CROP BREEDING AND APPLIED BIOTECHNOLOGY

PLANT BREEDING PROGRAMS

A brief history of the forty-five years of the Epagri apple breeding program in Brazil

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Abstract: *The E'AppleBP is the largest Brazilian apple breeding program in activity in Brazil, with Brazilian and international contributions to breeding of apple cultivars, under public funding. The main objectives are development of new apple cultivars with good local climate adaptation, disease resistance, high yield, high fruit quality, good fruit storability and lower demand for orchard hand labor. Twenty-seven apple cultivars have been released, including 15 from local breeding crosses and four sport mutations. 'Fuji Suprema', 'Monalisa', 'Venice', 'Daiane', 'Luiza', and 'Kinkas' are most promising for commercial use. The other eight cultivars were released for use as pollinizers.*

Keywords: *Malus x domestica Borkh., climate adaptation, disease resistance, fruit quality.*

INTRODUCTION

Since the beginning of apple cultivation in Brazil, one of the main challengers for apple growers has been deficient adaptation of high chilling apple cultivars to the local climate, including Gala and Fuji and their sport mutations, which have been imported from colder regions of the world. Plant health management of the orchards is also a considerable challenge, since the local climate and the susceptibility of the current commercial apple cultivars highly favor the development of diseases. Deficient climate adaptation and high susceptibility to major diseases lead to low yield, low fruit quality and high production costs, which constitute the main shortcomings of the cultivars currently planted in Brazil. For these reasons, at the beginning of the 1970s, the first studies on apple breeding in Brazil were initiated, giving rise to the Epagri Apple Breeding Program - E'AppleBP. The initial objectives were to develop and/or introduce new apple cultivars well adapted to southern Brazilian climates that are resistant to the main apple diseases and have high yield potential and high fruit quality. Over time, additional objectives were added, such as: a) selection of good sport mutations of 'Gala' and 'Fuji', considering that these two have been the main cultivars for Brazilian growers and consumers; b) high storability and trees with a natural tendency to grow horizontal lateral branches, aiming to reduce hand labor on tree training; c) better equilibrium between vegetative and reproductive structures on trees, aiming to reduce the need for fruit thinning; and d) an increase in options of commercial cultivars in order to diversify the alternatives in the apple sector. The text that follow takes a historical approach to the E'AppleBP, reporting the routine involved in development of new genotypes and the main advances obtained over the last 45 years of work.

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AN OVERALL VIEW OF THE E'APPLEBP

In 1973, when the Empresa Brasileira de Pesquisa Agropecuária - Embrapa (Brazilian Crop and Livestock Research Agency) was established, public research on temperate-zone fruit was initiated in Brazil. It also marked the beginning of the E'AppleBP through partnership with the fruit breeding researcher from Rutgers University and New Jersey University - USA, Ph.D. Leon Fredric Hough, who gave assistance to the E'AppleBP up to the end of the 1980s. In 1975, the newly established Empresa de Pesquisa Agropecuária de Santa Catarina – Empasc (Santa Catarina State Agricultural Research Institution), replaced by the Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina – Epagri (Santa Catarina State Crop and Livestock Research and Rural Extension Agency) in 1991, assumed this responsibility, continuing the research at the Videira Experimental Station in the state of Santa Catarina. From 1975, research on apple breeding was transferred to the Caçador Experimental Station – EECd, also in Santa Catarina, where the work on apple breeding continues to develop. In 1975, the Apple Gene Bank was also established with the objective of concentrating all the available apple gene pools throughout Brazil and genotypes introduced from other countries, a research activity that also involved the American breeder Dr. Hough. Beginning in 1982 in the Highland Region of Santa Catarina, which has more severe winters, there was a considerable increase in apple production. For that reason, the Epagri Experimental Station of São Joaquim – EESJ, also became involved in the E'AppleBP.

The first segregate populations of apple evaluated by the E'AppleBP came from crosses made by Dr. Hough in the USA. The seeds were sent to Brazil for germination, and subsequently the seedlings were evaluated for the Rvi6 Scab resistance gene, formerly known as the Vf gene (Shay and Hough 1952). Some crosses from the USA have been made aiming to obtain genotypes with low chilling requirements involving the Anna cultivar as a parental line, which was released in Israel because of its very low chilling requirement. Among the hybrid populations imported from the USA, many promising selections have been identified and five new cultivars have been released from them, namely, Princesa, Primícia, Duquesa, Fred Hough, and Joaquina (Table 1). This last cultivar was released by the EESJ. Among these five apple cultivars, Primícia, Fred Hough, and Joaquina carry the Rvi6 gene for vertical resistance to apple Scab and also genes for a moderate chilling requirement. 'Duquesa' has the Rvi6 gene and low chilling requirement. 'Princesa' is a Scab susceptible cultivar, but with a low chilling requirement. It is now being used as a pollinizer of others cultivars of low chilling requirement, among them Condessa, also released by the E'AppleBP (Table 1), and Eva, a cultivar bred at the Instituto Agronômico do Paraná (IAPAR).

