell or other resonant tool held close to the flower and rung to shake pollen loose onto a

collecting plate or slide, then dip your forceps into the pollen and paint it onto the exposed stigma of your emasculated flower. Multiple pollinations to the same flower can improve fruit set, and involves collecting and transferring pollen from several mature flowers of the male parent to the single emasculated flower of the female parent. Tomato pollen is long-lived and stores well in the event you need to collect it for future use. The pollen can remain viable for weeks at room temperature (out of direct sunlight), and if dried down and refrigerated can last for several months.

Label each cross with a tag identifying the female and male parents and the date the cross was performed. Small white merchandise tags are effective and inexpensive for labeling crosses. It is also advisable to remove all other flowers from the inflorescence bearing the controlled cross. This step eliminates confusion at fruit set and focuses all of the plant resources for fruit development into that one fruit on that particular inflorescence.



It is important to rinse your forceps in rubbing alcohol when you move from working with one variety to another. This should be done both when

you are doing emasculation and pollen collection. Rubbing alcohol will kill any pollen remaining on or sticking to your tool. This step is very important when you are making controlled crosses so as to avoid unintended self-pollination or contamination. Make sure to allow time for your forceps to fully dry before collecting pollen so that what you collect is not rendered useless by the alcohol still on your tool.

Emasculated flowers do not need to be covered to prevent contamination. At this point, the colorful and showy petals and anthers have been removed, so there is nothing left to attract potential pollinators. If you are conducting controlled pollinations outside in the field you may need to cover emasculated flowers with glassine bags to prevent them from drying out and desiccating under hot, dry, or windy conditions. Glassine bags are quite inexpensive and can easily be located with a quick Internet search. If possible, it is ideal to make crosses outdoors when the weather is cool and overcast with little to no wind.

Controlled pollinations can be effectively done in all daylight hours; however, some studies suggest a decline in effectiveness in the late afternoon. Each seed represents a separate act of pollination and fertilization and many varieties will set fruit if only a single seed is formed. Some varieties are *parthenocarpic*, meaning they can set seed without fertilization. Making crosses with these types of varieties is possible but may require multiple pollinations to ensure successful fertilization and seed set.

VI. Breeding Methods

Whether you begin with a quality hybrid, a genetically diverse pool of material, or a controlled or natural cross, your breeding work will follow a similar course. In general, you will need to grow out the largest populations you can for the first several years followed by a few years of single plant selections and family row evaluations. You will then bulk together the seed of desirable plants that meet your needs and goals. There is some debate about the minimum and ideal numbers of individuals per *family* that will maximize your chances of success. You will be better off planting fewer individu-

als from a greater number of families rather than planting lots of individuals from only a few families. Planting fewer than five individuals per family is not recommended. The more individuals you plant per family the more you will see the breadth of genetic potential and expression each family is capable of. Always keep some remnant seed in reserve each year so that all is not lost in the event of a crop failure or unintended cross-pollination.

The closer you can replicate the recommendations in this guide, the more grounded your work will be in the genetic foundations of plant breeding. That said, plant breeding is an art as well as a science, and the best plans are not immune to change and adaptation. Strive to maintain flexibility in your breeding work so you can adapt and change course in response to what you see in the field.

The pedigree method of plant breeding involves making a controlled cross followed by several successive generations of single plant selections. The goal is to develop at least one new variety from a single cross. Breeders and companies that employ this method are generally making many crosses each year and often have large programs. The fact that they are always working on multiple breeding projects simultaneously helps to ensure their success. The following example of a pedigree breeding method is based on the program at a public university that produces new tomato varieties for both the fresh market and the processing industry. This particular public program is relatively small in scale, so the numbers presented below may be smaller when compared to other commercial tomato breeding programs.

Although the pedigree method is a reliable and successful means to developing new tomato varieties, it can be genetically restrictive for on-farm and diverse system breeding work. A finished variety from a pedigree program is known as a *pure-line*, meaning the commercially released variety comes from a succession of single plant selections that result in a high degree of genetic uniformity. The development of *multiline varieties* is a more useful and genetically resilient approach to creating new varieties for organic and diverse farming systems. A variety released as a multiline is composed of several similar but genetically diverse

pureline materials. This can be accomplished by following OSA's approach to breeding self-pollinated crops, referred to as the "OSA method" in the section below.

