

Transgenic-Based Solutions for Citrus Disease Management in Argentina

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Core ideas:

- GMO strategies were implemented in Argentina for citrus disease management
- Local regulations for GMO have evolved
- Further actions need to be taken to improve GMO implementation

Abbreviations: AMP, antimicrobial peptide; avr, avirulence; *Ca. L.*, *Candidatus Liberibacter*; CP, coat protein; CONABIA, National Advisory Commission on Agricultural Biotechnology; CPsV, citrus psorosis virus; CTV, citrus tristeza virus; GAIN, Global Agricultural Information Network; GMO, genetically modified organism; HLB, huanglongbing; ihp, intron-hairpin; MAGyP, Ministry of Agriculture, Livestock and Fisheries; mRNA, messenger ribonucleic acid; PDR, pathogen-derived resistance; R, resistance; SAGyP, Secretariat of Agriculture, Livestock and Fisheries; SENASA, National Food Safety and Quality Service; USA, United States of America; USDA, United States Department of Agriculture; *Xcc*, *Xanthomonas citri* subsp. *citri*

ABSTRACT

Citrus is a major fruit crop with economic importance worldwide, with citriculture historically threatened at times by a diverse array of pathogens. As a leading producer and exporter, Argentina

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has been dealing with endemic and quarantine diseases of citrus by implementing conventional management strategies. In recent decades, the pursuit of pathogen-resistant transgenic citrus has been explored in the country as part of a long-term and sustainable disease management strategy. Successful genetically modified organisms (GMOs) were created locally, engineered to resist viruses and bacteria for the control of tristeza, psorosis, canker and huanglongbing diseases of citrus. While the Argentine regulatory system accommodated these developments, there were also difficulties that demand further recognition and analysis. In the present work, we describe four major diseases affecting Argentine citriculture and a series of GMO-oriented strategies for their management. We explore the methodologies behind these strategies, including transgenic-based approaches, the current state of regulations, and what further actions may be taken to ensure continuing protection for citriculture.

Argentina has a strong, dynamic citrus industry, and is a world-leading producer of fresh fruit for domestic consumption and exportation. Production is concentrated in two main geographical areas: the Northeast (Entre Rios, Corrientes, Misiones) and the Northwest (Tucuman, Salta, and Jujuy), alongside other citrus-growing provinces with lesser economic impact (Stein, 2019; GAIN, 2020). The primary citrus fruits cultivated in these regions are lemons (*Citrus limon* L. Burm. f.), oranges (*Citrus sinensis* L. Osb), mandarins, and grapefruits (*Citrus maxima* L. Osb) (Futch & Singerman, 2017). Argentina is the world's largest producer of lemons, with the leading citrus crop in the country mostly grown in the province of Tucuman (Stein, 2019). The most recent records, from 2020, indicate that Argentine fresh citrus production amounted to 1.6 million tons of lemons, 650,000 tons of oranges and 310,000 tons of mandarins (GAIN, 2020). The main lemon varieties are Genoa and Eureka; while Hamlin, Pineapple, Robertson, Navel, Salustiana and Valencia are the predominant orange cultivars (GAIN, 2019). The main mandarin varieties in this country are: Clementine (*Citrus clementina* Hort.); Clemenvilla, Ellendale, Murcott, Ortanique and Malvasio (*Citrus reticulata* Blanco); and Montenegrine (*Citrus deliciosa*) (GAIN, 2019). Grapefruit is the least produced citrus

crop with a yield of 110,000 tons in 2018, continuing an overall decrease in production over the last decade (Stein, 2019). In 2020, Argentina exported 354,000 tons of citrus (254,600 tons lemons), with the main destinations for exports being the European Union (55%) Eastern Europe (28%), and a recently resumed market in USA (USDA, 2016; Federcitrus, 2020; GAIN, 2020).

