

The Brazilian Pear Breeding Program

P.R.D. de Oliveira, P.S. Ritschel,
A.D.R. Rufato and J.C. Fioravanço
Embrapa Uva e Vinho
Bento Gonçalves
Brazil

I.D. Faoro and G.B. Leite
Epagri
Estação Experimental de Caçador
Caçador
Brazil

L.F. Dutra and N.A. Mayer
Embrapa Clima Temperado
Pelotas
Brazil

J. Degenhardt-Goldbach
Embrapa Florestas
Colombo
Brazil

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Abstract

Pear production in Brazil reached 17,391 tons in 2008, enough to provide for a mere 20% of the local market. The lack of adapted cultivars is a major obstacle to self-sustainable pear production in the country. In 2006, a novel nationwide pear breeding program was initiated, encompassing several research institutions and aiming to develop cultivars adapted to local growth conditions in an attempt to reverse the current pear importer status of Brazil. The program is structured in distinct research lines, including: enlargement of the genetic variation available in germplasm collections; formation and evaluation of segregating populations, hybrids and selections of scion and rootstock materials; network cultivar agricultural evaluations; screening of gamma-irradiated mutants of scion materials; and genetic transformation employing disease resistance genes. The main results obtained so far include: 12 scion populations consisting in a total of approximately 1,000 individuals; 220 rootstock seedlings (*Pyrus communis*) under mass selection in Pelotas, RS; 200 *P. communis* seedlings for rootstocks under field selection in Lages, SC; selection of six promising *P. communis* rootstocks; establishment of a cultivar evaluation network consisting of five cultivars ('Abate Fetel', 'Packham's Triumph', 'Rocha', 'Santa Maria' and 'William's') shared by all evaluation sites, and other five locality-specific cultivars; and 475 putative mutants under field evaluation.

INTRODUCTION

Pear production in Brazil reached 17,391 tons in the year 2008, from an orchard area of 1,609 ha (IBGE, 2010). The major producing state was Rio Grande do Sul, followed by the states of Paraná, Santa Catarina and São Paulo. In total, the production from the four states corresponded to approximately 95% of the total harvested during the season.

Historically, pear production in Brazil has been insufficient to supply the local market, requiring massive imports. In 2008, for example, 139,778 tons were imported, which is equivalent to eight times the amount produced in the country (ALICEWEB, 2010). Among the countries exporting to Brazil, Argentina is one of the most important, being solely responsible for more than 86% of the total imports of pear in the referred year.

The vast majority of the pear breeding programs is conducted in Europe and focus on using *Pyrus communis* and *P. nivalis*, aiming to develop materials displaying pyriform and aromatic fruits, with buttery texture and fine flavor (Bell et al., 1996). In Asia, cultivars have been produced directly from *P. pyrifolia*, *P. ussuriensis*, *P. × bretschnideri* and *P. pashia* or from hybrids derived from these species. These materials are different from the European cultivars due to their round fruit shape, smaller emission of aroma and crispy texture (Faoro, 2001). North-American pear breeding programs aim to develop hybrids between European and Asian materials to introduce disease resistance

maintaining the acceptable fruit quality. In the Southern Hemisphere, pear breeding programs are recent and have generated only a few results of commercial significance to date, with a notable exception being the release of the cultivar ‘Packham’s Triumph’, in Australia (Bell et al., 1996).

In countries exhibiting climates similar to Brazilian conditions, such as South Africa and Mexico, the breeding programs have focused on several aspects associated with climatic adaptation (Human, 2005; Rumayor et al., 2005). The joint program conducted by IRTA-P&FR is noteworthy due to its wide-ranging scope aimed at the development of scion materials of the European type, displaying high-quality fruits and able to produce efficiently under warmer conditions (Batlle et al., 2008).