Our recommended population sizes and number of plants allow you to better observe the genetic expression of traits and make effective selections. As you modify numbers into smaller population sizes, your ability to distinguish between the genetic contribution to a trait versus the contribution of environmental effects diminishes. This does not mean you will not be successful, but rather the chance your selections will be based on non-genetic, and thus non-heritable, factors will increase. Strive to plant the largest populations of plants that you can practically handle.

It is also helpful to know if any of the traits of interest are dominant or recessive in nature. Once a recessive trait appears in a self-pollinated crop like tomatoes, you can be confident that it is fixed and will be carried on through future generations. Dominant traits, on the other hand, require progeny observation to identify whether or not the trait is fixed or still in a heterozygous form. In this case, if the trait is fixed, the offspring will all express the dominant form of the trait; whereas, if the trait is not fixed most of the offspring (approximately 75%) will express the dominant form of the trait and a few (approximately 25%) will express the recessive form. For more on dominant and recessive trait expression and inheritance, refer to OSA's *Introduction to On-Farm Organic Plant Breeding*. See table 1 at the end of this section for a list of traits commonly selected for and when to select for them, along with their gene symbols and the type of inheritance they exhibit. For a comprehensive list of known tomato genes, refer to the C.M. Rick Tomato Genetics Resource Center's list of gene names and symbols, found at <http://tgrc.ucdavis.edu/Data/Acc/Genes.aspx>.

Below we provide instructions for both the OSA method and pedigree method by breaking down the first six years of a tomato breeding project.

Year 0

Define your breeding goals and identify potential parent material. Use variety trials to help you

evaluate and determine which varieties or other germplasm to use as parent material in your breeding work to best meet your breeding goals.