Although the two geographical regions have different climatic conditions, overall rainfall in Argentina is abundant, and both evince optimal characteristics for the spread of a number of pests and diseases (Futch & Singerman, 2017). Pests and pathogens have reduced citrus production and even resulted in export bans: USA only recently resumed the importation of Argentine lemons after more than a decade-long ban, mainly due to black spot infestation caused by the fungus *Phyllosticta citricarpa* (USDA, 2016). Historically, citrus disease management has relied mainly on chemical applications, but over time this practice raised environmental concerns and, in some cases, threatened natural balances (Sarandon & Marasas, 2017). Moreover, consumers began to demand high-quality food produced in sustainable agricultural systems (Caserta et al., 2019). Biotechnology could bring innovative and sustainable disease management solutions by developing genetically modified organisms (GMOs) that can contribute to crop protection (Caserta et al., 2019), significantly reducing chemical applications in some cases (Qaim & De Janvry, 2005; Burachik et al., 2010). In this perspective, we discuss four important citrus diseases and the solutions that were developed, focusing on GMOs (Table 1).

VIRAL DISEASES OF CITRUS AND GMO MANAGEMENT STRATEGIES

The impact of graft-transmissible diseases, including all viral diseases, has been reduced in Argentina over the past two decades, largely due to a successful certification program for pathogen-tested propagative materials like budwood (Anderson, 2000). However, these measures are not wholly sufficient for disease eradication as some viruses are naturally transmitted. This is the case for the two most severe viral pathogens in Argentina: citrus tristeza virus (CTV), associated with several syndromes, such as quick decline, stem pitting, and seedling yellows (tristeza complex); and citrus psorosis virus (CPsV), causing psorosis disease.

CTV, within the genus *Closterovirus*, is one of the most destructive citrus pathogens (Folimonova, 2020; Patil, 2020). With a large positive RNA genome, relatively fragile virions, and a niche limited to phloem-associated cells, this pathogen has a complex biology. CTV induces different syndromes depending on the citrus host, including yellowing, stem pitting, decline, and death (Folimonova, 2020). Sour orange (*Citrus aurantium*) was previously the most commonly grown citrus rootstock (Castle et al., 1987), with excellent horticultural and phytophthora resistance characteristics until tristeza quick decline struck, and approximately 30 million citrus trees grafted to this rootstock were lost in Argentina and Brazil between 1940 and 1960 due to CTV susceptibility (Patil, 2020).

A common approach for virus resistance is pathogen-derived resistance (PDR): the presence of a gene from the pathogen in the host can be deployed to disrupt the infection. The pathogen-derived gene can be modified such that it is dysfunctional, under excessive expression, or at the wrong developmental stage (Khalid et al., 2017). PDR-mediated approaches for control of CTV employed expression of the CTV p25 coat protein gene (Domínguez et al., 2002) as well as fragments derived from the p23 silencing suppressor (Soler et al., 2012). These transgenics were successfully tested in greenhouse trials, showing different levels of CTV-resistance. Ten potentially CTV-resistant sour orange transgenic lines were tested as rootstocks for non-transgenic Valencia sweet orange scions under field conditions in the Entre Rios province of Argentina (Anderson et al., 2015). CTV is efficiently transmitted by the brown citrus aphid *Toxoptera citricida*, endemic to this area, and manifesting all severe syndromes. Initially, the tested GM trees exhibited reduced CTV infection in the first and second years after planting. However, after 3.5 years the rate of CTV-positive scions increased considerably to 47.3%, and by the fourth year nearly 100% of the trees were infected and symptomatic with stunting, vein clearing, leaf cupping, chlorosis, and some of them died (Anderson et al., 2015). This was the first attempted local field trial of transgenic citrus being used against diseases and the contradictory results obtained between greenhouse and field conditions highlight

the importance of these varied tests. This study also set a significant precedent for future field trials testing GM citrus.

