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Review

Transformation systems, gene silencing and gene editing technologies in oomycetes

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ABSTRACT

Oomycetes are spore-forming eukaryotic microbes responsible for infections in animal and plant species worldwide, posing a threat to natural ecosystems, biodiversity and food security. Genomics and transcriptomics approaches, together with host interaction studies, give promising results towards better understanding of the infection mechanisms in oomycetes and their general biology. Significant development and progress in oomycetes genomic studies have been achieved over the past decades but further understanding of molecular processes, gene regulations and infection mechanisms are still needed. The use of molecular tools such as CRISPR/Cas and RNAi helped elucidate some of the molecular processes involved in host invasion and infection both in plant and animal pathogenic oomycetes. These methods provide an opportunity for accurate and detailed functional analysis involving various fields of studies such as genomics, epigenomics, proteomics, and interactomics. Functional gene characterisation is essential for filling the knowledge gaps in dynamic biological processes. However, every method has both advantages and limitations that should be considered before choosing the best method for investigating a particular research question. Here we review transformation systems, gene silencing

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and gene editing techniques in oomycetes, how they function, in which species and what are their main advantages and disadvantages.

1. Introduction

The oomycetes are a diverse group of filamentous spore-forming organisms, that comprises hundreds of notorious pathogens. Several of them are on worldwide quarantine lists and strictly regulated by national and international laws to help prevent their spread (Rossmann *et al.*, 2021). Hosts include major cultivated fish and plant species, as well as numerous animal and plant species in natural ecosystems (Cao *et al.*, 2012; Fernández-Benéitez *et al.*, 2008; Kamoun *et al.*, 2015; van den Berg *et al.*, 2013). Oomycetes form a taxonomically distinct and large group of eukaryotic microorganisms that shares some physiological and morphological features with fungi (e.g., formation of hyphae and different purpose spore types) but are phylogenetically related to heterokont algae (Baldauf et al., 2000; Latijnhouwers *et al.*, 2003). Oomycetes and true fungi can be distinguished by several biochemical and cytological characteristics that only oomycetes possess: a) cellulose as the major microfibrillar component of their hyphal walls; b) cytoplasmic dense body/fingerprint vacuoles containing phosphorylated β-(1,3)-mycolaminarin glucans; c) diploid thalli with meiosis preceding gamete formation; d) mitochondria with tubular cristae; and finally e) utilization of the α-ε-diaminopimelic acid lysine synthetic pathway (Beakes *et al.*, 2012). The large spectrum of environmental conditions and hosts that oomycetes thrive in is reflected in their phylogenetic diversity (Thines, 2014).

In the last decades, host–oomycete interaction studies combined with genomics and transcriptomics have given considerable understanding on how oomycetes infect their hosts (Burra *et al.*, 2017). Realizing the role of the many interacting molecules is important for targeted development of management strategies. It has been established that oomycetes secrete a battery of effector proteins that modify the immune system of their host to facilitate infection (Bozkurt *et al.*, 2012; de Bruijn *et al.*, 2012; Fabro *et al.*, 2011). However, a huge number of molecules produced by the different oomycete pathogens during infection are yet not accounted for. For functional analysis of these *in vivo*, techniques to genetically modify the oomycetes, such as RNAi (Saraiva et al., 2014; Whisson *et al.*, 2005), stable transformation (Judelson *et al.*, 1993) or CRISPR/Cas (Fang and Tyler, 2016) are of crucial importance. The development of molecular techniques has progressed more slowly for oomycetes than for fungi, and is currently limited to relatively few species, and with low efficiencies compared to fungi. Because of the heterogeneity within the oomycetes, transformation protocols need to be optimized for each species and, within the species, often for each strain. It is therefore a challenging task to transform new, unknown strains with optimal efficiency.

2. Established transformation systems in oomycetes

2.1. *PEG-mediated protoplast transformation*

Polyethylene glycol (PEG)-mediated transformation is a simple and efficient method that relies on three key steps: protoplast preparation, DNA uptake, and protoplasts regeneration on selective media. Transformable protoplasts can be prepared through digestion of the mycelium or germinating spores using cell wall degrading enzymes. It has been reported that different batches of lytic enzymes may vary in the effectiveness of cell wall degradation, therefore testing each batch and the combination of different lytic enzymes to determine protoplasting efficiency is highly advisable (Liu and Friesen, 2012). The digestions are usually performed in an osmotic buffer containing sorbitol or high salt to stabilize the resulting protoplasts. The DNA uptake by protoplasts is carried out by incubating protoplasts with highly concentrated DNA followed by the addition of up to 10 volumes of a 40–60% PEG 4000, which is assumed to function by condensing the plasmid DNA molecule to prevent attack by nucleases and to facilitate the penetration of DNA into the cells. The selection of transformants can be achieved by using a drug resistance screening depending on the selectable marker gene used in the DNA vector (Ruiz-Díez, 2002).

The PEG-mediated transformation protocol using protoplasts was first described for an oomycete pathogen by Judelson *et al.* (1991). At the time, the method was a significant breakthrough. Since then, the PEG-mediated transformation method was successfully used in several other oomycete species including *P. sojae* (Judelson *et al.*, 1993), *Saprolegnia monoica* (Mort-Bontemps and Fèvre, 1997), *P. parasitica* (Bottin *et al.*, 1999), *P. palmivora* (van West *et al.*, 1999), *P. brassicae* (Si-Ammour *et al.*, 2003), *Pythium aphanidermatum* (McLeod *et al.*, 2008), *P. capsici* (Dunn *et al.*, 2013) and *P. cactorum* (Chen *et al.*, 2016).