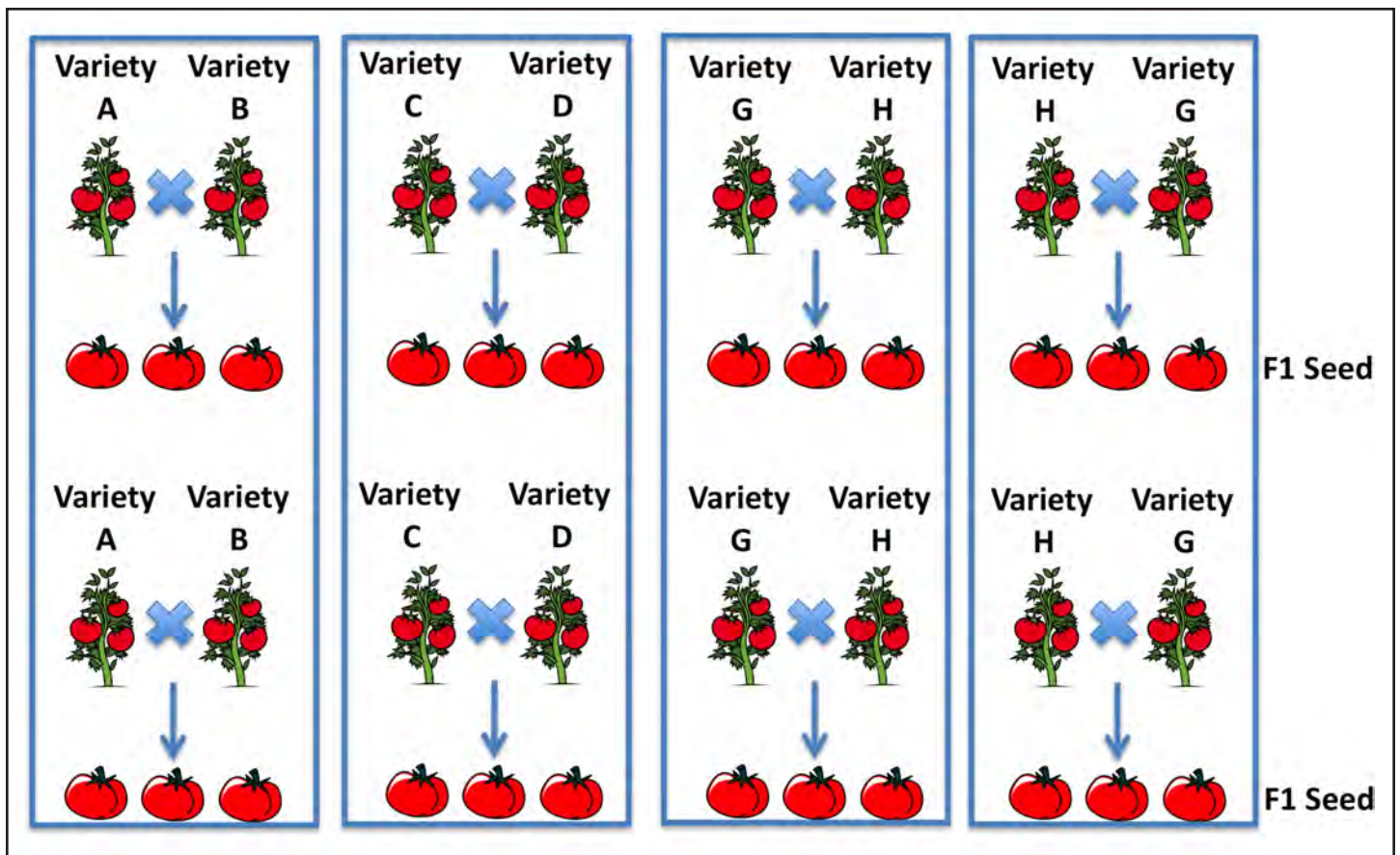
Year 1

OSA Method

Make crosses between several pairs of varieties, ideally between as many different parent varieties as is practical for you (shoot for 3 to 6). Increasing the number of parent combinations will increase the range of possible new genetic combinations to select from. It is more efficient to grow out and evaluate smaller populations of progeny from a number of different crosses than to look at a large population of progeny from a single cross. This approach increases the probability of finding your desired *ideotype* and combination of traits, and assumes you have promising parent material. Making crosses with inferior material just to increase the number of parent combinations will not be beneficial.

There are two approaches to making these crosses. The first is to make *reciprocal crosses* between the same set of parent varieties. The second is to make multiple crosses between different sets of parent varieties. A reciprocal cross uses two parent varieties as both the female and male in the cross: variety A crossed to variety B (A x B, the female plant is always listed first in a cross) and variety B crossed to variety A (B x A). The second approach is to make multiple crosses between multiple sets of different parents. For example, if you plan to make six controlled crosses, you could either make reciprocal crosses between three different sets of parents or make crosses between six different sets of parents. Reciprocal crossing requires fewer plants and generally results in a narrower range of variation to select from.

Making multiple crosses between different types of tomatoes is the most efficient way to create a diverse pool of genetic material from which to begin your breeding work. For example, if you are interested in crossing an orange slicer with a red grape type, source three varieties of interesting orange slicers and three varieties of red grapes and make multiple crosses between them. These crosses would give you nine different combination options and 18 potential reciprocal crosses.



To ensure success, make controlled crosses between at least two sets of plants for each variety combination you are working with. Make crosses on at least 4 to 6 flowers on different *inflorescences* between every set of parents. Collect seed from two or more fruits from each cross. It is OK to combine seed collected from crosses between more than one set of the same parents, but keep seed from crosses between different sets of parents separate. If making reciprocal crosses, keep seed from each cross separate (i.e., do not combine seed of A x B with seed of B x A). The following diagram illustrates this setup with crosses made between two sets of parents for each variety combination. The boxes represent where it is acceptable to combine seed. The two boxes on the right represent a reciprocal cross.