Citrus psorosis disease is caused by another citrus virus with a long history in Argentina, CPsV, a type member of the genus *Ophiovirus*. CPsV virions are filamentous, with a genome comprised of three negative RNA fragments (Moreno et al., 2015). Psorosis disease is graft-transmissible, and likely spread by an unidentified natural vector. This disease has been endemic in Argentina and Uruguay for decades, reducing fruit production and causing economic losses (Anderson, 2000; Zaneck et al., 2006). Typical psorosis symptoms include bark scaling, pustules, and gum accumulation on the trunk, flecking and chlorotic spots in leaves, and necrotic shock of young shoots. There are two psorosis syndromes (PsA and PsB), with PsB being the more aggressive, causing rampant bark lesions that expand rapidly even to thin canopy branches, and sloughing large strips of bark (Moreno et al., 2015). The lack of knowledge about its vector and the lack of naturally resistant citrus cultivars have motivated development and deployment of GMO strategies for psorosis management.

Beginning with the PDR approach, sweet orange transgenic lines were generated expressing one of three genes encoded in the CPsV RNAs: the *cp*, *54k* or *24k* (Zaneck et al., 2008). Several lines were obtained and evaluated for their acquired resistance against PsA and PsB viral isolates, but all were proven susceptible to both (Reyes, Zaneck, et al., 2011). In the case of CP-transgenic lines, the expression of the *cp* gene was low, variable and exhibited no correlation between copy number and mRNA accumulation (Zaneck et al., 2008). A newer approach was tested, using intron-hairpin RNA transcripts (ihpRNA) to trigger post-transcriptional gene silencing against the same three CPsV genes from the Argentine isolate CPsV 90-1-1 (Reyes, De Francesco, et al., 2011). For this purpose, ihpCP, ihp54k and ihp24k sweet orange transgenic lines were obtained and challenged with the same viral isolate used for construct generation and transformation. A 100% resistance, identified as undetectable viral titers and no expression of symptoms, was observed in greenhouse trials for most of the ihpCP lines (Reyes, De Francesco, et al., 2011). Two highly resistant ihpCP lines were tolerant

to CPsV 90-1-1 isolate through time and repeated propagation, with cross-resistance to the more virulent PsB-CPsV isolate (De Francesco et al., 2017). Recently, these two ihpCP lines were successfully combined by grafting with non-transgenic sweet orange scions, to evaluate the feasibility of conferring resistance to a non-transgenic scion (De Francesco et al., 2020). This work showed that resistance-related molecules translocated through the graft union and provided resistance against CPsV in the interstock, with varying levels of protection in the non-transgenic scion, depending on the virus inoculation site (De Francesco et al., 2020). Taken together, research on ihpCP citrus transgenic lines indicates that they could be part of a long-term solution for psorosis management and that their use as rootstocks with non-transgenic scions could protect the trees from infection while producing non-GM fruits. This promising biotechnological alternative requires further tests and field trials before large scale implementation by the citrus industry.

BACTERIAL QUARANTINE DISEASES OF CITRUS AND GMO MANAGEMENT STRATEGIES

The two most important bacterial diseases affecting citrus nationwide are huanglongbing (HLB) and citrus canker. HLB is associated with the unculturable phloem-limited bacteria *Candidatus Liberibacter* spp. and transmitted by psyllid vectors. This citrus disease is the most destructive in the world, with no effective control means (Wang, 2019). HLB symptoms include blotchy mottling of leaves, premature fruit drop, and tree decline (Bové, 2006). Three putative *Liberibacter* species were described, although none have been cultured axenically: *Candidatus Liberibacter* (*Ca.* L.) asiaticus, *Ca.* L. americanus and *Ca.* L. africanus; the first two are transmitted by *Diaphorina citri* Kuwayama and the third by *Trioza eritreae* (Bové, 2006; Wang, 2019). *Ca.* L. asiaticus was first detected in Argentina in 2012 and is to date the only HLB-associated species reported in the country (Badaracco et al., 2017). The vector is distributed throughout the entire northeastern and parts of the northwestern growing regions. Two northwestern provinces (Salta and Jujuy), and six northeastern provinces have reported HLB cases recently (SENASA, 2020). To protect the national citrus industry and prevent the spread of the disease, the Argentine government created the “National Program for Prevention of Huanglongbing” (PNPHLB). This program is coordinated by the National Food Safety

and Quality Service (Spanish: Servicio Nacional de Sanidad y Calidad Agroalimentaria, SENASA), public and private entities as well as several national institutions looking for new HLB-resistant germplasm obtained via transgenesis or other breeding strategies. Currently, the main limitation to exploring GMOs for HLB management in Argentina is the restriction of experimental pathogen inoculation, due to the low prevalence of the disease and the risk of accidental spread. However, GMO strategies involving expression of antimicrobial peptides from different origins are being designed (Furman et al., 2013; Conti et al., 2020) and will be tested against *Candidatus Liberibacter* as soon as regulations allow.