Hybridization in Brazil started in 1982 aiming at climate adaptation and disease resistance as special traits. For adaptation to the mild climate of midwestern Santa Catarina, parents were selected from the Apple Gene Bank, among them 'Mollie's Delicious'. For the Rvi6 Scab resistance gene, focusing on the highlands of the São Joaquim region, where Scab is more severe, pollen from resistant genotypes from the USA was used. Under this scheme, the main traits at EECd were low to moderate chilling requirements and horizontal resistance to the main diseases, using the Gala cultivar as female parent, aiming at high fruit quality. Meanwhile, at EESJ, the main focus was high Scab resistance, originally found in *Malus floribunda* var. 821 (carrier of the Rvi6 gene), using the Fuji cultivar as female parent, aiming at good flavor and long storability. The E'AppleBP has been conducted in an integrated scheme involving both Experimental Stations: EECd and EESJ. It is noteworthy that the E'AppleBP was the first to apply for plant variety rights for an apple cultivar in Brazil, in this case Daiane. It is the first apple cultivar subject to plant variety rights (PVR) in Brazil. Currently, the cultivars of the E'AppleBP that are also under PVR in Brazil are Kinkas, Joaquina, Monalisa, Luiza, Venice, and Elenise (the official full name of these cultivars is shown in Table 1).

Organization and breeding strategies of the E'AppleBP

Since traditional Gala and Fuji clones are not adapted to the climate in important regions of temperate-zone fruit production below 1,200 MASL in southern Brazil, the E'AppleBP is being developed in the EECd, midwestern Santa Catarina, at 960 MASL under a mild winter climate – 1,017 chilling hours on average. In this climate, the choice of parents for hybridization based on local climate adaptation (lower chilling requirement than 'Gala' and 'Fuji') augments the chances of obtaining advanced selections with good adaptation to the local climate in both regions simultaneously – in the highlands with severe winters and the midwestern part of the state with mild winters.

Figure 1 shows the current organizational scheme of the E'AppleBP involving both the EECd and the EESJ. In the EECd (mild winter climate), the initial breeding activities involving the Apple Gene Bank study, pre-breeding research, hybridizations, all the initial steps of seedling pre-selection, and field evaluation for advanced selection are developed. This strategy increases the chances of obtaining advanced selections carrying all the pre-established traits of the E'AppleBP, including simultaneous local climate adaptation in both aforementioned regions. This strategy avoids the need to develop an additional breeding program at the highlands in the EESJ, reducing breeding costs of the E'AppleBP.

Choice of parents, hybridizations and development of seedling populations

The importance of a suitable choice of parents for hybridization in apple is directly related to the chances of success of an apple breeding program (Janick et al. 1996). As apple is a typical temperate-zone crop with self-pollination being prevented by the gametophytic self-incompatibility system (Mir et al. 2016), it requires cross pollination. This condition results in high heterozygosity in nature, a common feature of the apple species (Hanke et al. 2007). Many characteristics of high agronomic value are under polygenic control (Janick et al. 1996), and the complexity of segregation for these characteristics requires careful choice of parents based on previous studies of heritability, a concern that has been

Table 1. General description of apple cultivars released by the E'AppleBP since the 1970s