Pedigree Method

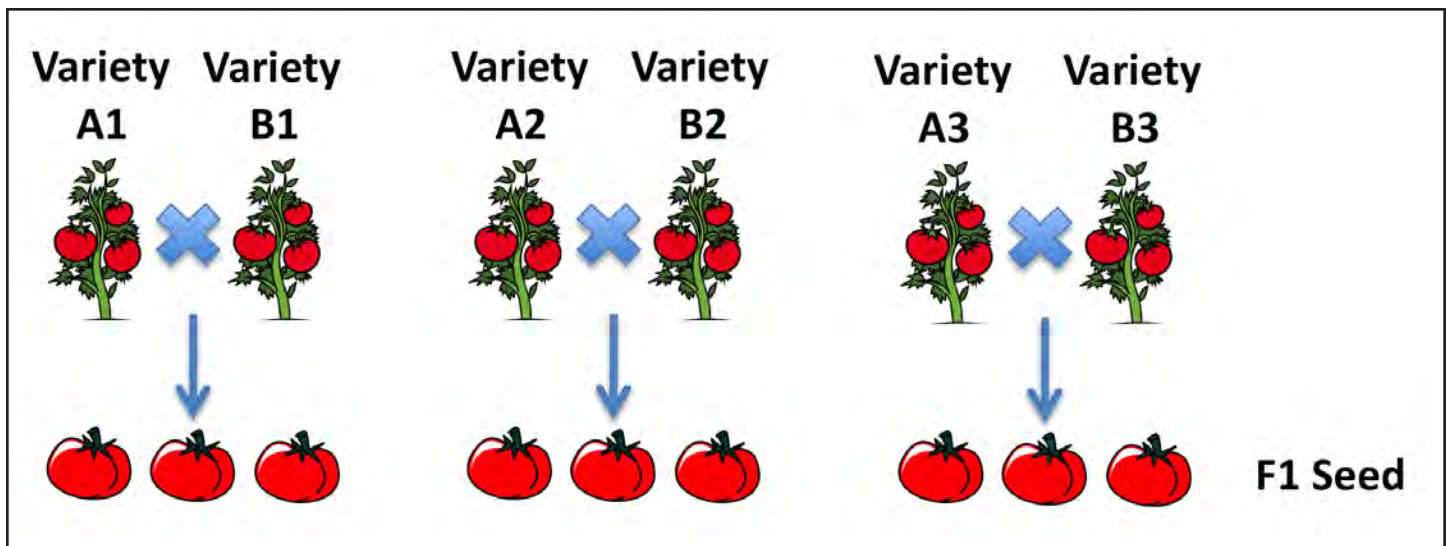
Make controlled crosses between three pairs of plants, using the same variety for the female and the same for the male in each cross. Make crosses on at least 4 to 6 flowers on different inflorescences between each set of parents. Collect seed from at least 2 or 3 fruits from each female parent plant. For example, say you have three plants (1, 2,

and 3) from variety A that you have chosen to be your female plants, and three plants (1, 2, and 3) from variety B that are your male plants. Make four crosses on four different flowers on plant A1 using pollen from plant B1 (A1 x B1). Follow the same procedure using plants A2 and B2 in a cross (A2 x B2) and plants A3 and B3 in a cross (A3 x B3). When you collect, process, and clean the seed from these crosses, keep all of the seed from each cross together but separate from the rest (i.e., the seed from A1 x B1 is collected, processed, and cleaned separately from the seed from A2 x B2 and A3 x B3). This is illustrated in the following diagram.

Year 2

OSA Method

The generation following your cross is known as the first filial, or F1, generation. All of these plants should look pretty identical, assuming the parent varieties were highly uniform. Plant 3 to 6 F1 offspring from each cross you have made. The progeny plants should look distinctly different from the parent varieties. Planting out the parent varieties alongside the progeny can be very helpful as a visual comparison. If self-pollination has occurred



instead of a cross, the offspring will resemble the maternal parent. Collect and bulk seed from several fruit of each original cross, keeping seed of each cross separate from the others.

Pedigree Method

Plant a half-dozen plants using the seed from one of your crosses. Keep the seed from the other two crosses as **remnant seed** in case of a crop failure or unforeseen problem. Collect seed from several fruits from each plant and bulk the seed.

Year 3

OSA Method

This next generation is known as the second filial (F2) or first selfed (**S1**). This year you will begin to see the expression of genetic variability created in the cross. However, the genetic variability won't be fully expressed in this generation, so intensive selection should be avoided. Any selections should be based on highly heritable traits, such as growth habit, fruit size, shape, color, maturity, and disease or pest resistance.