Citrus canker is caused by *Xanthomonas citri* subsp. *citri* (*Xcc*), a gram-negative bacterium affecting most citrus species (Brunings & Gabriel, 2003; FERENCE et al., 2018). Originally detected in Asia, the disease has spread to most of the main citrus producing areas around the world (Brunings & Gabriel, 2003). Since first reported in 1928, it has been considered an endemic disease in Argentina, affecting 10% of commercial citrus cultivated in the northeastern and northwestern areas of the country (Canteros et al., 2017). Symptoms appear on leaves, stems, and fruits, including corky spots surrounded by chlorotic haloes on leaves and larger corky lesions on branches and fruits (Brunings & Gabriel, 2003; Graham et al., 2004). Severe infections result in defoliation, premature abscission of fruits, and reduction of the photosynthetic activity (Graham et al., 2004). These symptoms lead to production losses and regulatory restrictions on the sales of plants and fruits including restrictions in international trades.

Bacterial disease management involves specific nutrition (fertilization), periodic inspection, propagation of pathogen-free plant materials, windbreaks, and removal of infected trees. Additionally, the control of Asian citrus leaf miner (*Phyllocnistis citrella*) that can exacerbate disease spread and symptoms, and copper sprays are used for canker disease management (FERENCE et al., 2018). Several biotechnological approaches are currently available for conferring resistance against plant bacterial infections (Caserta et al., 2019). In Argentina, efforts oriented towards achieving

canker resistance via transgenesis can be grouped in two categories: 1) expression of antimicrobial peptides (AMPs); and 2) expression of genes inducing plant defense mechanisms.

AMPs are part of innate immunity, establishing a first line of defense against pathogens. Most of them are cysteine (Cys)-rich peptides expressed in nearly all organs constitutively or in response to infections. For these reasons they have a broad-spectrum activity against different bacteria, mainly acting through interactions with membrane lipids (Stotz et al., 2013). Transgenic expression of AMPs has been successfully implemented in citrus worldwide (Boscariol et al., 2006; Cardoso et al., 2010; Fu et al., 2011; Yang et al., 2011; Huang et al., 2021). Dermaseptins exhibit a broad-spectrum activity against Gram-positive and Gram-negative bacteria (Navon-Venezia et al., 2002; Yaron et al., 2003; Osusky et al., 2005; Rivero et al., 2012). A dermaseptin present in *Phyllomedusa* spp., comprising of 27–34 amino acids, was constitutively expressed in sweet orange plants and a considerable degree of resistance to *Xcc* was obtained: plants showed symptom reduction levels of up to 50% in *in planta* assays performed under controlled conditions (Furman et al., 2013).

The most recent example of the first group (i.e., AMPs) is the over-expression of the peptide Snakin-1 (SN1) in a Troyer citrange rootstock. SN1 is an AMP isolated from potato (*Solanum tuberosum*) which exhibits remarkable antimicrobial activity *in vitro* (Almasia et al., 2008) and *in vivo* in potato plants (Almasia et al., 2020); as well as in wheat (*Triticum aestivum*) (Faccio et al., 2011) and lettuce (*Lactuca sativa* L.) (Darqui et al., 2018). SN1 shows antimicrobial activity against very different pathogens such as *Blumeria graminis*, *Gaeumannomyces graminis* (Faccio et al., 2011; Rong et al., 2013), *Rhizoctonia solani* and *Sclerotinia sclerotiorum* (Darqui et al., 2018). The SN1-coding sequence was constitutively expressed in transgenic Troyer citrange and challenged with *Xcc* under controlled inoculation assays. The transgenic plants showed symptom reductions of up to 75%, as measured by the frequency of canker development, as well as smaller size of cankers and a delay of symptom onset, suggesting a significant decrease of disease severity (Conti et al., 2020). Constitutive expression

of the mentioned AMPs would also allow transferring the technology among bacteria colonizing different cell types such as *Candidatus Liberibacter* spp.