The PEG–mediated transformation allows for simultaneous processing of many samples. Potential disadvantages of PEG-mediated transformation include difficulty in obtaining high concentrations of viable protoplasts, low transformation efficiency, high percentages of transient transformants, and frequent multiple loci integration. However, due to its simplicity in technical operation and equipment required, the PEG-mediated method remains the most used method to conduct transformation.

2.2. *Electroporation*

Electroporation, also known as electropermeabilisation, is a transformation technique that uses electrical current for macromolecular uptake by creating temporary pores in the cell membrane (Arena *et al.*, 2014). The electric field also drives negatively charged DNA strands from the cathode (negative) towards the anode (positive) of an electric field. Therefore, an electric pulse causes some of the DNA to enter the cell. After the electric field is switched off, the cell membrane reseals, trapping some of the DNA within the cell (Carter and Shieh, 2015). The electrical conditions such as voltage, field strength, and the length and shape of the pulse are important conditions that needs to be optimized for each species and strain, to avoid irreversible injury to the cell (Li *et al.*, 2017).

Electroporation in oomycetes was successfully established in *P. infestans* by Latijnhouwers and Govers (2003). In this study they determined that the G-protein β subunit is involved in sporangium formation. The authors observed that the integration of plasmid DNA by electroporation resulted in a few transformants. Later the method was improved by Dong *et al.* (2015), also using *P. infestans*. Other oomycete species have been genetically transformed using electroporation since then, including *Pythium aphanidermatum* (Weiland, 2003) and *P. capsici* (Huitema *et al.*, 2011).

Although the principle of electroporation is applicable to all cell types, its efficiency depends on the electrical conditions as well as buffer, temperature, and DNA concentration (Li *et al.*, 2017). DNA formulation with certain types of polymers has been found to enhance electroporation efficiency, for instance transformation of DNA complexes of cationic liposomes revealed to be more effective than naked DNA (Anwer, 2008). The DNA delivery by electroporation is not target-specific.

2.3. *Microprojectile bombardment*

Microprojectile bombardment uses high velocity metal particles to deliver DNA into cells. Klein *et al.* (1987) observed that tungsten particles could be used to introduce RNA and DNA into epidermal cells of onion with subsequent transient expression of foreign genes encoded by these nucleic acids. One year later, Christou *et al.* (1988) demonstrated that the process could be used to deliver DNA into soybean embryos and recovered stable transformants. Microprojectile bombardment has later proved effective for transforming other plant species, prokaryotes, mammalian cells, and fungi (Guo *et al.*, 1996; Herzog *et al.*, 1996; Hilber *et al.*, 1994; Mäenpää *et al.*, 1999).

The first report of oomycetes transformed using the biolistic method date from 1993, where the species *Phytophthora capsici, P. citricola, P. cinnamomi* and *P. citrophthora* were transformed with plasmids containing the β-glucuronidase gene and hygromycin B resistance (Bailey *et al.*, 1993). Later, Cvitanich and Judelson (2003) optimised the protocol using *P. infestans*. They reported transformation of several different isolates and target tissues, including germinated sporangia, germinated zoospore cysts and mycelia.

Both biological and physical factors affect the transformation efficiency in particle bombardment. Cell type, growth condition, and cell density as well as particle type and size, vacuum and pressure level and target distance are key factors (Gouka *et al.*, 1997). Among all the genetic transformation methods, particle bombardment is the most powerful one. However, it is only used when all the other methods have failed due to very expensive equipment and consumables.

2.4. Agrobacterium-*mediated genetic transformation*

The plant pathogenic bacterium *Agrobacterium tumefaciens* (syn. *Rhizobium radiobacter*) genetically manipulates its hosts by transferring a fragment of DNA (T-DNA) from its plasmid (Ti plasmid) into the genome. The ability of *A. tumefaciens* to target a wide range of hosts, allows it to be applied to several other eukaryotic species (Lacroix and Citovsky, 2016). Integration of T-DNA in fungi occurs by non-homologous end joining, but also targeted integration of the T-DNA by homologous recombination (HR) is possible (Gelvin, 2003). *Agrobacterium* harbouring a binary plasmid with T-DNA borders can efficiently transfer and integrate a target gene into the oomycete genome (Vijn and Govers, 2003). *Agrobacterium*-mediated transformation (AMT) is simple, low cost and has the additional advantage that it integrates only one or two copies of the target gene compared to multiple copies integration in the genome with other transformation systems (Vijn and Govers, 2003; Wu *et al.*, 2016). Although, a higher transcript level and gene silencing may be achieved through multiple copies introduction (Ah-Fong *et al.*, 2008), this can disrupt the functionality of other genes, therefore complicating functional genomic studies.

Agrobacterium tumefaciens is widely used to transform plants, yeasts, filamentous fungi and oomycetes (De Groot *et al.*, 1998; Gogoi *et al.*, 2019; Hwang *et al.*, 2017; Piers *et al.*, 1996; Vijn and Govers, 2003). Among the oomycetes, three *Phytophthora* species: *P. infestans* (Vijn and Govers, 2003), *P. palmivora* (Ochoa *et al.*, 2019; Pettongkhao *et al.*, 2020; Vijn and Govers, 2003; Wu *et al.*, 2016), *P. nicotiana* (Dalio *et al.*, 2016), and two *Pythium* species: *P. ultimum* (Vijn and Govers, 2003) and *P. oligandrum* (Wang *et al.*, 2017) have been successfully transformed using AMT (Table 1).

