Stone fruit breeding

AGROCIENCIA URUGUAY

Overall situation of peach breeding

Visón general sobre el mejoramiento genético del duraznero

Visão geral sobre o melhoramento genético do pessegueiro

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Abstract: Genetic breeding work requires continuity as well as a significant amount of time, work and resources (human, genetic and financial). Despite this, the development of new cultivars has become a fairly good business in the United States and in several European countries. Thus, most large American and European nurseries have their own breeding program. On the other hand, public institutions in several countries are experiencing a strong tendency to obtain and maintain germplasm, genetic studies and breeding techniques. Certainly, joint efforts, such as those mainly by the RosBreed group in the United States and Fruit Breedomics in Europe, are of great importance, as they provide important tools for classical breeders, mainly regarding to molecular markers. The most used method in cultivar development is still the controlled hybridization, followed by phenotypic selection and clonal propagation. Among the priority objectives of most peach breeding programs are: adaptation to climate change (low chilling genotypes; tolerant to high or very low temperatures); productivity; fruit appearance (without pronounced tip or bulge; attractive color, and large size), and fruit quality (sweeter flavor, and flesh firmness); stone adherence (free or semi-freestone for fresh consumption); fruit firmness and conservation (resistance to handling and transportation, low ethylene production) and disease resistance.

Keywords: breeding objectives, breeding methods, market trends.

Resumen: La actividad de mejoramiento genético requiere continuidad, así como mucho tiempo, trabajo y recursos (humanos, genéticos y financieros). A pesar de esto, en Estados Unidos y en varios países europeos el desarrollo de nuevos cultivares se ha convertido en un negocio. Así es que la mayoría de los viveros norteamericanos y europeos tienen su propio programa de mejoramiento genético. En las instituciones públicas de varios países existe una fuerte tendencia hacia un mayor esfuerzo para enfatizar la obtención y el mantenimiento del germoplasma, estudios genéticos y técnicas de mejoramiento. Evidentemente, los esfuerzos conjuntos, principalmente por grupos como RosBreed en Estados Unidos y Fruit Breedomics en Europa, son muy importantes, ya que proporcionan herramientas para el mejoramiento genético clásico, especialmente con respecto a los marcadores moleculares. El método más utilizado en el desarrollo de cultivares es aún la hibridación controlada, seguida de la selección fenotípica y la propagación clonal. Entre los objetivos prioritarios de la mayoría de los programas de mejoramiento de duraznero están: adaptación al cambio climático (baja necesidad de frío, tolerancia a temperaturas altas o muy bajas); productividad; apariencia de la fruta (sin punta en el ápice, ni sutura muy pronunciada, color atractivo y buen tamaño) y calidad de la fruta (sabor más dulce y textura de pulpa); adherencia del carozo (para frutas tipo mesa, preferiblemente priscos o semilibres); firmeza y conservación de la fruta (resistencia al manejo y el transporte, baja producción de etileno); y resistencia a enfermedades.

Palabras clave: objetivos del mejoramiento genético en *Prunus*, métodos de mejoramiento, tendencias del mercado.

Resumo: A atividade de melhoramento genético exige continuidade bem como, muito tempo, trabalho e recursos (humanos, genéticos e financeiros). Apesar disso, nos Estados Unidos e em vários países europeus, o desenvolvimento de novas cultivares passou a ser um negócio. Assim, a maioria dos grandes viveiros americanos e europeus tem seu próprio programa de melhoramento. Nas instituições públicas de vários países há uma forte tendência de um esforço maior no sentido de enfatizar obtenção e manutenção de germoplasma, estudos genéticos e técnicas de melhoramento. Evidentemente que esforços conjuntos, principalmente por parte de grupos como RosBreed nos Estados Unidos e do Fruit Breedomics na Europa, são muito importantes, por fornecerem importantes ferramentas ao melhoramento clássico, principalmente no que se refere a marcadores moleculares. O método mais utilizado no desenvolvimento de cultivares é, ainda, a hibridação controlada, seguida por seleção fenotípica e propagação clonal. Dentre os objetivos prioritários da maioria dos programas de melhoramento do pessegueiro estão: adaptação às mudanças climáticas (baixa necessidade em frio; tolerância a altas ou muito baixas temperaturas); produtividade; aparência dos frutos (forma mais arredondada, coloração atrativa e tamanho grande) e qualidade dos frutos (sabor mais doce e textura da polpa); aderência do caroço (solto ou semi-livre para frutas tipo mesa); firmeza da polpa e conservação da fruta (resistência ao manuseio e transporte; baixa produção de etileno); e resistência a doenças.

