Plant Science Technologies: New Cultivar Development

The application of plant science technologies for the development of new mint cultivars

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Plant science development programs have had a traditional focus of seeking new cultivars or varieties that target improved quality and yield, plants with enhanced resistance to disease and pests, and, finally, specialty products having new quality characteristics and performance traits. This paper will present an overview of one approach and the steps taken during the development and commercialization process.

The Development, Evaluation and Commercialization of New Plant Cultivars

The A.M. Todd Company began developing new mint cultivars in the mid 1940s. In the late 1970s, using mutation breeding techniques with Black Mitchum peppermint, Merritt Murray released new cultivars to the industry having enhanced tolerance to *Verticillium* wilt, a devastating soil disease for peppermint. These cultivars allowed for the sustained production of peppermint in areas of the United States where *Verticillium* wilt would previously have prevented commercial activity.

The maintenance and development of a germplasm library is central to such a program. The oil quality and agronomic traits of more than 800 essential oil-containing plant species and varieties have been characterized, and more than 400 plants are maintained within the company's greenhouse system. The genetic diversity contained within this collection has been used to create new commercial cultivars for many different applications.

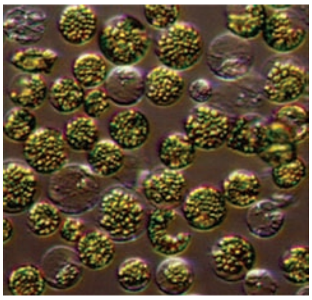
Cultivar and Variety Development

Industry practice for the development of new cultivars and varieties is approached in many different ways where both GMO (genetically modified organism) and non-GMO techniques are employed. For the purpose of this overview, a standard non-GMO approach of somaclonal variation will be presented. Importantly, variation through chromosomal changes is often inherent to plants produced through standard plant tissue culture techniques. By taking germplasm candidates with known and interesting traits, it is this variation that one attempts to capture as the basis and start of an interesting new cultivar or variety.

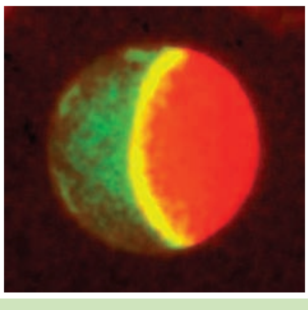
The non-GMO techniques of somaclonal variation, protoplast fusion and irradiation-induced mutations are all techniques that can be used to create new plants. Importantly, screening these new plants can lead to new cultivars having improved performance characteristics along with novel new properties. **F-1** is an example of a plant protoplast preparation that can be managed in several different ways, including direct culturing and protoplast fusion. **F-2** is an example of a protoplast fusion experiment where a dying treatment has been used to demonstrate a successful fusion of two distinct protoplast isolates. **F-3** is an example work to induce mutations through ultraviolet irradiation.

Once the protoplast preparations have been produced, the plant regeneration phase is initiated in order to produce a healthy plant. The process involves culturing the preparation in order to produce a growing colony or plant callus. Once a shoot emerges from the callus, it is transferred to a test tube containing a root inducing media. The evaluation phase begins once the new plant is healthy enough to be transferred to the greenhouse. A minimum

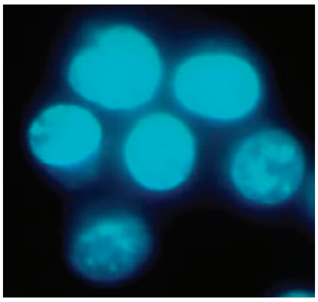
F-1 An example of a plant protoplast preparation that can be managed in several different ways, including direct culturing and protoplast fusion



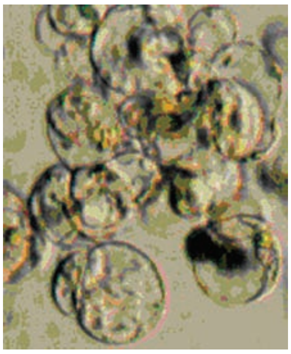
F-2 An example of a protoplast fusion experiment where a dying treatment has been used to demonstrate a successful fusion of two distinct protoplast isolates



An example work to induce mutations through ultraviolet irradiation treatments **F-3**



New plant development **F-4**



Field plot farm **F-5**

of six months is required for this process. Examples of the typical steps are shown in **F-4**.