Transformation of oomycetes using *Agrobacterium* is influenced by several factors including: i) bacterial density; ii) acetosyringone concentration; iii) co-incubation time of *Agrobacterium* and the oomycete cells; and iv) bacterial and oomycete strains used. Although a gradual increase in bacterial density may correlate with a higher frequency of integration events, densities beyond optimum (e.g. A600 = 0.8 for *P. palmivora*) can inhibit zoospores encystment and germination. The presence of the inducer molecule acetosyringone activate expression of *vir* genes that facilitates transfer of T-DNA. During incubation with zoospores, *Agrobacterium* infection is influenced by exposure time, which is interconnected with bacterial density and acetosyringone concentration. Low bacterial cell density means reduced physical points of contact with the zoospores and thus need more incubation time for successful infection. However, longer incubation time may result in false positive colonies. For example, in *Pythium guiyangense*, incubation time of more than 48 h resulted in more false positive colonies, making it difficult to screen true transformants (Zhao and Su, 2008). Increase in

Table 1 – Summary of different transformation methods optimised for oomycetes.

Transformation approach	*Oomycete species* (strain)	Starting material (unit number)	Average regeneration rate (%)	DNA/At strain (amount/optical density-A600)	Vector	Marker gene[a]	Average number of transformants obtained	TF[b] (%)	Reference
PEG-$CaCl_2$ mediated	*P. infestans* (Race 0)	Protoplast (10^8)	5	plasmid (30 μg)	pHAMT34H pHAMT34N pTH210 pTH209	*nptII/hpt*	5.33	1.066×10^{-4}	Judelson *et al.* (1991)
	S. monoica (53967)	Protoplast (10^6)	7.5	plasmid (20 μg)	pCM54 pTH210 pHAM34H pBT6	*hpt/bmI*	3.375 or 25 (with carrier DNA)	4.5×10^{-4} 33.33×10^{-4} (with carrier DNA)	Mort-Bontemps and Fèvre (1997)
	P. citricola	Protoplast (10^6)	17.5	plasmid (13 pmol)	pDBHAMT35G pHAMT35G pEXO pPPT pPSACTIN pATPase	*gus*	–	–	McLeod *et al.* (2008)
	Py. aphanidermatum	Protoplast (10^6)	40	plasmid (13 pmol)	pDBHAMT35G vpDCHAMT35G pHAMT35G	*gus*	–	–	McLeod *et al.* (2008)
	P. sojae	Protoplast (10^6)	17.5	plasmid (13 pmol)	pHAMT35G pDBHAMT35G pPSACTIN	*gus*	–	–	McLeod *et al.* (2008)
	P. palmivora (P6390)	Protoplast (10^6)	15	plasmid (30–45 μg reporter + 15 μg selectable pTH209)	pHAMT35G + pTH209 pVW2 + pTH209	(*gfp* + *nptII*)	26	1.73×10^{-2}	van West *et al.* (1999)
	P. sojae (P6497-race 2)	Protoplast (2×10^7)	3.5	plasmid (25–50 μg)	pHAMT34H + pTH210	*hpt*	4	5.7×10^{-4}	Judelson *et al.* (1993)
	P. ramorum BBA9/95, BBA 26/02 BBA MSOD03-02 BBA PR01	Protoplast (10^7)	–	plasmid (20 μg)	p34GFN	*nptII/gfp*	40 5	–	Riedel *et al.* (2009)
	P. nicotiana (Tucker-race 0)	Protoplast (10^7)	–	plasmid (20 μg each)	pTH210 + pAHB6	*gfp/hph*	15 (hph) 2 (gfp)	–	Bottin *et al.* (1999)
Agrobacterium-mediated	*P. infestans* (98014/98027, H30P02)	Zoospores (10^7)	–	LBA1100 (0.25)	pNptII + pBBR1MCS.virGN54D	*nptII*	1 24.4	0.1×10^{-4} 2.44×10^{-4}	Vijn and Govers (2003)
	P. palmivora (P1)	Zoospores (10^7)	–	EHA105 (0.4)	pCB301TOR pCB301TOR-GFP	*nptII* *gfp*	30 23	2×10^{-4} 2.3×10^{-4}	Wu *et al.* (2016)
	P. nicotiana (IAC 01–95)	Mycelium	–	EHA105 (0.4)	pCAMDsRed	*hph* *dsRed*	–	–	Dalio *et al.* (2016)
	G. ultimum (CBS 219.65)	(10^7)	–	LBA1100 (0.25)	pNptII + pBBR1MCS.virGN54D	*nptII*	3	0.3×10^{-4}	Vijn and Govers (2003)
	Pythium guiyangense	Mycelium	–	LBA4404 (0.15)	pCambia-Pnos-PNN-hphl	*hph*			Zhao and Su (2008)