Palavras-chave: objetivos do melhoramento, métodos de melhoramento, tendências de mercado.

1. Introduction

Peach is one of the temperate climate species that has experienced high expansion in the world. Important production centers are generally located between latitudes 30º and 45º N and S[1]. At higher latitudes, problems with frosts or even bud freezing are a limiting factor and, at lower latitudes, there is usually lack of cold accumulation in winter and excessively high spring temperatures for the crop. However, genetic

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improvement programs combined with specific sites of altitude and differentiated plant management allow orchards to be found at latitudes as low as 18° S (as in Espírito Santo, Brazil) and even close to 0° latitude, in Ecuador.

Usually, the low chill cultivars produce inferior fruit quality (considering flavor, shape and even skin color) as compared to high chilling requirement cultivars. However, cultivars developed by breeding programs through controlled hybridizations have greatly improved those(2).

It is known that the first breeding program for low or medium chilling requirements was in Riverside, California, in 1907. In Brazil, the first one started in the State of São Paulo, around 1950. Throughout all these years, hundreds of cultivars were developed.

In an article on the history and characteristics of genotypes introduced in the United States, before the 1980s(3), Werner and Okie presented a table with 69 accessions in which only five had their chilling requirement below 600 chilling hours, and only two of them needed below or equal to 400 chilling hours. None of the accesses had a chilling requirement below 300 hours, considering temperatures <7 °C.

The situation has changed, and, as already mentioned, in the last decades, breeding for developing low chilling cultivars has allowed peach cultivation in subtropical areas such as the South of the USA, Southeastern Brazil, Southeast Asia, Australia, South Africa and areas of the Mediterranean(4).

Worldwide, hundreds of cultivars have been registered(5)(6), aiming mainly for adaptation to different regions with different climatic conditions.

This paper presents a summarized review of the current status of breeding programs, their objectives, trends, available marked assisted selection traits and a short discussion about cultivar protection.

2. Breeding programs

Peach breeding programs other than those in the United States were limited by the middle of the 20th century. In many countries, American cultivars were imported, tested and recommended, replacing local cultivars. However, many programs around the world have started or expanded since the mid-1980s(7). In contrast, several American public programs have either closed or moved more towards basic studies than to cultivar development. In the last 20 years, there has been a marked trend of the new cultivars' development being pursued mainly by private programs since there is no interest in investing public resources in long-term programs.

In the United States, breeding programs which still remain active are generally financed by royalties from the obtained cultivars(8). In 1998, private American programs devoted more than 90% of their efforts to the development of new cultivars, while public programs devoted only 36%, with the remainder effort divided among genetic studies, improvement of breeding and maintenance techniques and germplasm characterization. Probably for this reason, as horticulturist breeders (more connected to the field and traditional breeding) retire, they are usually replaced by geneticist breeders. These can better use methods to accelerate the whole process applying molecular markers or searching for new ones, and find the reactions that involve each process. Programs from Florida, California, South Carolina, New Jersey, Arkansas, Michigan, Georgia and Texas (which is planned to close in the coming years) are still very active in developing cultivars. But when looking at the total of registered American cultivars in 2018(9) the participation of public American programs decreased to less than 20%. Presently, peach breeding programs are found in several countries besides the United States, such as Bulgaria, Canada, France, Greece, Hungary, Italy, Poland, Romania, Serbia, Spain, Ukraine, China, Japan, Korea, New Zealand, South Africa(7), and also Australia , Brazil, Mexico, Taiwan(10), Uruguay and Argentina.