Among the second group (i.e., plant defense genes), an approach that has been implemented is the expression of resistance (R) genes: plant-derived genes that recognize specific pathogen-derived compounds. These genes interact with the product of a pathogen avirulence gene (*avr*) in the context of effector-triggered immunity (Gassmann & Bhattacharjee, 2012). Using this approach, transgenic citrus plants expressing the *Xa21* R gene from rice have been generated (Mendes et al., 2010). In Argentina, sweet orange transgenic plants expressing pepper *Bs2* R gene were developed (Sendín et al., 2017). The *Bs2* R gene, isolated from pepper cv. Early Cal Wonder, specifically recognizes and confers resistance against *X. campestris* pv. *vesicatoria* containing the corresponding *avrBs2* gene (Tai et al., 1999). The *avrBs2* gene confers an adaptive advantage to the pathogen (Kearney & Staskawicz, 1990; Swords et al., 1996). Further, this gene is highly conserved in other pathovars of *Xanthomonas* such as *Xcc*. Therefore, *Bs2* gene was introduced in citrus to recognize the *avrBs2* of *Xcc*, thus activating the plant defense mechanisms. Canker symptoms were reduced up to 70% in sweet orange transgenic lines expressing the *Bs2* gene (Sendín et al., 2017). These results indicate that the pepper *Bs2* resistance gene is also functional in a family different from *Solanaceae*, and that it could be considered useful not only for citrus canker control but also for other diseases caused by members of the *Xanthomonas* genus.

GMO REGULATORY SYSTEM IN ARGENTINA

The GMO era started quite early in Argentina, in the early-1990s (Burachik, 2020). The country currently has over 25 million hectares of GM crops, 19.2 of which are soybean (almost 100% of the overall cultivated crop), 5.5 maize (96%), and 0.3 cotton (almost 100%) (Vicién & Trigo, 2017; Burachik, 2020). The high level of adoption for this novel agricultural technology in Argentina can be explained as a combination of factors, including political support (from agricultural officials), the ability to address prevalent needs among farmers, economic and environmental factors, and the

early implementation of effective regulations (Burachik, 2010). The Argentine state institution charged with establishing and maintaining the regulatory framework for GMOs is the Secretariat of Agriculture, Livestock and Fisheries (SAGyP is the Spanish acronym), consistent with the production profile of Argentina as an agricultural commodities exporter (Burachik, 2012). The agencies that enforce GMO regulations are: SENASA, the National Advisory Commission on Agricultural Biotechnology (Spanish: Comisión Nacional Asesora de Biotecnología Agropecuaria, CONABIA), and the Agricultural Markets Office from SAGyP. The mandated regulations include risk assessments, confined field or greenhouses trials, and off-season production of regulated seed for export to countries where GMO cultivation is permitted. The confined trials are conducted under strict management practices to prevent dissemination of the modified species outside the growing area (Garcia-Alonso et al., 2014). Minimum isolation distances and periods of post-harvest land use restrictions depend on the crop and the environmental conditions (Oroño & Vesprini, 2018). Approval for commercialization requires food evaluation, environmental risk assessment, and assessment of economic impact on Argentine agricultural production and commerce (Orroño & Vesprini, 2018). Fig. 1 shows the series of steps that follow the deregulation of GMOs in Argentina.