Electroporation	*P. aphanidermatum* 898B	Protoplast (10^6)	12.5	plasmid (20 μg)	pHAMT35N/SK	*nptII*	21	1.68×10^{-2}	Weiland (2003)
Particle bombardment	*P. infestans* (1306, 88-1-1, 88069, 6636)	Sporangiazoospores (10^6)	–	10 μg (1 μg plasmid coated over 0.6 mg of 0.6 μm gold particles)	pTH209−35G, pTH209−35G-3 pTH209−35G-6 pTH209 + pHAMT35G	*nptII/gus*	11 12 9 14	1.1×10^{-3} 1.2×10^{-3} 0.9×10^{-3} 1.4×10^{-3}	Cvitanich and Judelson (2003)

a *nptII* – neomycin phosphotransferase gene; *hpt* – hygromycin-B phosphotransferase gene; *hph* – hygromycin resistance gene; *BmI* – β-tubulin gene; *gfp* – green fluorescence protein gene; *gus*: β-glucuronidase gene.
b TF – transformation frequency – number of positive transformants obtained/total number of regenerated protoplasts or spores used × 100.

acetosyringone concentration was correlated with higher transformation efficiency in both *P. infestans* and *P. palmivora* (Vijn and Govers, 2003; Wu *et al.*, 2016). In *P. guiyangense* and *P. infestans*, no transformants were obtained when *Agrobacterium* was grown in the absence of acetosyringone (Vijn and Govers, 2003; Zhao and Su, 2008). However, the introduction of a mutant *virG* gene (*virGn45D*) that act as a constitutive inducer of vir genes could complement the absence of acetosyringone in the *Agrobacterium* strain LBA1101 and gave similar number of transformants in *P. infestans*. Nevertheless, a combination of *virGn45D* and acetosyringone treatment enhanced transformation efficiency using the same *P. infestans* stain H30PO2 (Vijn and Govers, 2003). Different bacterial and oomycete strains also influence the transformation efficiency. In *P. palmivora*, so far the three *Agrobacterium* strains EHA105 (Gumtow *et al.*, 2018; Wu *et al.*, 2016), LBA1101 (Vijn and Govers, 2003), and AGL1 have been successfully used to transform zoospores, while transformants obtained via LBA4404 and GV3101 strains showed abnormal growth (Ochoa *et al.*, 2019). Similarly, different *P. infestans* strains used for transformation by LBA1101 carrying plasmid pNptII gave varied transformation frequencies with lowest in strains 98014 and 98027 (1 transformants per 10^7 zoospores) and highest in H30P02 strain (average 24.4 transformants per 10^7 zoospores) (Vijn and Govers, 2003). Thus, efficiency of the AMT method is determined by these aforementioned factors that have been optimised for transformation of several oomycetes.

2.5. *Gene expression control*

Gene expression is governed by transcription and translation. The promoter elements present upstream of a gene determine the level and timing of transcription (Fig. 1). The RNA polymerase binds to the core promoter to initiate transcription. Promoter sequences are therefore essential parts of any vector for functional gene studies, whether it controls a native gene or a marker gene like green fluorescence protein (*gfp*) (Fig. 2). Judelson *et al.* (1992) identified three promoter sequences from the phytopathogenic oomycetes *Bremia lactucae* (*Ham34* and *Hsp70* genes) and *P. sojae* (*actin* gene) that resulted in accumulation of high levels of the β-glucuronidase (GUS) reporter in three oomycete species. Furthermore, they concluded that the transcriptional machinery of the oomycetes differs significantly from that of fungi (Judelson *et al.*, 1992). Of promoters from other oomycetes, *P. infestans* genes *Piexo1, Piexo2, Piexo3, Piendo2, Piypt, PiATPase*, and actin genes from *P. sojae* (*Psactin*) and *Aphanomyces cochlioides* (*Paactin*), only a chimeric combination of the *Piexo1* and the *Ham34* promoter sequences combined with terminator from *Ham34* superseded GUS activity relative to the native *Ham34* promoter (McLeod *et al.*, 2008). The chimeric regulatory sequences in the pDBHAMT35G vector were shown to give high levels of GUS activity in the oomycete species *P. infestans, P. sojae, P. citricola*, and *Pythium aphanidermatum* (McLeod *et al.*, 2008). The promoter sequence derived from the glyceraldehyde 3-phosphate gene (*PgpdA*) and the terminator sequence of the *trpC* gene from *Aspergillus nidulans* have also been used for constitutive expression of selectable marker genes, *DsRed*-Express and hygromycin resistance (*hph*), in *P. nicotianae* and *Pythium guiyangense* (Dalio *et al.*, 2016; Zhao and Su, 2008). The constitutive and universal role of the regulatory sequences from *Ham34 and*