2.1 Public vs. private programs

Terrer[11] refers the following countries as having both public and private active programs: Spain, France, Italy and the United States, and cites Australia, Brazil, China, Taiwan, Thailand, Chile, Mexico and South Africa, with only public active programs. In fact, in addition to public programs, Brazil has one private program (Clone Viveiros, in the State of Paraná).

Some private programs are well known all over the world, such as Zaiger, Bradford, Maillard, Frutaria, Sunworld, Agromillora, among others[11]. In reference to low chill cultivars, breeding efforts are mainly being developed in Australia, Brazil, Mexico, South Africa, Taiwan and, in some American States like Florida and California. Recently, a new program started in Jujuy, Argentina.

In Europe, according to applications for cultivar registrations with the Community Plant Variety Office (CPVO), only 9% are from public institutions[11]. In Brazil, based on the records of the Ministry of Agriculture, Livestock and Supply (mapa), the figure is over 98%.

2.2 Objectives

In general, all breeding programs have at least some common objectives, such as to obtain cultivars adapted to the soil and climatic conditions to which it is intended to be planted, improving fruit quality to meet consumer's preference, increasing productivity, simplifying management, increasing disease resistance in the new material and reducing production costs. However, there are distinct peculiarities between different countries and even regions, which are reflected in the priorities of the respective breeding programs.

2.2.1 Climate adaptation

Although some programs, located in areas with very cold and regular winters, have bud resistance to cold damage as one of the priorities, currently, in a generalized way, one of the objectives of most breeding projects is the reduction of chilling requirement in the new cultivars. This is a consequence of the expansion of cultivation to subtropical areas and also under protected environments (greenhouse).

In 2002, Byrne[12] already referred to the trend in developing medium chill (350 to 650 chilling units) and low chill cultivars (less than 350 chilling units), either for cultivation under plastic greenhouse as in China, or for subtropical and tropical conditions, mainly in South America and North Africa.

In addition, climate changes that have been occurring, notably global warming, make the search for low chilling cultivars even more important[13]. Average temperatures have increased by about 0.74 °C during the last 100 years, and the estimate is that in 2100 the global average temperature will be between 1.8 °C to 4 °C higher, and it could even reach a difference of 6.4 °C[14]. This would result in less chilling units accumulation in the winter and as a result some cultivars will have delayed sprouting, prolonged flowering, low productivity and/or will produce undesirable shaped fruits with protruded apex, large suture and elongated shape[15][16][17], which justifies the need to develop new genotypes with lower chilling requirement.

But insufficient winter cold accumulation is not the only problem that comes with global warming. In some regions, temperature peaks near 30 °C during pre-flowering or flowering period have being occurring fairly often. High temperatures during these phases can cause low percentage of fruit set, either by negatively influencing pollen viability, or by shortening the stigma receptivity period or preventing fertilization[18][19][20][21]. However, there are differences between sensitivity of different genotypes to temperature stress. There are genotypes with greater plasticity and hence the importance of testing them in different environments.

2.2.2 Reduction of production costs

This is another important objective which can be achieved in different ways mainly by developing higher disease resistance cultivars —which means less losses and less need for spraying or developing cultivars with a different architecture of the plant in order to facilitate handling and reduce labor. Cost decrease could allow higher profit, benefiting the fruit grower and all the chain links.