In Brazil, pear cultivars were released by the Instituto Agronômico de Campinas - IAC and Embrapa Clima Temperado, although these were not able to provide plant material for commercial production in the country (Raseira and Nakasu, 2001; Nakasu and Faoro, 2003). Studies on pear adaptation carried out in the states of Rio Grande do Sul and Santa Catarina have showed that the commonly grown cultivars exhibit low and irregular yields when grown in those locations, indicating that a careful evaluation of the behavior of these genotypes is required, and also reinforcing the importance of investments to develop adapted cultivars with high commercial value (Simonetto and Grellmann, 1999; Faoro and Hentschke, 2000; Faoro, 2004).

Thus, the lack of cultivars capable of regularly providing high-quality yields under Brazilian conditions remains one of the major obstacles for self-sustainable pear production in the country. This fact, associated with the requirement for commercially acceptable alternatives to the existing crops for fruit producers, has posed novel challenges to horticultural research. In response to these needs, the research project “Development of cultivars to allow viable pear production in Brazil” was conducted from October 2006 to March 2010, aiming to provide adapted cultivars to the pear growth conditions in Southern Brazil, capable of high-quality regular yields. The research project was elaborated by organizing joint articulated actions converging to a multi-institutional effort in pear breeding. In order to continue the previous project, a new proposal has been initiated, entitled “Consolidation of the Brazilian pear breeding program”, starting in April 2010 until March 2014 (Oliveira et al., 2010). The current work presents a summary of the activities carried out in both projects, whilst also reporting their major results.

MATERIALS AND METHODS

The research activities were organized around four lines: 1) broadening of the germplasm available for the breeding program; 2) formation and evaluation of the scion and rootstock segregating populations; 3) installation of network trials to agronomically evaluate commercial cultivars and advanced selections from the breeding program, in order to survey their adaptation and to generate information to recommend their use to the growers; and 4) studies on mutation induction and genetic engineering to increase the genetic variation and to provide support technologies to the breeding program.

Broadening of the Germplasm Available to the Breeding Program

The Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina - Epagri maintains the Brazilian collection of pear germplasm at the Estação Experimental de Caçador. It consists of approximately 200 accessions, mainly of Asian origin, kept under field conditions. In order to provide enough support to the breeding program it requires the incorporation of European plant material, mainly those exhibiting potential for low requirements of winter chilling. Therefore, the goals of the current research line are the introduction of accessions requested from foreign germplasm banks of consolidated international importance, such as the pear germplasm bank maintained by USDA/ARS - National Clonal Germplasm Repository - NCGR - *Pyrus* Germplasm (Pear, 2010), and the molecular, morphological and agronomical characterization of the Epagri collection.

Development of Scion Segregating Populations

The goals of the present research line are: general plant adaptation, fruit quality, early fruiting and yield stabilization. In order to develop scion segregating populations, pollen was collected from commercial orchards and germplasm collections. Due to the diversity in the life cycle of the materials of potential interest, which exhibit distinct flowering times, a pollen bank was created. Populations of approximately 100 individuals were obtained, with the choice of parents based on agronomical features. The seeds resulting from the controlled crosses were germinated and the vegetative material of the scions was grafted to quince (*Cydonia oblonga*) for further evaluation.

For the current project, “Consolidation of the Brazilian pear breeding program”, two novel research line works are included under the topic: 1) evaluation of five scion selections, developed by Embrapa Clima Temperado, under the responsibility of Embrapa Transferência de Tecnologia; and 2) formation and evaluation of scion hybrids, under the responsibility of Epagri/Estação Experimental de Caçador, consisting of evaluating hybrids between European and Asian materials.