Plant several small populations from your multiple crosses, aiming for a total population size somewhere between 50 to 200 plants. Plant as many plants from each cross as you have the time and space for, as evaluation and selection will be minimal at this point. For example, if you started with three crosses, grow a population of at least 15 to 30 plants from each in this generation. However, consider that as you progress to **family selection** in the next several generations your total population size can substantially increase. If you are working

with a cross between two distinctly different types of tomatoes (i.e., an orange slicer and a red grape) and/or are aiming to combine a suite of multiple traits, you will need to maintain large populations. If you are working with a cross between two similar tomatoes (i.e., two red slicers) and want to improve only a few traits, you can work with smaller population sizes.

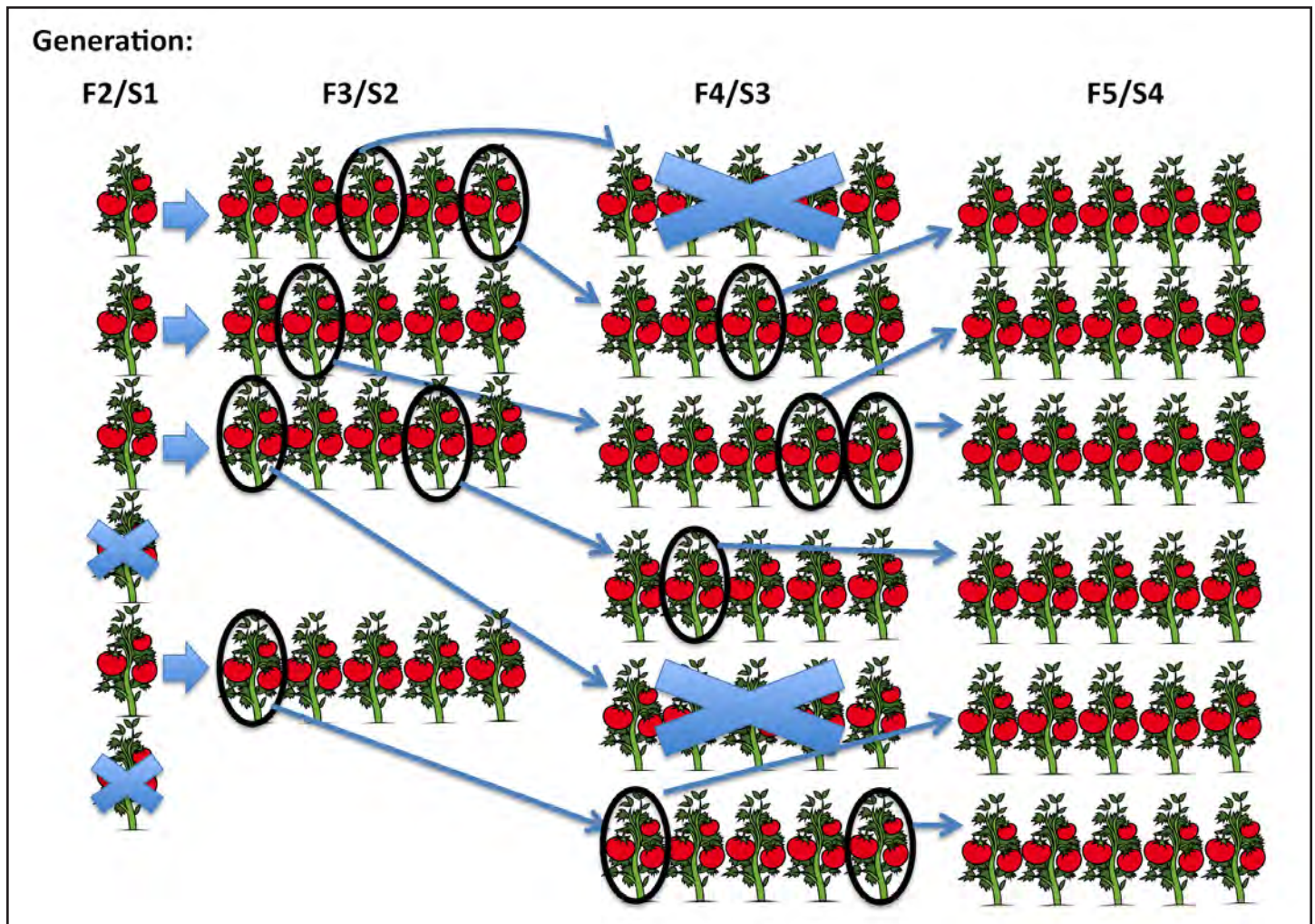
Discard plants that are obviously or fatally flawed or easily succumb to disease. Taste test fruit from every plant and discard any plants with terrible or off tasting fruit. Strive to maintain the breadth of diversity and variability you see in this generation in your selections. Save seed from several fruits from as many of the remaining plants as you can practically handle, and keep seed from each plant separate. In the next season you will begin family observation. Each plant you save seed from in this season will become a family with five or more plants in the next season, so plan the number of your selections accordingly. See the diagram below.