Scion cultivars				
Cultivar	**Parents (♀ x ♂)**	**Year of release**	**Ripening period**	**Resistance to main diseases**
Princesa[1]	NJ-56[2] x Anna	1986	1st ten days/Jan.	GLS
Primícia[1]	D1R101T117[2] x D1R103T245[2]	1986	2nd ten days/Jan.	Scab[7]; GLS
Epagri 402-Catarina[4]	Fuji x PWR37T133[3]	1996	1st ten days/Apr.	Scab[7]; GLS
Epagri 403-Fred Hough[1]	NJ-76[2] x Coop-14[2]	1994	2nd ten days/Feb.	Scab[7]; GLS
Epagri 404-Imperatriz	Gala x Mollie's Delicious[2]	2000	1st ten days/Feb.	Scab[8]; GLS
Epagri 405-Fuji Suprema[5]	Sport mutant of Fuji	1997	3rd ten days/Mar.	GLS
Epagri 406-Baronesa	Fuji x Princesa	1997	2nd ten days/Apr.	Scab[8]; GLS
Epagri 407-Lisgala[5]	Sport mutant of Gala	1997	1st ten days/Feb.	-
Epagri 408-Condessa	Gala x Malus 41[9]	1998	3rd ten days/Dec.	Scab[8]
Epagri 409-Duquesa[1]	D1R100T147[2] x Anna	1998	2nd ten days/Jan.	Scab[7]; GLS
Daiane	Gala x Princesa	1998	1st ten days/Mar.	GLS
Joaquina[1]	NJ-76[2] x Coop-14[2]	2003	2nd ten days/Feb.	Scab[7]
Castel Gala[6]	Sport mutant of Gala	2005	3rd ten days/Dec.	-
SCS413 Fuji Precoce[6]	Sport mutant of Fuji	2009	3rd ten days/Feb.	GLS
SCS416 Kinkas[4]	Fuji x PWR37T133[3]	2009	3rd ten days/Mar.	Scab[7]; GLS
SCS417 Monalisa	Gala x Malus 4[9]	2009	1st ten days/Feb.	Scab[7]; GLS
SCS425 Luiza	Imperatriz x Cripps Pink	2015	1st ten days/Feb.	GLS
SCS426 Venice	Imperatriz x Baronesa	2015	1st ten days/Mar.	Scab[8]; GLS
SCS427 Elenise	Imperatriz x Cripps Pink	2015	3rd ten days/Apr.	Scab[8]; GLS

Pollenizer cultivars[10]						
Cultivar	**Parents (♀ x ♂)**	**Year of release**	**Blooming**			**Resistance to main diseases**
			Initial	**Period**	**Intensity**	
SCS429 SMC1	Fred Hough x Imperatriz	2015	Sept. 25	20 days	High	Scab; GLS; Mildew
SCS 431 Felix 1	Baronesa x Open Pollination	2015	Sept. 18	27 days	Very high	Scab; GLS
SCS 432 Felix 2	Baronesa x Open Pollination	2015	Sept. 10	28 days	Very high	Scab; GLS; Mildew
SCS 433 Felix 3	Baronesa x Open Pollination	2015	Sept. 1	30 days	High	Scab; GLS
SCS 434 Felix 4	Baronesa x Open Pollination	2015	Oct. 5	20 days	High	Scab; GLS; Mildew
SCS 435 Felix 5	Baronesa x Open Pollination	2015	Sept. 25	27 days	Very high	Scab; GLS; Mildew
SCS 436 Felix 6	Baronesa x Open Pollination	2015	Oct. 10	15 days	High	Scab; GLS; Mildew
SCS 430 Felix 7	Baronesa x Open Pollination	2015	Sept. 25	30 days	Very high	Scab; GLS

GLS: Glomerella Leaf Spot; [1] Cultivars selected from seeds imported from the USA; [2] Parents from the USA; [3]Parent for which pollen came from the USA; [4] Cultivars developed by using pollen from the USA; [5] Sport mutations for better red skin color; [6] Sport mutations for early fruit ripening; [7] Vertical resistance (Rvi6 gene); [8] Good level of horizontal resistance (minor resistance genes); [9] Local selections: Malus 4 is a descendant of Prima x Anna; Malus 41 is a descendant of NJ-56 x Anna; [10] The Felix series was selected from open pollination of the Baronesa cultivar as female parent with the wild sub-species pollinizers *Malus eley and Malus baccata,* and the ornamental wild apple *Golden Gem*, with the objective of offering apple growers cultivars that are highly efficient as apple pollinizers for commercial apple cultivars.