Development of Rootstock Segregating Populations

In order to investigate the effect of gibberellin concentrations and of period of seed imbibition in gibberellin solutions on the seed germination and initial development of *P. communis* seedlings, a sample of seeds was submitted to germination tests at the Laboratory of Seed Technology of the Faculdade de Agronomia Eliseu Maciel - FAEM/Universidade Federal de Pelotas - UFPel and of the Centro de Ciências Agroveterinárias - CAV/Universidade do Estado de Santa Catarina - UDESC. After the initial germination test, the seeds were submitted to distinct periods (24 and 48 hours) of seed imbibition in gibberellin solutions, and distinct gibberellin concentrations, from 0, 500 and 1000 mg L⁻¹, for a period of three weeks. The experimental design consisted of randomized blocks with 100 seeds per replicate. The emergence capacity and plantlet survival were evaluated. The recovered plants were transferred to the field for mass selection. The main observed characteristics were plant vigor and disease incidence. Less vigorous plants, evaluated by their stem diameter, were grafted and the effect of the mass selection was evaluated on the scion. Plants exhibiting disease symptoms under natural infection conditions were considered susceptible and eliminated. The evaluations were carried out under two contrasting climatic conditions, in Pelotas, RS (FAEM/UFPel), and in Lages, SC (CAV/UDESC).

Controlled crosses, open-pollinations and self-fertilization were carried out for *P. communis* and *C. oblonga* aiming to supply genetic material for the development of novel rootstocks, employing parents showing favorable genetic traits. The hybridizations were carried out with plants from UFPel and Embrapa Clima Temperado orchards. The definition of traits to be evaluated in the progenies was based on the traits of interest displayed by the parents used in each cross.

Agronomical Evaluation of the Cultivars

The network of agronomical evaluation comprised cultivars considered as of the highest interest and commercial potential, based on current knowledge. All scions were grafted on ‘Adams’ quince (*C. oblonga*), characterized by its low vigor and precocious production. The network was established in seven locations, representing a broad range of climatic and soil conditions. The participating locations were: in the state of Rio Grande do Sul: Eldorado do Sul (Estação Experimental Agrônômica - EEA/Universidade Federal do Rio Grande do Sul - UFRGS), Pelotas (Embrapa Clima Temperado), Vacaria (Estação Experimental de Fruticultura Temperada - EEFT/Embrapa Uva e Vinho) and Veranópolis (Centro de Pesquisa da Serra/Fundação Estadual de Pesquisa Agropecuária - Fepagro); in the state of Santa Catarina: Caçador (Estação Experimental de Caçador/Epagri), Lages (CAV/UDESC) and São Joaquim (Estação Experimental de São Joaquim/Epagri). The experimental design was complete randomized blocks, consisting of 10 treatments (cultivars), three repetitions and five plants per plot, supported by simple fencing system

and planted at the spacing of 4.0×1.0 m. Five of the cultivars are shared by all locations; ‘Abate Fetel’, ‘Packham’s Triumph’, ‘Rocha’, ‘Santa Maria’ and ‘William’s’, whereas the remaining five were chosen according to their adaptation expectations. It is planned to install, in 2011, one additional location in Itaquí (Universidade Federal do Pampa - UNIPAMPA), in the state of Rio Grande do Sul.

In the current project “Consolidation of the Brazilian pear breeding program”, two new trials investigating hybrid *P. communis* × *P. pyrifolia* material were included under the topic: 1) evaluation of five cultivars previously released by IAC (‘Centenária’, ‘Primorosa’, ‘Seleta’, ‘Tenra’ and ‘Triunfo’), carried out by the aforementioned institution; and 2) evaluation of five cultivars (‘Cascatense’, ‘Kieffer’, ‘Smith’, ‘Teen’ and ‘Triunfo’) in the region Serra Gaúcha, carried out by Fepagro.

Mutation Induction and Genetic Engineering

The experiments on mutagenesis, by gamma irradiation of in vitro scion shoots, were carried out by a partnership between Embrapa Clima Temperado and UFPel. The radiation doses used were 0, 5, 10, 20 and 30 Gy. The plants regenerated from irradiated shoots will be field evaluated, from the growing season 2010-2011 onwards, for vegetative and fruit features and for their response to biotic stresses.