2.2.3 Disease resistance

There are several pathogens that cause damage on peach. But when it comes to seeking genetic resistance to diseases, brown rot (in South America mainly caused by *Monilinia fructicola*) is probably the most studied and for which the most attention has been driven in breeding programs(22)(23)(24)(25). Although there are no sources of immunity to this disease, cultivars such as Bolinha (Embrapa Temperate Agriculture, Brazil) and Contender (Raleigh, Agr. Exp. Sta. North Carolina USA) are among the most used and considered as capable of transmitting a certain level of brown rot resistance to their progenies. Under some conditions, the fungus is unable to produce spores or the sporulation is truly little, which is important to prevent rapid disease spread in the orchard.

Another priority of most peach breeders is resistance to bacteriosis, caused by *Xanthomonas arboricola* pv. *pruni*. Research on this subject is well advanced and the RosBreed researchers group(26), mainly Dr. Ksenija Gasic and Dr. John Clark, from Clemson University and University of Arkansas respectively, with their teams and a team of phytopathologists have been working to combine *Xanthomonas* resistance in the peach leaf and fruit as well as in the development of DNA-based tests to identify potentially resistant genotypes to this pathogen. The Ppe-Xap DNA test, developed by RosBreed, has a prediction capacity of approximately 35% of the phenotypic variation for bacterial spot resistance in the fruit. Ppe-Xap consists of a single multiplex PCR test using four SSR markers that cover two genomic regions located in chromosomes 1 and 6, respectively. The test is already widely used in pre-breeding to select and combine parents with resistance alleles to one or both *loci*, and also in the early selection of seedlings with favorable allele combinations. In Brazil, Thurow and Castro(27), working with broad genomic association (GWAS) for resistance to bacterial spot in the leaf, identified several genes involved in defense mechanisms against the pathogen infection, with greater emphasis on two genomic regions, located on chromosomes 1 and 2, both harboring several resistance genes (R genes).

Field evaluations and laboratory inoculation were also carried out, being the genotypes Norman, Cristal Taquari, La Feliciana and Precocinho considered to be highly resistant and probably effective for use in breeding programs for resistance to *Xanthomonas arboricola* pv. *pruni*(27). Resistance to other diseases may be more or less important for a breeding program of one peach growing region or another, but they will not be as cosmopolitan.

2.2.4 Fruit appearance and quality

Consumer preferences for fruit vary from region to region and even among individuals. Generally, according to information from wholesales people of ceagesp (Companhia de Entrepostos e Armazéns Gerais de São Paulo), the largest gross market for fruits and vegetables in Brazil, preferences are for large peach fruits, white flesh and sweet flavor with low acidity. On the other side, a survey made through a questionnaire, consulting more than 600 people(28), concluded that the Brazilian peach and nectarine consumers prefer fruits with red skin and yellow flesh, followed by white flesh with red skin and, in any case, freestone fruits. The criteria used by most people when purchasing fruits would be appearance, absence of defects, followed by their price and fruit size. Nevertheless, worldwide, today's preferences move to sweeter fruits, with higher soluble solids. European countries and the United States show several different trends when compared to Brazil, where consumers still prefer peaches over nectarines, and round shaped over flat peach fruits.

Several Italian and Spanish authors point out that in the last 20-30 years breeding programs have focused on classic fruit-shaped patterns and were mainly dedicated to the extension of the harvest period(5)(29)(30). Sansavini and others(13) consider that the 1920s coincided with the golden years of peach breeding, in which American programs managed to achieve what the market wanted at that time: high yields of large, yellow and firm peaches. Some European breeders(29)(30) refer to the importance of obtaining a cultivar with desirable characteristics and being easily recognized by the consumer. They cite as main objectives the development of new types of peaches, such as flat shaped, low acidity, completely anthocyaninless (like the Ghiachios, from Italy) and/or with bloody flesh. In Spain, flat fruits, whether peaches or nectarines, are very appreciated for their sweet and aromatic flavor, they are easy for consumption and consumers can easily recognize them.

A steady supply of a series of cultivars producing very similar fruit in appearance and often in flavor makes it difficult to recognize each one, even though they may have different productivity and rusticity, and differ according to their region of origin and adaptation(29).