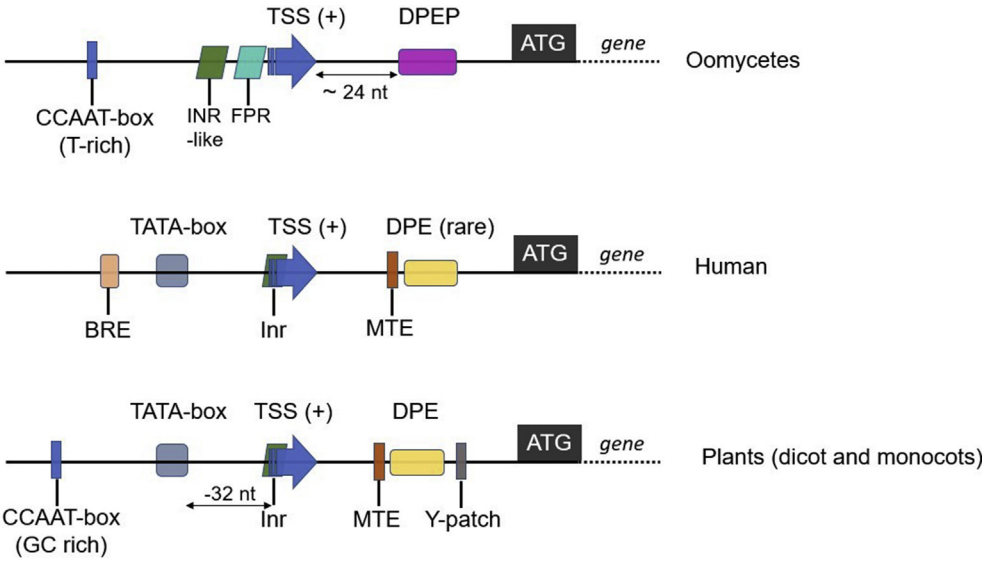

Fig. 1 – Schematic representation of core promoter and distal elements from oomycetes, human and plants. The initiator-like element (INR), flanking promoter region (FPR), downstream promoter element Peronosporales (DPEP) present upstream and downstream of transcription start sites (TSS) detected in oomycetes differ from the motifs present in humans and plants (Haberle and Stark, 2018; Juven-Gershon and Kadonaga, 2010; Kumari and Ware, 2013; McLeod *et al.*, 2004; Roy *et al.*, 2013). The oomycete core promoters are deprived of canonical TATA-box and other elements such as motif ten element (MTE), TFIIB recognition element (BRE), downstream promoter element (DPE) and pyrimidine rich region (Y-patch) necessary for transcription activity in human or plants.

Hsp70 genes have been well documented in many oomycetes including *P. infestans* (Judelson and Michelmore, 1991), *P. sojae* (Fang and Tyler, 2016; Judelson *et al.*, 1993), *P. nicotianae* (Bottin *et al.*, 1999; Narayan *et al.*, 2010), *P. palmivora* (Gumtow *et al.*, 2018; van West *et al.*, 1999), *P. ramorum* (Riedel *et al.*, 2009), *P. cactorum* (Chen *et al.*, 2016), *P. citricola* (Mcleod *et al.*, 2008), *Saprolegnia monoïca* (Mort-Bontemps and Fèvre, 1997), *P. litchii* (Jiang *et al.*, 2017), *Pythium aphanidermatum* (Weiland, 2003), *Globisporangium ultimum* (Vijn and Govers, 2003). However, overdependency on these two regulatory sequences may complicate gene functional studies, mainly because of transcriptional interference for multiple genes introduced under the same regulatory sequences (Shearwin *et al.*, 2005).

The bidirectional and constitutive nature of viral promoters have been extensively studied and used for functional analyses in plants (Alok *et al.*, 2019; Mitsuhara *et al.*, 1996), fungi (Sharma *et al.*, 2006) and animals (Liu *et al.*, 2008). Many viruses are known to infect and replicate within oomycetes (Cai and Hillman, 2013; Cai *et al.*, 2013; Kozlakidis *et al.*, 2010; Poimala and Vainio, 2020). Recently, the regulatory sequences from *Tobacco mosaic virus* (TMV) were showed to express green fluorescence protein (GFP) in mycelium and sporangia of *P. infestans* (Mascia *et al.*, 2019). To investigate the possibility of using viral promoters for expression of foreign genes in oomycetes, we searched for the presence of core promoter elements from oomycetes in three viral genomes: *Pythium splendens RNA virus 1* PsRV1-425508 (NCBI Accession No. LC467965.1), *Phytophthora cactorum RNA virus 1* (PcRV1; MN956531.1) and *Tobacco mosaic virus* (TMV; NC_001367.1) that can infect *Pythium* and *Phytophthora* species (Mascia *et al.*, 2019; Poimala and Vainio, 2020; Shiba *et al.*, 2019). The initiator motif CCACTCC was detected in the antisense (–) strand of all three viral genomes with high-confidence ($P < 0.001$; based on FIMO motif scan using the MEME Suite 5.3.3; http://meme-suite.org/tools/fimo),

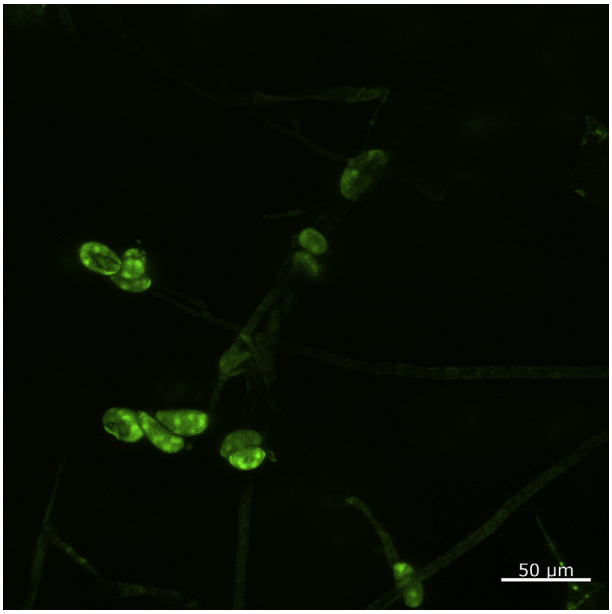

Fig. 2 – Sporulating *Phytophthora infestans* hyphae constitutively expressing enhanced Green Fluorescent Protein (eGFP). The image is a maximum intensity projection obtained using a laser scanning microscope (LSM 880, Zeiss) equipped with an Airyscan detector. Courtesy Sophie M. Brouwer.

suggesting transcriptional activity inside oomycetes. Motifs of flanking promoter region (ATTTTAC) and downstream promoter element (GAAGAAG) were also detected near initiator sequences, but with low confidence. This preliminary study suggests that oomycete viral promoters can be used to engineer vectors for further improvement of functional studies in oomycetes.