Regarding the genetic engineering experiments, in the initial project, leaf disc explants, obtained from in vitro shoots, were used for regeneration experiments employing mannose as a selective agent. Gene transfer was carried out by *Agrobacterium tumefaciens* co-cultivation employing kanamycin as a selective agent. Additionally, in order to provide plant material for the genetic transformation and mutagenesis assays, in vitro plant propagation protocols were adjusted. The subsequent steps of the project will involve the stable transfer of disease resistance genes to commercially important scion and rootstock pear materials, employing *Agrobacterium*-mediated transformation.

RESULTS AND DISCUSSION

Broadening of the Germplasm Available to the Breeding Program

During a visit to the USDA/ARS NCGR-Corvallis - *Pyrus* Germplasm, a list of 150 pear accessions of potential interest to the Brazilian breeding program was prepared by the local researchers and the curator of the pear collection in the USA, Joseph Postman. The choice of the accessions was based on information on their low chilling requirement. The material to be imported will be solely constituted of in vitro plants to avoid the introduction of fire blight, caused by *Erwinia amylovora*. In 2009, 70 accessions were introduced and are currently being maintained in vitro, propagated and ex vitro acclimated for the establishment of a field collection.

The accessions from the Epagri collection are being characterized for their phenology and agronomical features. Moreover, the accessions will be fingerprinted by simple sequence repeats (SSR) molecular markers. The results from the characterization of the accessions provide support to the establishment of germplasm collections and to the choice of parents to be employed in the formation of segregating populations.

Development of Scion Segregating Populations

Throughout the four years of the project, 12 crosses were carried out: ‘Housui’ × ‘Abate Fetel’ and ‘Abate Fetel’ × ‘Housui’, in 2006; ‘Housui’ × ‘Rocha’, ‘Abate Fetel’ × ‘Packham’s Triumph’ and ‘Packham’s Triumph’ × ‘Abate Fetel’, in 2007; ‘Santa Maria’ × ‘Packham’s Triumph’, ‘Santa Maria’ × ‘Rocha’ and ‘Packham’s Triumph’ v ‘Rocha’, in 2008; and ‘Packham’s Triumph’ × ‘Kieffer’, ‘Packham’s Triumph’ × ‘Smith’, ‘William’s’ × ‘Kieffer’ and ‘Red Sensation’ × ‘Kieffer’, in 2009. Approximately, 1000 seedlings were obtained and the plants are undergoing distinct evaluation steps according to the development stage of the material. The first field for hybrid evaluations was established in October 2009, adopting the spacing of 4.0×0.5 m.

Development of Rootstock Segregating Populations

The significant influence of gibberellins on plantlet emergence has been reported, along with the linear increase of the germination percentage in response to progressive increases in gibberellins, ranging from 0, 500 and 1000 mg L⁻¹ concentration. The investigated periods (24 and 48 hours) of seed imbibition in gibberellins solutions did not appear to influence the germination of *P. communis* seeds. 220 plantlets were produced and transferred to the mass selection field at UFPel, in Pelotas, RS. At the CAV/UDESC, in Lages, SC, two areas were installed with *P. communis* plants, consisting of two populations of two and three years of age, respectively, both constituted of 100 plants. Plant evaluation was carried out from field trials at UFPel by applying 21 parameters described by the Ministry of Agriculture, Livestock and Food Supply, and from the trials at CAV/UDESC, employing two descriptors of plant height and diameter. From the evaluation of the material by mass selection, six individuals of *P. communis* exhibiting potential traits of interest for rootstock were selected. The selected plants are currently under vegetative propagation to supply material to establish a selection field.

Hybridizations by controlled crosses, open-pollinations and self-fertilization were carried out for *P. communis* and *C. oblonga*. In 2008, eight populations from distinct *C. oblonga* crosses were obtained, each population consisting of, at least, 150 individuals. The number of recovered seeds of *P. communis* hybridization was not sufficient to generate a population due to the low levels of fruiting after the crosses and self-fertilizations.