In summary, following the needs or preferences of producers and consumers, presently most breeders seek the following objectives: to extend the harvest period by anticipating or delaying harvest, in order to offer fresh fruit for the longest possible period; to reduce the chilling requirement in new cultivars; to modify the growth plant habit changing the architecture of the canopy in order to reduce the need for labor, which is now so little available in the field; to develop cultivars that are more resistant to the main diseases; to look for novelties (differentiated type fruits) and achieve the advances, in relation to the genetics and genome of the peach tree, or at least learn about them(8)(13). Added to the above, other goals involve the increase in the fruit size and skin color, ease management, long shelf life; taste improvement and "slow melting" texture(31), and for some regions, late flowering.

3. Marker-assisted selection

It is common to hear, in a plant breeding course, the master saying that genetic breeding is both: art and science. History shows that the further breeding advances as a science, the closer it must be to other research areas and the closer it brings together different groups of breeders. Genetic improvement has to be integrated with research areas such as phytopathology, physiology, ecophysiology, statistics, and surely, it is or should be in connection with those areas considered as basic or pure science such as genetics, genomics and bioinformatics.

Breeding programs can only evolve as quickly as the modern world needs, when a link is established between scientific genetic research and its application to the applied field, that is, to breeding programs. It was with the intention of establishing this connection that the Rosbreed project(32) in the United States and the Fruit Breedomics(33) in Europe started. With an integrated, multidisciplinary strategy, these groups were organized in order to improve the efficiency of *Rosaceae* breeding programs by the American group, and apple and peach by the European group.

Fruit Breedomics was devoted to the development of new tools or adapting existing ones; to study a wider range of characteristics to improve the selection criteria; to analyze and explore the genetic diversity; to generate innovative results (like methodologies, tools or vegetative material) that could be directly used by the breeders, and to establish a users´ network. Rosbreed followed a similar alignment and mainly emphasized search for disease resistance coupled with fruit quality, with a focus on developing and applying modern DNA tests and related breeding methods to obtain cultivars of eight main *Rosaceae* fruit species: apple, blackberry; peach, pear, rose, strawberry, sweet cherry and acid cherry. The first project of this group, entitled "Rosbreed: Enabling Marker-Assisted Breeding in Rosaceae", was developed between 2009 and 2013. It was renewed from 2014 to 2019 ("Rosbreed: Combining Disease Resistance with Horticultural Quality in New Rosaceous Cultivars"). Those projects resulted in a series of publications, the Genome Database for Rosaceae (GDR); new genotypes to be used as parents with valuable alleles; enrichment of germplasm, promising new selections, tools for DNA-based diagnostic tests and training of human resources, in addition to the formation of a researchers network[34] .

A very important part of those results is related to markers to be used in assisted selection, as the available tests for the presence of "blush", red on the fruit skin (Ppe-Blush); acidity (Ppe-Acid); flesh texture (Ppe-texture); peach × nectarine (indelG); white × yellow flesh (CCD4-SSR) and maturation season (G4Mat-SSR), in addition to the previously mentioned for resistance to bacterial spot (Ppe-Xap). For Xanthomonas the prediction capacity is 35%; for maturation 80%; flesh color, 100%; and red on the epidermis, 70%. Regarding the number and type of markers, already validated, for selected traits, for red on the epidermis (blush) it is one SSR type marker; for acidity two, of the same type; for texture, two SSR markers (endo-PG and SMF); for Xanthomonas, four SSR markers; for peach × nectarine there are also four SSR as well as for maturation, whereas for flesh color is one SSR marker (conversation with Dr. Ksenija Gasic, Clemson University; unreferenced).

Based on data from three breeding programs from three different countries, markers for sterility —an undesirable characteristic in peach— have been validated, and recently published[35].