Pedigree Method

Plant a minimum of 100 plants, preferably 200, from the F1 seed. Selections are made to encompass the range of diversity expressed in the population. Select approximately 30% of the population: at least 30 individual plants from a population of 100. Save seed from 2 to 3 fruits on each. Keep seed from each plant separate. These plants now represent 30 or more distinct families. Depending on resources, time, and facilities, some public and private breeding programs will select more severely in this generation, keeping only 1 to 10% of plants in the population.

The following diagram illustrates how to set up and use family selection in the F2/S1 through F4/S3 generations. Selected plants from the F2/S1 generation are planted out to family rows the following year to create the F3/S2 generation. Each selection (plants that are circled) made from each of these families will become its own family in the following year to create the F4/S3 generation. Entire families can be eliminated in this generation and desirable individuals are selected. This cycle is continued for as many generations as necessary to arrive at material that reflects the desired ideotype and meets the breeding goals of the project.

each of these families. At this point you still want to maintain a high degree of diversity and variability in your selections, and you still want to discard plants that are obviously or fatally flawed, taste terrible, or easily succumb to disease. Again, you only want to select for highly heritable traits. Collect seed separately from as many plants as you can reasonably work with as families in the next generation. Try to collect seed from the same number of plants as in the previous year so that your population size does not become unmanageable.



Year 4

OSA Method

For the third *filial* (F3) or second selfed (S2) generation, plant each of your previous generation selections to single family rows with as many plants per family row as is practical. Since you potentially saved seed from 50 or more individual plants in the previous generation, plant 5 to 20 individuals from

Pedigree Method

Plant 15 to 25 plants from each family into 30 separate rows. This generation will display the greatest range of expression of genetic diversity. In this round of selection the aim is to maintain the full range of variation in the population while striving to maintain the same approximate population size. Select 30 individuals and save seed from 2 to 3

fruits. Keep the seed from each plant separate.

Year 5

OSA Method

In this generation, F4/S3 individuals within each family should start to look similar and differences between families should start to become more obvious and distinct. Discard entire families that do not meet breeding goals or are performing poorly.

From each F3/S2 selection, again plant family rows with 5 to 20 individual plants per family. At this stage, you can begin a more rigorous selection process. You can start to select for more **quantitative traits**, such as concentration of fruit set, yield, complex disease resistance, canopy cover and plant architecture, and quality traits, such as flavor, texture, and resistance to cracking. Select plants with favorable trait combinations and again save seed separately from each. Keep the number of selected plants similar to the previous two years.

Pedigree Method

Plant 10 to 20 plants from each plant selected the previous year into separate family rows. Make another series of single plant selections from within the most desirable families. Discard entire families that do not meet breeding goals or are performing poorly. Keep seed from each individual plant separate.

Year 6 (and beyond)

OSA and Pedigree Method

In the F5/S4 generation, begin replicated trials. Planting three randomized replications allows for analysis and evaluation of quantitative traits

at this stage. Measurements can be taken and/or scores can be assigned to desirable traits. These numbers are then averaged over the three replications to illustrate and tease out the differences between families. If desired, basic statistical analysis can be performed at this point to determine which, if any, of the families are statistically different (rather than different due to chance) from the others. An **analysis of variance** (ANOVA) is the most commonly used statistical test for this type of evaluation.

Plant three family rows of 5 to 10 plants from each F4/S3 single plant selection. Discard undesirable families that do not meet breeding goals. Base selections on quantitative traits, such as yield, productivity, fruit distribution, set concentration, and fruit quality. Families should be uniform enough to rouge out any underperforming individuals. Bulk the seed from remaining individuals within each family. Sometimes single plant selections are continued for a few generations past the F5/S4, depending on the stability and degree of uniformity in the material at this stage. Replicated trials are continued in the F6/S5 and F7/S6 generations to determine the most desirable and robust selection for an eventual variety release.