Agronomical Evaluation of the Cultivars

A network for cultivar evaluation was established, coordinated by the systematic application of similar management and cultural practices in all locations. Five cultivars, shared by all locations, 'Abate Fetel', 'Packham's Triumph', 'Rocha', 'Santa Maria' and 'William's', were planted in September 2008. The remaining cultivars were planted in October 2009 (Eldorado do Sul: 'Conference', Selection 6.93, Selection 9.93, 'Pera Branca' and 'Kieffer'; Pelotas: 'Conference', 'Forelle', 'Cascatense', 'Pera Branca' and 'Clapp's Favorite'; Vacaria: 'Conference', 'Forelle', 'Max Red Bartlett', 'Cascatense' and 'Clapp's Favorite'; Veranópolis: 'Conference', 'Forelle', 'Cascatense', 'Pera Branca' and 'Clapp's Favorite'; Caçador: 'Conference', 'Forelle', 'Max Red Bartlett', 'Pera Branca' and 'Clapp's Favorite'; Lages: 'Conference', 'Forelle', 'Max Red Bartlett', 'Pera Branca' and 'Clapp's Favorite'; and São Joaquim: 'Conference', 'Forelle', 'Max Red Bartlett', 'D'Anjou' and 'Beurré Bosc'). From the growing season 2010-2011 onwards, the phenology, yield and fruit quality evaluations will be carried out.

Mutation Induction and Genetic Engineering

The cultivars 'Ya Li' and 'Carrick' were submitted to in vitro propagation. Moreover, the cultivar 'Cascatense' was successfully established in vitro and is currently under propagation, employing MS medium supplemented with 30 g L⁻¹ sucrose, 0.1 g L⁻¹ myo-inositol, 1.1 mg L⁻¹ 6-benzylaminopurina and solidified with 7 g L⁻¹ of agar. These results will provide support to the mutagenesis and genetic engineering experiments.

Gamma irradiation trials were conducted according to the program established. The shoots of the cultivars 'Ya Li' and 'Carrick' regenerated after the treatment with gamma irradiation were acclimated in a greenhouse and 475 plants were transferred in August 2009 to field to be evaluated for growth and disease susceptibility.

The response of calli and shoots regenerating from leaf discs of in vitro cultivated plantlets to the selective agent mannose was tested. The concentration interval adequate for selection providing some regeneration, aimed at obtaining low rates of escapes was determined to range between 2.0 and 5.0 g L⁻¹ of the selective agent. A regeneration protocol was established for 'Ya Li' and 'Carrick'. Plant transformation experiments were conducted using the alternative binary vector pMOG402GUS, carrying as selective agent a gene conferring resistance to the antibiotic kanamycin. More than 10 transformation trials were conducted, but no transformed shoots were recovered.

CONCLUSIONS

The projects “Development of cultivars to allow viable pear production in Brazil” and “Consolidation of the Brazilian pear breeding program” constitute an effort to re-establish pear breeding research in Brazil. The institutions IAC and Embrapa Clima Temperado conducted pioneer work in this area of research. IAC released ‘Primorosa’, ‘Centenária’, ‘Seleta’, ‘Triunfo’ and ‘Tenra’ (Campo-Dall’Orto et al., 1996), while Embrapa Clima Temperado released ‘Cascatense’ (Nakasu and Leite, 1992). More recently, Epagri/Estação Experimental de Caçador was responsible for researches in pear breeding employing Asian materials.

From the initial efforts and from the current work carried out by several institutions, a novel nationwide breeding program was elaborated; it is constituted by a complex institutional arrangement that will provide promising results, even considering the difficulties imposed by the species biology. Therefore, it is expected that the planned and ongoing results of these two projects, coordinated by Embrapa, will help to enrich the germplasm available for the breeding program, form and evaluate segregating and hybrid populations of scion and rootstock material and provide network information on the agronomical performance of pear cultivars, thus constituting a solid supporting framework for the breeding program. In order to complement the aforementioned group of actions designed as classical breeding actions, the execution of research lines involving mutagenesis and genetic engineering were devised to increase the germplasm variation. The innovative aspect lies on the application of these tools to a temperate fruit breeding program developed in Brazil.

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