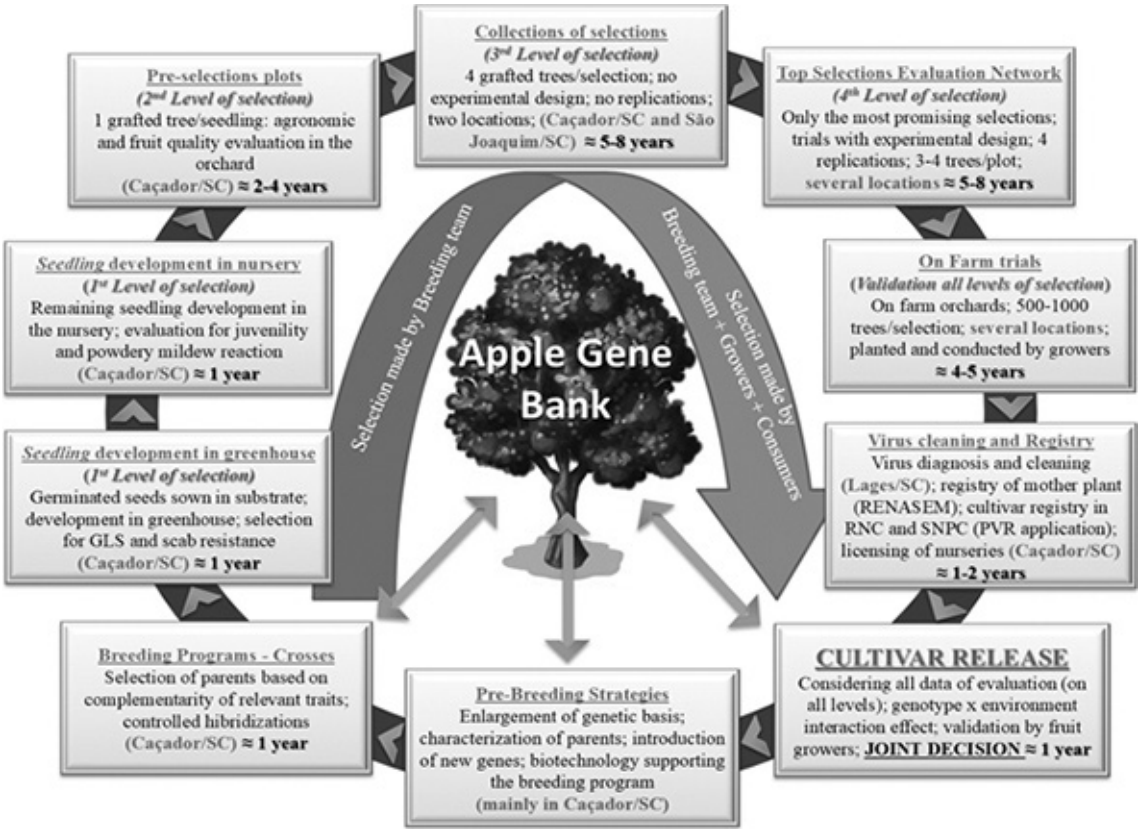

Figure 1. Scheme showing the organizational structure and the phases of the Epagri Apple Breeding Program (E'AppleBP), now involving the Epagri Experimental Stations of the municipalities of Caçador, São Joaquim, and Lages. An average of 22 to 31 years is spent on developing, releasing, and recommending a new apple cultivar for commercial use.

always a focus of the E'AppleBP. Previous and detailed knowledge of each available parent and its ability to transmit good characteristics to its progenies is of fundamental importance for decision-making regarding parental combinations. Any mistake in this step will certainly compromise the quality of the final results of long years of expensive research. For example, despite its limitations concerning deficient climate adaptation in Brazil and its susceptibility to many diseases, 'Gala' was one of the parents most used at the beginning of the E'AppleBP, because its seedlings obtained by artificial crosses tend to show superior phenotype, mainly for traits related to good flavor and attractive fruit skin finish. The E'AppleBP released four cultivars from crosses involving 'Gala': 'Imperatriz', 'Condessa', 'Daiane', and 'Monalisa' (Table 1); the last three are already commercially planted in the south of Brazil, and 'Daiane' and 'Monalisa' are already under PVR in the European Community.

As some traits important for apple breeding are determined by many genes, it is important to have large number of small populations to obtain information on the segregation pattern of each parent. Thus, in the E'AppleBP routine, we initially made a large number of limited crosses (no more than 500 seedlings per cross). However, when the parents are known to be good from previous evaluations, a smaller number of extensive crosses (1,000 to 3,000 seedlings) are usually made. The results obtained from big populations involving some previously studied parents of the E'AppleBP, such as 'Imperatriz', 'Baronesa', and 'Princesa', have been very successful. Many cultivars of commercial importance, including Daiane, Luiza, Venice, and Elenise, have been released from crosses involving these parents (Table 1).

However, whenever open pollination (OP) was used, where the male parent is unknown, the results obtained up to now have not been promising. From 13 OP progenies, for a total of 12,417 seedlings, even with an average of 955 seedlings per progeny, it was possible to get selections from only two of them (Imperatriz x OP; Baronesa x OP). But, unfortunately, no one new cultivar for commercial fruit production was released from them.