From this point on you can either maintain the variety by continuing to preserve the families separately and make composite mixtures when needed or maintain the variety as a single population. If you have not achieved the desired combination of traits and qualities at this point, consider making controlled crosses again using the most desirable plants.

	Trait	Inheritance	When to Select
<i>Agronomic</i>	Growth habit	Indeterminant dominant, determinant recessive. Modifiers can result in semi-determinance	Early generations (F2 and beyond)
	Plant stature	Tall dominant, dwarf recessive	Early generations (F2 and beyond)
	Leaf shape	Regular dominant, potato shape recessive	Early generations (F2 and beyond)

	Trait	Inheritance	When to Select
	Canopy cover	Complex	Late generations (F4 and beyond)
	Maturity	Complex	Early generations (F2 and beyond)
	Pedicle attachment	Jointless recessive	Early generations (F2 and beyond)
<i>Defensive</i>	Disease resistance	Some dominance and some recessiveness depending on disease	Early generations (F2 and beyond)
	Drought tolerance	Complex	Late generations (F4 and beyond)
<i>Fruit quality</i>	Skin color	Yellow dominant, colorless recessive	Early generations (F2 and beyond)
	Flesh color	Red dominant. Red combined with green flesh produces purple/brown flesh. Green ripe gene results in bright green flesh.	Early generations (F2 and beyond)
	Fruit stripe	Dominant	Early generations (F2 and beyond)
	Green striping	Recessive. Color combination depends on alleles at red color gene location.	Early generations (F2 and beyond)
	Skin texture	Smooth dominant, peach recessive	Early generations (F2 and beyond)
	Flavor	Complex	Early and late generations (F2 and beyond)
	Texture	Complex	Late generations (F4 and beyond)
	Shape and size	Complex	Early generations (F2 and beyond)
	Green shoulder and uniform ripening	Green shoulders dominant, uniform ripening recessive	Early generations (F2 and beyond)
	Parthenocarpic fruit	Recessive	Early generations (F2 and beyond)

Table 1. Traits commonly selected for and when to select for them, along with their gene symbols and the type of inheritance they exhibit.

Glossary

Allele: one of two or more forms of a gene. Tomatoes have two alleles at each gene location.

Analysis of Variance (ANOVA): a common statistical test used to determine differences between group means.

Dehiscence: release or discharge of pollen.

Emasculation: removal of the male structures (anthers).

Exserted: thrust out.

Family: a group of genetically related plants.

Family selection: selecting individual plants or families based on the overall performance of a family.

Filial: relating to a generation or the sequence of generations following the parental generation.

Germplasm: the entire collection of genetic material for any given crop species.

Heterzygous: having different alleles at a given gene location.

Heterozygosity: state of having different alleles at one or more gene locations.

Homozygous: having the same alleles at a given gene location.

Homozygosity: the state of having the same alleles at one or more gene locations.

Hybrid: the product of a cross between genetically distinct parents.

Ideotype: an idealized vision of the perfect combination of all desired features for the growth, appearance, production and performance of a crop.

Inbred: a variety produced by successive inbreeding over a number of generations.

Inflorescence: a group of flowers arranged on a stem.

Locule: a chamber within the tomato fruit where the seeds and placental tissue are found. The number of locules in tomatoes can vary from 2 to 25, but is most commonly between 5 to 9.

Multiline variety: mixture of a number of pureline varieties or populations with similar appearances and qualities.

Parthenocarp: the natural production of fruit without fertilization of the ovaries.

Pedicel: the stem that attaches a single flower (and eventual fruit) to the stem of the inflorescence.

Phenotype: the observable, outward appearance of the crop.

Polygenic trait: relating to or controlled by multiple genes.

Pureline: genetically uniform variety developed by selecting a series of superior individual plants from a single cross.

Reciprocal cross: Two mirrored crosses made between a single set of parents where each parent serves as both the female and the male.

Remnant seed: seed reserved for planting at a later date.

S1 family: a family structure where the plants in the family all resulted from the same self-pollination.

Quantitative trait: synonymous with polygenic trait (*See polygenic trait definition*).

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