3. Gene silencing strategies

Extensive studies have been carried out in the past decades to deploy several gene silencing strategies to control important oomycetes diseases and for functional studies of oomycete genes.

3.1. *RNAi for functional studies*

Historically RNA-interference (RNAi) have been known under different names such as post transcriptional gene silencing or quelling. The method was first described in the nematode *Caenorhabditis elegans* (Fire *et al.*, 1998). The discovery of the method granted Andrew Fire and Craig Mello the Nobel prize in physiology or medicine in 2006. RNA-interference is a naturally occurring process in many eukaryotes and is protecting the organism's cells from transposons and viruses and regulates gene expression (Mascia *et al.*, 2019). The discovery of the process was ground-breaking since it allows reduced specific gene expression. RNAi has rapidly become a powerful reverse genetic tool, especially in organisms where gene targeting is inefficient and/or time-consuming (Nakayashiki and Nguyen, 2008).

The RNAi mechanism is based on the introduction of double stranded RNA (dsRNA) which activates an endonuclease called Dicer. The Dicer cuts the molecule into short 21–25 nucleotides fragments that are called small interfering RNA (siRNA). The siRNA then binds to a group of proteins from the Argonaute family, which are major components of the RNA-induced silencing complex (RISC), and the strands of the siRNA is separated. The sense strand is degraded immediately while the antisense strand is used by the RISC-complex as a template for recognising the complementary mRNA sequence which finally is degraded (Montgomery, 2002). The introduction of a synthetic complementary dsRNA to protoplasts will silence target genes at the post-transcriptional stage (Whisson *et al.*, 2005). In RNAi the dsRNA is only introduced to the protoplast at one point in the transformation process, which essentially means that the template will be depleted in time. The RNAi efficacy peaks around 14 days post introduction after which the efficacy rapidly decreases and the organism will return to normal gene expression (Whisson *et al.*, 2005).

The sequence-specific characteristics of RNAi could be effectively used to analyse functionally redundant gene families since family members sharing high sequence similarity can be simultaneously silenced by a single RNAi construct (Grenville-Briggs *et al.*, 2008).

For oomycetes, RNAi offers a valuable gene analysis tool since the RNAi machinery is known to degrade cognate mRNA in the cytoplasm and, therefore, is likely to be operative against any mRNA in multinuclear heterokaryotic mycelia. RNAi or transient gene silencing was first proven in *P. infestans* using the marker gene, *gfp*, and two *P. infestans* genes, *inf1* and *cdc14* (Whisson *et al.*, 2005). The study showed that transient gene silencing can be used to generate detectable phenotypes in *P. infestans* and could provide a high-throughput tool for *P. infestans* functional genomics. Grenville-Briggs *et al.* (2008) successfully used RNAi to silence a complete gene family from *P. infestans*. Silencing of a family of four cellulose synthase genes impaired appressorium differentiation and plant infection. Also in 2008, Walker *et al.* showed by RNAi that a DEAD-box RNA helicase is required for normal zoospore development in *P. infestans*. Zhao and collaborators (2011) described the first transient silencing protocol for *P. sojae*, and successfully silenced *PsCdc14* which is normally highly expressed during sporulation, zoospore, and cyst life stage. Silencing of *PsCdc14* resulted in low sporangial production and abnormal development of the transformants. In 2014, Saraiva *et al.* successfully used RNAi to silence a gene that encodes a tyrosinase – *SpTyr*, in the fish pathogenic oomycete *Saprolegnia parasitica*. This gene is involved in cell wall melanisation, and silenced lines showed abnormal sporangia formation. Recently, Iberahim *et al.* (2020) described RNAi silencing of an *Aphanomyces invadans* chaperone gene – *AiLhs1*. Silencing this gene, considerably reduced the number of virulence factors in the secretome and thereby the virulence of *A. invadans*, while proteins involved in the general production of proteins increased in abundance.

RNAi causes only a knockdown but not a complete loss (knockout) in gene expression. However, incomplete gene suppression can sometimes be beneficial. For example, partial silencing by RNAi makes it possible to investigate the effects of an essential gene. A negative aspect of sequence specific silencing by RNAi compared to knockout mutations, is that it is not possible to verify the RNAi results by genetic complementation.

3.2. *Cross-kingdom RNAi*

Cross-kingdom RNAi is a form of communication between two interacting organisms such as a host and its pathogen, pest, parasite, or symbiont. This implies that a translocation of gene silencing signals occurs between the two interacting organisms. Eukaryotic regulatory small RNAs (sRNAs) that induce RNAi are involved in a variety of biological processes, including host immunity and pathogen virulence. These immune regulatory sRNAs operate through distinct RNAi pathways that trigger transcriptional or post-transcriptional gene silencing (Weiberg and Jin, 2015). Bidirectional cross-kingdom RNAi can be exploited to genere silencing effects through the introduction of RNA molecules in transgenic plants that can suppress fungal and oomycetes virulence genes (Wang *et al.*, 2016b).