In following, some contrasting results based on parent choice and size of seedling populations are described: a) from crosses made from 1983 to 2007 using randomized parental combinations, totaling 157 crosses and 86,469 seedlings, 74 (47%) of the combinations did not generate any selection; b) from 110 small parental combinations (≤ 500 seedlings each), even though 74 selections were obtained, no one was released as a new cultivar; and c) from 47 extensive crosses (> 500 seedlings each), made according to previous knowledge regarding parental value, 374 selections from a total of 55,797 seedlings were obtained, and six of them were released as new apple cultivars. The best progeny results were obtained from the crosses Mollie's Delicious x Gala, Imperatriz x Cripps Pink, Imperatriz x Baronesa, and Imperatriz x Fred Hough. From these four combinations alone, 128 selections were obtained from a population of 12,630 seedlings, and four new cultivars were released: Imperatriz, Luiza, Venice, and Elenise. From them, 'Imperatriz' (descendent of 'Mollie's Delicious'), is not of commercial value, but it has shown great efficiency in segregating for fruit quality (size, shape, skin color, and flavor), good disease resistance (Scab, Glomerella Leaf Spot, Mildew, and Bitter Rot), and an even lower chilling requirement then 'Gala'. Another important parent is 'Princesa'. From 12 cross combinations using this cultivar as one of the parents, 111 selections were obtained from a population of 9,987 seedlings, and two were released as new cultivars, Baronesa and Daiane (Table 1). More recently, the parental combinations most effective for creating new cultivars carrying the desired traits were Imperatriz x Baronesa and Imperatriz x Cripps Pink. From these two crosses, 'Luiza', 'Venice', and 'Elenise' were released, classified as "apples for the future" according to the concept of Sansavini et al. (2004), describing current tendencies in commercial apple use worldwide. For these authors, "apples for the future" are characterized as having firm, crisp, juicy flesh with high sugar and high acidity content, also known as "JFC apples"- Juicy-Firm-Crispy Apples.

In addition, it should be noted that the apple breeding process tends to lead to the same situation observed in other plant species, a narrowing genetic base over time - endogamy. In order to avoid genetic problems from endogamy, risks of deleterious effects caused by an increase in parental similarity (Brown and Maloney 2009), the introduction of foreign pollen with different genealogy is very important and has recently been adopted by the E'AppleBP.

Development of seedling populations and disease inoculation

The methodology of performing crosses in the field, seed collection and germination, and the first steps of growing seedlings in the greenhouse is described in Denardi et al. (2013). Screening for disease resistance carried out under artificial inoculation were used only for populations with at least one parent carrying vertical resistance to Scab (Rvi6 gene) and/or to Glomerella Leaf Spot - GLS.

Routine followed for pre-selecting seedlings in the nursery (Level 1)

Pre-selection for short juvenile period

The juvenility of apple seedlings is the period from seed germination to first fruiting and is considered to be the main factor that slows release of new apple varieties (Hanke et al. 2007). It can extend up to eight years or even more. This trait is positively correlated with the number of spines along the main stem of the seedlings and with the time that adult trees take to come into fruiting after grafting, known as the vegetative period. This means the fewer spines, the shorter the juvenile period of the seedling (Hanke et al. 2007). Since this trait is only expressed in the young phase of the seedlings, the first step of selection in the E'AppleBP is carried out while seedlings are still in the nursery, discarding all those with a large number of spines along the main stem. Three contrasting examples can be cited regarding this trait: a) Princesa: with a very short vegetative period, it efficiently transmits this trait to its progenies, and there is practically no presence of spines; b) Gala: with an intermediate vegetative period, it transmits few spines to its progenies; c) Fuji: with a long vegetative period, it generally transmits many spines to its progenies. Among the 15 scion cultivars obtained by crossing and released by E'AppleBP (Table 1), 10 have a shorter vegetative period than 'Gala', an indication that the indirect pre-selection process based on the presence of spines on seedlings has been efficient. Another important trait related to shortening of the juvenile period is seedling vigor, conversely related to the transition from the juvenile to the adult phase in apple (Hanke et al. 2007). The faster the growth, the shorter the time for the seedling to achieve the adult reproductive phase. This is another criterion used for selection by the E'AppleBP, aiming to shorten the fruitless period of the seedlings and, consequently, to shorten the time necessary for selecting seedlings in the field and releasing new cultivars. This is to emphasize the importance of ensuring the best environmental conditions for growing the seedling

populations in the field. Under this postulate, the vegetative material for grafting is always collected at the highest part of the main stem of the pre-selected seedlings.