The Argentine regulatory system has a strong scientific and technical basis (Vicién & Trigo, 2017; Lewi & Vicién, 2020). Thanks to its academic members and its track record in the field, in 2014, CONABIA was recognized as a Reference Center for the Biosafety of GMOs by the Food and Agriculture Organization (FAO). From this perspective, Argentina should have been more successful in the deregulation of its local biotechnological releases. However, only two out of the sixty-one releases that have been approved for commercialization in Argentina were developed locally: the abiotic stress-resistant and herbicide tolerant Soybean HB4, and the virus Y-resistant Potato (Lewi & Vicién, 2020; MAGyP, 2020). The different obstacles faced by GMO developers in Argentina could explain the unsuccessful deregulation of their local biotechnological products. Lack of awareness around the general regulatory process is one of the main restrictions. On the other hand, the costs of the deregulation process, sometimes estimated as being ten times higher than the GMO

development itself, presents another major difficulty. The existence of specific funding programs such as FONREBIO (Biotechnological Products' Regulation Funding), organized by the National Agency for Scientific and Technological Promotion (Spanish: Agencia Nacional de Promoción Científica y Tecnológica, ANPCyT) in 2015 (Lewi & Vicién, 2020) and the educational programs such as Argenbio (2020), among others, should provide a helpful framework for local GMO deregulations. However, the implementation of existing subsidies suffered from procedural changes. Formerly, there was a non-reimbursable funding for public institutions. Now, a reimbursable loan is supplied, which requires a private source of partial backing or a commitment to quick insertion into the market. Those modifications made it almost impossible for public institutions to execute these subsidies. Only private developments, with sufficient funding and adequate resource management, can pay for the required studies and their certification. The foundation of a government agency for GMO regulatory affairs, as well as the flexibilization of subsidy implementation, would be a few good steps to address the GMO deregulations issues in Argentina. The new agency could act as a shared platform to expedite the process, making it more streamlined and efficient (Lewi & Vicién, 2020).

Currently, there is one precedent for an approved GMO conferring resistance to pathogens developed in the country: the potato plants resistant to PVY (Bravo-Almonacid et al., 2012). Regarding transgenic citrus resistant to pathogens, the ongoing research efforts mentioned in this paper, and summarized in Table 1, are mainly in the early stages of the process, with only one having undergone field trials. Further efforts and funding are required to obtain the deregulation of these GM citrus (Fig. 1).

Public perception of GMOs in Argentina is another concern. Even though GMOs find high levels of support among state officials and growers (Burakich, 2010, 2012, 2020), influencers in the agroecology movement (Pengue, 2005; Sarandon & Marasas, 2017) could play a role in the public acceptance of GMO-derived citrus. Pathogen-resistant GM citrus could improve sustainability by reducing pesticide applications (Qaim & De Janvry, 2005; Burachik et al., 2010), but GM citrus carry other concerns. Additional research for the effects of GM citrus on humans and the environment is

required to move forward with public acceptance in Argentina. This includes: the risk of transgene dissemination by pollen, the potential damage to local production and small growers who cannot adopt the new technology, and risks to human health due to the consumption of citrus fruits produced from GM trees.

FURTHER ACTIONS

Even though a large number of diverse strategies have been applied in Argentina, in attempts to control citrus pathogens, the horizon of exploration remains broad. The expression of stacked antimicrobial genes or the overexpression of peptides from citrus origin to obtain cis-genic or intragenic varieties could be promising strategies, considering the issues around the public perception of transgenic plants and the need to address the streamlining of the deregulation process (Holme et al., 2013). When the genetic sequences to be introduced to citrus originate in closely related species, instead of phylogenetically distant ones, regulatory processes become easier to complete. Expressing AMPs in specific plant tissues where the pathogen is replicating, or the induction of their expression at specific developmental stages, are other approaches that could be explored in the case of citrus (Zhou et al., 2017; Tavano et al., 2019; Huang et al., 2021). Advances in the study of plant-microbe interaction could reveal specific cellular pathways to be modified for resistance such as key components of the salicylic or jasmonate pathways (Bari & Jones, 2009).