Anyway, advances in genomic selection are promising and studies are rapidly emerging to define mathematical models for genomic prediction, as well as the optimum number of markers and plants to be used. However, phenotyping is still one of the limiting aspects for using these DNA-based tools and developing new markers. "Phenotypic and genotypic data are essential to link genetic variation with biological function, and thus document the function of the gene"[36].

4. Cultivar protection and intellectual breeder property rights

In European countries, as well as in the United States, cultivar protection and charge of royalties has existed for many years. These rules facilitate the import of foreign cultivars by nurseries and producers and even by private improvement programs, but on the other side, at least in Brazil, it makes it difficult to access to these genotypes by public institutions. Nevertheless, it is true that breeders' rights are an instrument that provides a safe business environment that allows breeders to receive recognition and compensation for their investments[37]. Considering data from 1950 to 2014, regarding plant breeders' rights (PBR) and more specifically royalties on peaches and nectarines[37], it was concluded that the adoption of these laws was very important in South Africa to allow access of foreign superior genotypes by the local breeding programs, contributing to increasing genetic diversity and access to superior materials. Analysis of those data showed that the strengthening of the legislation related to cultivars contributed to the development of new cultivars; reduced the concentration in the planting of one or a few cultivars; made it possible to extend the cultivation areas by using genotypes adapted to different conditions and improving fruit exports to other markets.

It is also known that, under certain situations, these royalties help to maintain active the breeding programs. However, on the same work above mentioned, the author warns about the danger of concentrating breeding efforts on crops that are more profitable in terms of royalties. For example, annual crops whose seeds are annually purchased could be preferred, in comparison to perennials crops such as peach trees. It is also pointed out that the system gives some advantage to producers with better financial conditions, advising that the provision of public or private funds for cultivar development programs would be the most correct way to support cultivars development.

Controversial or not, cultivar protection with payment of royalties is a measure that is here to stay, given the time that it has been maintained in several countries.

Protection of peach cultivars is quite recent in Brazil. The first application occurred in 2009, being brs Kampai (table peach cultivar) and brs Libra (a processing type peach) the first protected Brazilian cultivars(38). The complete list of protected peach cultivars in Brazil can be found on the website of the Ministry of Agriculture, Livestock and Supply(39).

Generally speaking, in Brazil, royalties are collected from nurseries that, through the necessary documentation, are licensed to propagate a specific cultivar. And as it was emphasized on the above mentioned South African paper, problems arise when the leaders of institutions and companies compare the royalties of large crops like soybeans with those from perennial crops, such as peach.

5. Conclusions and perspectives

There was a great effort by breeders regarding the genetic improvement of the peach and the development of cultivars. In Spain, for example, more than 10 peach breeding programs have emerged in the last decades, which are estimated to provide over 130 cultivars(29). Despite all this, most European countries, including Spain, have had a low consumption of peaches. So, an important step is to recover, getting back this market share. Of course, economics is today a limiting condition, but there must also be a commitment from all links of the peach production chain to meet consumers satisfaction. Torrente(40) highlights the importance of making everyone aware that the products are increasingly sophisticated and that peach is not only consumed as fresh fruit and, therefore, different characteristics are important for one or another segment.

The ideal cultivar that would please consumers worldwide cannot be developed, taking into account the different regional market characteristic and fruit destination. However, over the years, breeders are trying to get as close as possible to the ideal cultivar for each region.

It is a dynamic, never ending process, each time with more difficulties to which one can add the resources shortage —both financial and human. It is relatively easy to develop a cultivar in a situation when there are no good ones available. But as the programs advance, there is a competition with good cultivars already in the global market, and the development of an excellent one, mainly under marginal climatic conditions, like in most areas of the American Southern Cone, is not easy. Happily, it is not impossible and the low chill cultivars are an advantage when facing climatic changes.

Breeders all over the world have challenges to win, goals to achieve and, in general, scarce resources. They may not be sure if they will completely reach those goals. But they certainly will try.

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