Routine for selecting seedlings grafted on rootstock (Pre-selections – Level 2)

Aiming for first fruiting at an earlier time, all pre-selections are grafted on a dwarfing rootstock. In this step, only one tree per hybrid is grafted and evaluated in the field, considering that the number of pre-selections evaluated annually is usually large (1,000 to 4,000 trees for each pre-selection block). The seedlings on dwarfing rootstock are evaluated for sprouting potential (climate adaptation), resistance to the main local diseases, and fruit quality. In this step, evaluation is carried out for maximum four years from the time of grafting up to the time of final decision on selection. Pre-selections that take more than four years from grafting to the first fruiting are considered to have an excessively long juvenile period, a condition that justifies discarding the pre-selection.

Screening for resistance to the main apple diseases

- Powdery Mildew (*Podosphaera leucotricha*): This is a spring disease that attacks the young parts of the tree and, consequently, limits the growth of branches. As it is a disease under polygenic control (Janick et al. 1996), and of low severity in most climate conditions in southern Brazil and rarely attacks the fruit (Boneti et al. 1999), screening for resistance is conventionally performed by using a visual numerical scale for spontaneous infection by the disease, from 1 = severe attack to 5 = absence of the disease. In the E'AppleBP, the minimum tolerable score is '3'. The results indicated 'Imperatriz', 'Baronesa', and 'Catarina' as good parents aiming at powdery mildew resistance, whereas 'Gala', 'Fred Hough', and 'Princesa' transmit high powdery mildew susceptibility to their progenies.

- Apple Scab (*Venturia inaequalis*): This was one of the most severe diseases in apple in the south of Brazil up to emergence and dissemination of Glomerella Leaf Spot (GLS) from the end of 1980s (Katsurayama et al. 2000). However, Apple Scab is still considered the most dangerous apple disease worldwide (Sansavini et al. 2004). In southern Brazil, it is more severe in orchards located in regions above 1,200 MASL, where the highly susceptible clones of 'Gala' and 'Fuji' are prevalent (Boneti et al. 1999). From 1982 to 2009, the E'AppleBP worked on two lines for Scab resistance: a) Horizontal resistance – under polygenic control: evaluations were carried out in the field and only at EECd, under natural infections over sequential years of evaluations. The cultivars Condessa, Imperatriz, Baronesa, and Venice can be included in the group with a good level of horizontal genetic resistance to apple Scab; b) Vertical resistance: this is conferred by a dominant allele of the gene Rvi6. Progenies from parents carrying this gene were screened in both EECd and EESJ through artificial inoculation of the disease at the young seedling phase (6-8 true leaves) under controlled temperature, moisture, and photoperiod conditions. Only resistant seedlings were transferred to the nursery, where all the remaining evaluations, for both types of resistance, were performed under the same methodology. Among the apple cultivars released, Duquesa, Primícia, Fred Hough, and Monalisa (in the EECd), and Joaquina, Catarina, and Kinkas (in the EESJ) carry a dominant allele for the gene Rvi6 for vertical Scab resistance. From 2009 until now, the E'AppleBP has concentrated all apple breeding efforts on vertical resistance to apple Scab (line 'b').

- Glomerella Leaf Spot - GLS (*Colletotrichum* spp): Studies carried out at Epagri show many apple genotypes at the Apple Gene Bank carrying high resistance to this disease (Furlan et al. 2010). However, very important genotypes in Brazil are highly susceptible to GLS, including Golden Delicious, a variety widely used as a breeding parent in the past, its descendant Gala, the most Gala-colored sport mutations, and Cripps Pink. The screening of hybrids for resistance to GLS is performed at the young seedling stage (10-12 true leaves) by using artificial inoculation of the disease under controlled temperature and moisture, according to the methodology explained by Furlan et al. (2010). Most of the many selections under evaluations in the EECd and 22 of the 27 scion cultivars released by the E'AppleBP carry vertical resistance to GLS. According to Katsurayama et al. (2001), resistance to GLS is under control of a recessive *genotype* for a single *gene*. Under this postulate, the last crosses in the E'AppleBP were made with both parents resistant, assuming that, in this case, all the progenies obtained will be resistant to the disease, excluding the need for artificial inoculation.

- Bitter Rot **(***Glomerella cingulata*): This disease has been a concern of the apple production chain in Brazil, especially during wet and hot years and in clones of high susceptibility, such as Golden Delicious. The evaluation for resistance is performed annually by a visual procedure based only on natural infection of the disease in fruit, identifying and discarding

trees with any symptom of the disease. Complementary evaluations have been carried out under artificial inoculation of the disease in the EECd. The results indicate high susceptibility of some of the main apple cultivars, among them Gala, Fuji, and Golden Delicious. The cultivars Monalisa and Fred Hough, and the local advanced selections M.13/00, M.15/01, and M.1/02, all developed by E'AppleBP, showed good level of resistance to this disease (Denardi et al. 2003). All these five genotypes obtained by E'AppleBP also have a dominant allele for the gene Rvi6 that confers resistance to Scab and show GLS resistance.