The RNAi machinery in plants has been demonstrated to also be functional against oomycete pathogens through a phenomenon known as host-induced gene silencing (HIGS). Typically, in this mechanism a sense, an antisense or a hairpin construct corresponding to a pathogen target gene is introduced into the plant. Ectopic expression of pathogen genes can then induce RNA silencing machinery and suppress the

target gene expression (Jahan et al., 2015). A significant reduction in *Phytophthora* spp. load and disease progression was recorded by HIGS targeting the *Avr3a* effector gene and the *G protein-β-subunit 1* (*PiGPB1*) gene of *P. infestans* and *P. capsici* in their host plants (Jahan et al., 2015; Sanju et al., 2015; Vega-Arreguín et al., 2014). In summary, HIGS can be used as a tool to functionally characterise oomycete genes (Vega-Arreguín et al., 2014).

Another aspect of HIGS is its possible use to control multiple pathogens by designing dsRNA and sRNA constructs that target several genes from different pathogens. Despite the promising results of HIGS, it involves the generation of genetically modified organisms (GMOs), which are currently banned in European agricultural productions (Cai et al., 2020).

4. CRISPR/Cas gene editing

Clustered regularly interspersed short palindromic repeats, CRISPR, associated with an enzyme, Cas, is a natural system of adaptive immunity against phage infection in bacteria and archaea (Deveau et al., 2010; Garneau et al., 2010; Horvath and Barrangou, 2010) co-discovered by Jennifer A. Doudna and Emmanuelle Charpentier who were awarded with the Nobel Prize 2020 in Chemistry for the discovery. The CRISPR/Cas system contains two components: Cas9 and single guide RNA (sgRNA) or a dual guide RNA (crRNA:tracrRNA). Cas9 is a nuclease from the type II CRISPR/Cas system of *Streptococcus pyogenes* that precisely cleaves DNA, resulting in double stranded DNA breaks (DSB) (Cong et al., 2013; Mali et al., 2013). The sgRNA contains a 20-nt sequence that guides Cas9 to a target DNA sequence directly upstream of any 5′-NGG sequence (protospacer adjacent motive; PAM) via Watson-Crick base pairing (Hsu et al., 2013). The DSB induced by Cas9 are repaired either by non-homologous end joining mechanism in absence of donor DNA or by homology directed repair (HDR) pathway in case of availability of a repair DNA template (Ran et al., 2013).

The CRISPR/Cas9 system is a powerful tool for obtaining precise mutations avoiding the potential undesirable effects from overexpression of target genes which seem more frequent in the traditional transformation method (Miao et al., 2018a,b). CRISPR/Cas9 based gene editing can produce homozygous mutants in diploid (polyploid) species (Pan et al., 2016; Wang et al., 2016a) which makes this technology highly promising for oomycete studies as obtaining homozygous mutants through sexual reproduction is very challenging (Lamour et al., 2007).

When setting up CRISPR/Cas9 the frequency of off-site target effects is a concern (Zhang et al., 2015). Expression of Cas9 or the sgRNA, non-working sgRNAs, efficiency of editing, selection of positive transformants, and toxicity are also concerns (Schuster and Kahmann, 2019). An additional limitation of the technology is the requirement for a PAM near the target site. Cas9 from the bacteria *Streptococcus pyogenes* (SpCas9) has a relatively short canonical PAM recognition site: 5′NGG3′, where N is any nucleotide. Nevertheless, CRISPR technology is inexpensive, relatively simple to use and reliable.

4.1. *Plant pathogenic oomycetes*

Establishment of the CRISPR/Cas9 system in the soybean root rot pathogen *P. sojae* through DSBs repaired by non-homologous end joining and HDR mechanisms was a breakthrough and has opened up for gene editing in oomycetes. Fang and Tyler (2016) conducted several experiments to verify efficient expression of *Cas9* using human-optimized codons (*hSpCas9*), previously used in diverse organisms (Cong et al., 2013; Peng et al., 2015; Zhang et al., 2014). Single guide RNA was generated using RNA polymerase II and transformants were screened using the geneticin (G418) resistance gene (*nptII*) as a marker driven by the *P. sojae* RPL41 promoter. After several experiments, they found increased editing efficiency when an “all in one” plasmid harbouring sgRNA, hSpCas9, and a selection marker in the same plasmid was used than when the necessary components were on different plasmids (Fig. 3A, B) The “all in one” CRISPR/Cas9 plasmid pYF515 constructed by (Fang et al., 2017) expresses both hSpCas9 and sgRNA, and in addition contains a site for cloning ribozyme-flanked sgRNAs by oligo-annealing and ligation. PEG-$CaCl_2$ mediated protoplast transformation was used for generating the gene-edited transformants in *P. sojae*. Single gene disruption was carried out by using sgRNA directing the Cas9 to cleave at the targeted position, while for a single gene replacement, the entire *nptII* open reading frame flanked by three different lengths of 5′ and 3′ sequences was used. The use of a sgRNA generated small indels at the Cas9 cleavage site, suggesting that the disruption of genes with large deletions probably require two or more sgRNAs or the use of HDR to replace the gene entirely. Moreover, CRISPR/Cas9 *AVR4/6* mutants showed no negative effects on growth of the pathogen while in-frame deletions of amino acids in the mutants impaired the avirulence effect (Fang and Tyler, 2016).