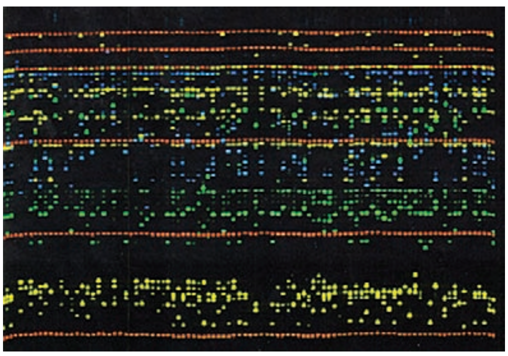
Plant Evaluation

The evaluation phase can be extremely challenging owing to the small amount of plant material that is available during the initial stages of its growth. Once the new plant has been established at the test tube stage, it is transferred to a four-inch pot for further development in the greenhouse. With mint, where the essential oil is the primary focus, the oil levels in these plants are very low and so alternate methods need to be used to make these preliminary assessments. Visual markers, leaf aroma and headspace analyses are all employed in order to make selections and reduce the number of candidates to a manageable number of test plants.

Moving forward, the test plants are then grown in two-gallon containers where, through the use of a special micro-distillation technique, sufficient essential oil can be collected for a compositional analysis and organoleptic assessment. Plants exhibiting interesting properties are then selected for field plot evaluation, and it is at this stage that yield and other agronomic measurements can be made. As a part of the field plot protocol, the plants are replicated and the studies are generally conducted over a two-year period.

F-5 shows one of three experimental farm sites where field plot studies are conducted. Importantly, vegetative propagation and rapid scale-up become critical aspects of the development process pursuant to the plant's commercialization.

Gene scan results **F-6**



Genetic fingerprinting of exceptional plants (**F-6**) begins at the time of the field plot phase with the data used to determine the plant's botanical classification. Importantly, the genetic fingerprint data are fundamental to a process of developing protection for the intellectual property pursuant to a future patent(s).

Building further on the matter of the botanical classification, plants within the genus *Mentha* often require several categories of data to be correctly identified and such categories would include taxonomy, essential oil chemistry and, as mentioned above, genetic profiling.

Classical taxonomy entails a study of the plant's physical features that are unique among other members of the group under consideration, i.e., other genera and species. Within the genus *Mentha*, physical characteristics of flower type, leaf structure and leaf shape and color are among the most important descriptors. The chemical profile of the oil is another important feature and, for this attribute, strong attention is given to the overall oil content and the interrelationships of the various ingredients within the oil. Finally, genetic profiling can be especially helpful with the identification of plants whose physical characteristics are very similar. Importantly, this work can give one the ability to distinguish different mint cultivars within the same species. Genetic profiling methodology involving micro-satellite markers has provided the best means for this level of discrimination.

Conclusion

Earlier sections have outlined a development process to create new cultivars of peppermint and cornmint and, in fact, these same disciplines may be relevant for many specialty herbaceous and other plants as well. In practice, plants with improved resistance to disease can be used to reclaim lost acreage. Additionally, these plants' essential oils may have optimized constituents. Finally, carefully selected plants can enjoy a superior yield, which helps growers address issues related to profitability in a market where high-input agricultural expense has become an issue. The application of plant science technologies is an opportunity to foster new product innovation through the introduction of new cultivars and varieties. These same technologies offer the industry the means to address market needs related to sustainability and improved economics.