Selection for local climate adaptation

The deficient climate adaptation of the current commercial apple cultivars is typical for most minor regions below 1,200 MASL in southern Brazil. The consequence is deficient bud sprouting and even reduction in fruit quality regarding size, shape, and overall skin finish. In this context, one of the main aims of the E'AppleBP is to develop varieties with lower chilling requirements than 'Gala' and 'Fuji' have. The evaluations in the E'AppleBP for sprouting potential of the advanced selections are being carried out in Caçador (EECd) according to the methodology described by Denardi et al. (2012). Results obtained by E'AppleBP indicate the cultivars Imperatriz, Baronesa, Fred Hough, Joaquina, Monalisa, and Princesa as good parents for medium chilling requirements. The gains obtained for this trait over the 45 years of the E'AppleBP are significant. Now Epagri has a wide range of cultivars and advanced selections with a bud sprouting index at least 60% superior to their ancestor Golden Delicious.

Selection for fruit quality and fruit storability

A post-harvest laboratory at EECd gives support to the studies and evaluations of apples from E'AppleBP. Considering Brazilian and worldwide trends, the E'AppleBP has given priority to select for red skin color, preferable with stripes as on 'Gala'. 'Gala' and 'Fuji' has been used as models of size and shape. For flavor and flesh texture, the priority is selection according to typical models of the "apple for the future", looking for fruit with firm, crisp, juicy flesh with both high sugar and acidity contents (Sansavini et al. 2004). Monalisa, Luiza, Venice, and Elenise can be included in this category among the cultivars that have been released by the E'AppleBP. The first step of selection for sensorial characteristics of the fruit is performed in the field on pre-selection blocks (Level 2), tree by tree. An overall score is attributed to each plant using a numerical scale from 1.0 to 5.0, where 1.0 = the lowest expression and 5.0 = the highest expression of the trait. For scores above 3.0, 10 to 20 fruit samples go to the lab for more detailed physicochemical analyses and also for first studying storability potential. As a rule, the minimum values tolerated are 14 lb. for firmness and 12 ºBrix for sugar. Values from 25 to 45 are tolerated for the ratio between total soluble solids and titratable acidity; currently, the storability of 'Gala' is considered to be at the minimum level tolerated. In this respect, the new cultivars of the E'AppleBP, Venice and Elenise have excellent storability and very promising sensory characteristics.

Selection for fruit ripening and picking time

One of the main concerns of apple growers in Brazil is the excessively high concentration of hand labor in a short time for harvesting, in view of the limited number of commercial cultivars being planted. This culminates in a very high concentration of hand labor for harvesting in only two months. 'Gala' and 'Fuji' are responsible for around 95% of total Brazilian apple production (Petri et al. 2011). In the south of Brazil, the winters are usually short, especially below 1,200 MASL. Consequently, the vegetative period (spring/summer/autumn) is longer than in Europe. This climate allows the harvest period to be expanded, beginning one month before 'Gala' and up to 1.5 months after the picking time of 'Fuji'. The ripening of the E'AppleBP cultivars begins with Condessa and Princesa just after Christmas (a month before 'Gala'). In January/February it continues with 'Monalisa' and 'Luiza' and in March with 'Daiane', 'Venice', 'Joaquina', and 'Fuji Precoce'. The beginning of April is the time of 'Kinkas' and 'Fuji Suprema', mid-April of 'Baronesa', and the end of April/beginning of May of 'Elenise' (Table 1). This range of distribution of cultivars allows growers to move up and to delay picking time up to a month, allowing optimization of the use of hand labor for harvesting over a period of 4.5 months.

Agronomic evaluation of advanced selections (Levels 3 and 4)

The selections from Level 2 are tested in local collections at EECd and EESJ, characterizing the beginning of Level 3 of evaluations (Figure 1). In these collections, 3 to 4 trees for each hybrid are sequentially planted without experimental design, using an evaluation scheme similar to that adopted in commercial orchards. In order to be more reliable, all the

results obtained over the previous levels of evaluations are compared with those of Level 3, aiming to make a good final decision on maintaining advanced selections for farther studies or not. Evaluations at this Level take 5 to 8 years of work.