CRISPR-Cas9 editing is an emerging genetic engineering approach which is being applied to different cultivars including citrus (Jia et al., 2019). The editing of host susceptibility genes could also lead to tolerance or resistance to some of the most important pathogens, generating the possibility of obtaining non transgenic but edited plants, thus falling within a different regulatory framework (Lema, 2019). The result of editing the citrus genome is comparable to that obtained by conventional mutagenesis if there are no leftovers of Cas9 and guide RNA inserted sequences. This means that after a case-by-case consultation with regulatory authorities and molecular characterization proving that there is no insertion of any "novel combination of genetic material", the edited citrus plant can be classified as non-GMO according to the Cartagena Protocol on Biosafety (Lema, 2019). The

scientific and technical expertise acquired through the last decades, and the advice from experienced organizations working on the regulatory framework, will certainly assure further advances in the field of plant genetic engineering. Once these promising alternatives evolve into concrete biotech-products, we can address new challenges regarding GMO regulations and policies as well as public opinion, in order to finally get these products to the market and reap the benefits of incorporating them within balanced and sustainable food production systems.

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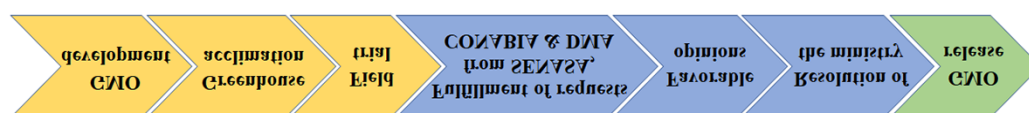
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Figure caption

Fig. 1. Scheme of the process for development and deregulation of GMOs in Argentina. Steps in orange arrows indicate current status of the transgenic events attempting citrus disease management described in this paper: GMO development, greenhouse acclimation and field trials. The steps in blue summarize the regulatory process: fulfillment of requests for CONABIA, SENASA and the Agricultural Market Office (Spanish: Dirección de Mercados Agrícolas, DMA) of SAGyP; favorable opinions from the three regulatory entities, and resolution of the Ministry of Agriculture, Livestock and Fisheries (MAGyP). The completion of these steps leads to the GMO commercial release (in green) (MAGyP, 2020).



Table

Table 1. Transgenic citrus engineered for pathogen resistance, developed and/or evaluated in Argentina.

Pathogen	Approach/ transgene	GMO development	Citrus variety	Trial status	Later references
<i>Citrus tristeza virus</i> (CTV)	Pathogen-derived resistance (PDR)/ CTV p25 (<i>cp</i>) and p23	Domínguez et al., 2002; Soler et al., 2012	Sour orange (<i>Citrus aurantium</i>)	field	Anderson et al., 2015
<i>Citrus psorosis</i>	PDR/ CPsV <i>cp</i> , 54k, 24k	Zanek et al., 2008	Pineapple sweet orange (<i>Citrus</i>	greenhouse	Reyes, Zanek, et al., 2011

<i>virus</i> (CPsV)		<i>sinensis</i> (L.) Osbeck)		
CPsV	PDR/ ihpRNA derived from CPsV <i>cp</i> , 54k, 24k	Reyes, De Francesco, et al., 2011	Pineapple sweet orange (<i>Citrus</i> <i>sinensis</i> (L.) Osbeck)	greenhouse De Francesco et al., 2017; De Francesco et al., 2020
<i>Xanthomonas</i> <i>citri</i> subsp. <i>citri</i> (<i>Xcc</i>)	Antimicrobial peptides (AMP)/ Dermaseptin	Furman et al., 2013	Pineapple sweet orange (<i>Citrus</i> <i>sinensis</i> (L.) Osbeck)	greenhouse
<i>Xcc</i>	AMP/ Snakin-1	Conti et al., 2020	Troyer citrange (<i>Poncirus trifoliata</i> . x <i>C. sinensis</i>)	greenhouse
<i>Xcc</i>	Resistance gene/ <i>Bs2</i>	Sendín et al., 2017	Pineapple sweet orange (<i>Citrus</i> <i>sinensis</i> (L.) Osbeck)	greenhouse

Note: The columns indicate: the targeted pathogen, the strategy and transgene respectively, references for the GMO development, the citrus variety and its scientific name, the latest status of the trials, and later references.