After that, the collections of advanced selections (Level 4) are set up under an experimental block design, with four replications, in climates and soils that represent the main apple production regions in southern Brazil. These regions are: a) the South Highlands of Santa Catarina, in EESJ, at 1,400 MASL with an average of 732 chilling hours ≤ 7.2 ºC; b) the Midwest of Santa Catarina, in the Fraiburgo region, at 1,000 MASL and 480 chilling hours ≤ 7.2 ºC on average; and c) the North Highlands of Santa Catarina, in the Papanduva region, at 800 MASL, with an average of 311 chilling hours ≤ 7.2 ºC. At the two first regions, advanced selections of medium to medium/high chilling requirements are evaluated, while in the last region, advanced selections of early fruit ripening – before 'Gala' - and low chilling requirements are evaluated. The advanced selections evaluated at Level 4 are all resistant to GLS. In addition to the local advanced selections, new cultivars or sport mutations with a commercial perspective for the Brazilian market from other breeding programs should be introduced in these collections.

In a complementary manner, a research line for evaluation of sport mutations is carried out as a parallel scheme to the E'AppleBP. For more details on the methodological procedure of this research line, see Faoro (2018). Among the 27 new apple cultivars of the E'AppleBP, four are sport mutations of 'Gala' ('Lisgala' and 'Castel Gala') and of 'Fuji' ('Fuji Precoce' and 'Fuji Suprema'), this last cultivar being one of the most planted in southern Brazil currently.

At Levels 3 and 4, the apple trees are evaluated for the following traits: tree vigor, bud sprouting level (climate adaptation), early fruit bearing, phenology of flowering and fruit ripening, and yield performance. For fruit, all traits related to visual and sensory quality are evaluated. In addition to these evaluations, physicochemical analyses of the fruit are performed in the post-harvest laboratory for the most promising advanced selections, including studies on storability.

Final evaluations, participatory research, and release of new apple cultivars

The final selections undergo further studies in on-farm trials, under a participatory research arrangement with apple growers, who assume responsibility for planting, management, and performance evaluations of the elite advanced selections for commercial purposes. The final objective is to validate all the information obtained by the E'AppleBP team on an experimental scale aiming to decide on a joint release of new apple cultivars. Additionally, a complementary objective of this step is to give experience to apple growers regarding new options of commercial apples. In this step, plant health diagnosis (virus status) is performed, and the mother-plant is registered in the Registro Nacional de Sementes e Mudas (RENASEM) [National Registry of Seeds and Seedlings], including the Distinctiveness, Homogeneity, and Stability (DHS) assay for registering the new cultivars in the Registro Nacional de Cultivares (RNC) [National Cultivar Registry] and in the Serviço Nacional de Proteção de Cultivares (SNPC) [National Cultivar Protection Service] for PVA application. Whenever necessary, the new cultivar(s) is (are) cleaned for viruses under lab conditions. The Epagri Experimental Station of Lages is currently assisting the E'AppleBP in this step (Figure 1). In any case, a sample of each new cultivar is introduced in the Apple Gene Bank in order to enrich genetic diversity for future use by apple breeders.

Main outreach activities developed over the 45 years of the E'AppleBP

Over the 45 years of the E'AppleBP, the following achievements are noteworthy: 1) Release of 27 new apple cultivars, including 15 local commercial obtained by controlled crosses, 4 sport mutations of 'Gala' and 'Fuji', and 8 cultivars for exclusive use as pollinizers for commercial apple cultivars (Table 1). One of these 8 cultivars, SCS429 SMC1, has normal apple traits, and the other 7, as Felix series, have wild traits (very small fruit, bitter flavor, and very intense blooming). Of these 27 cultivars, Fuji Suprema, Kinkas, Monalisa, Luiza, Venice, Elenise, and Daiane should be highlighted as most promising for commercial fruit production. 'Fuji Suprema' is now replacing the standard 'Fuji' in Brazil; 'Daiane' is being planted as an alternative for harvest in the gap between 'Gala' and 'Fuji' picking times; 'Monalisa' is already being planted as a good alternative for the same picking time of 'Gala', but with multiple disease resistance; and 'Luiza', 'Venice' and 'Elenise' are still under investigation by fruit growers for consumer acceptance of the fruit for decision making about future planting. 2) Numerous trainee positions for agronomists from Brazilian universities, research institutes, and fruit growing companies; openings for agronomists from Uruguay, Bolivia, Ecuador, Nicaragua, and the Philippines; and numerous assistance services for undergraduate and graduate students. 3) At least, 18 field days were carried out to show field performance of the new apple cultivars. 4) At least 11 foreign apple breeding programs were visited by the

E'AppleBP team, and numerous apple breeders from Brazil and other countries have visited the E'AppleBP over the last 45 years. 5) Hundreds of samples of E'AppleBP apple fruits have undergone quality evaluations by Brazilian and foreign evaluators in Brazil, involving public and private sectors.

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