Since then, several successful studies have been reported from other oomycetes (Table 2). The “all in one plasmid” reported by Fang et al. (2017) has been used for other studies in *P. sojae* (Lin et al., 2018; Miao et al., 2018b, 2020; Ochola et al., 2020; Peng et al., 2019; Wang et al., 2020), *P. capsici* (Chen et al., 2019; Miao et al., 2018a; Wang et al., 2019) and *P. litchii* (Kong et al., 2019; Wang et al., 2019).

To employ CRISPR/Cas9 in *P. palmivora* the “all in one plasmid” from Fang et al. (2017) was modified by cloning PsNLS-hSpCas into pCB301TOR (Wu et al., 2016) to form pCB301TOR-hSpCas9 and then the entire sgRNA expression cassette from PYF2.3G-Ribo-sgRNA (Fang et al., 2017) resulting in an “all in one plasmid” (Fig. 3C) later used for *Agrobacterium* mediated transformation in *P. palmivora* (Gumtow et al., 2018).

Lin et al. (2018) employed CRISPR/Cas9 to study the role of the MADs box transcription factor *PsMAD* in *P. sojae*. The study revealed that *PsMAD* is involved in zoosporogenesis and pathogenesis in *P. sojae*. In the same year, this gene editing and replacement method was used to confirm that the substitutions G770V, G839W and ΔN837 in PcORP1 conferred resistance to oxathiapiprolin in *P. capsici* (Miao et al., 2018a). These results were later supported by a study of the same substitutions in ORP1 in *P. sojae*, *P. capsici* and *P. litchi* by Wang et al. (2019). Recently, Miao et al. (2020) used the CRISPR/Cas9 based HDR method to investigate if the point mutations responsible

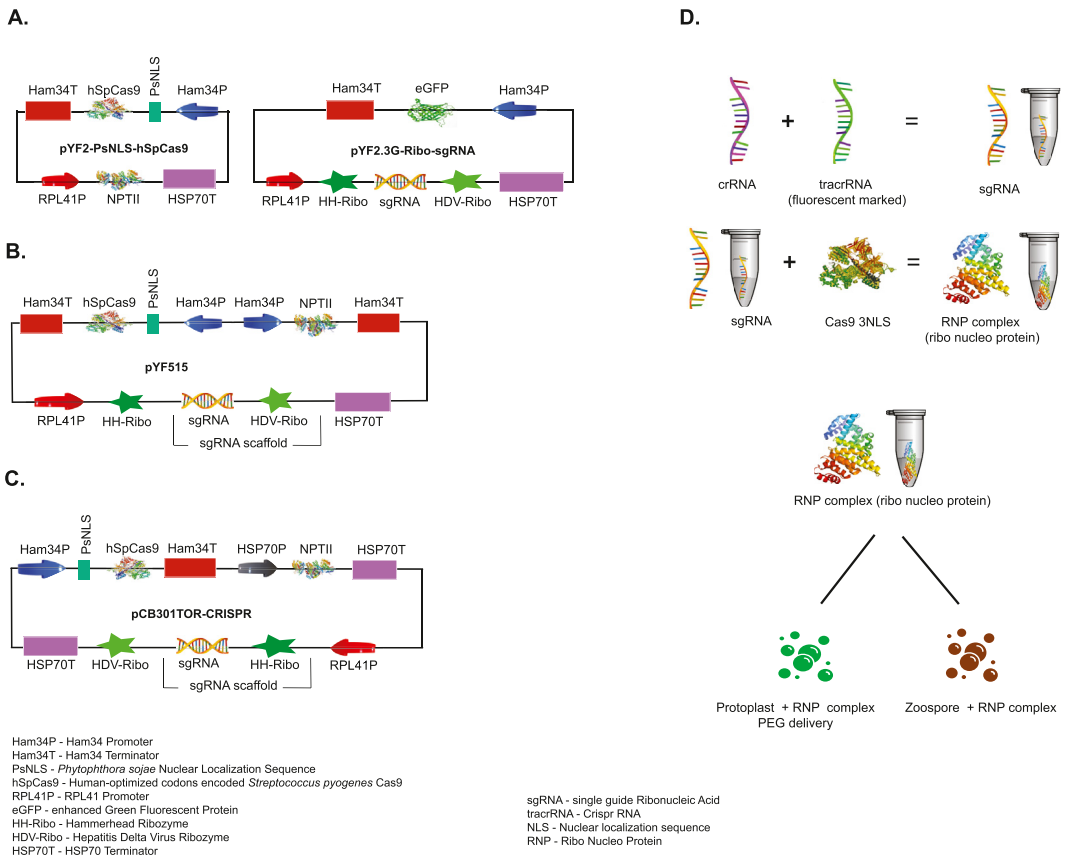

Fig. 3 – Schematic representations of CRISPR/Cas9 systems used in plant pathogenic oomycete (A–C) and animal pathogenic oomycetes (D). A) Two plasmid system in which Cas9 and sgRNA are expressed in two separate plasmids. B) Plasmid in which sgRNA, *Cas9* and *NPTII* resistant genes are in the same plasmid with individual promoters and terminators. C) All-in-one plasmid system for *Agrobacterium* mediated transformation of *P. palmivora*. D) Schematic representation of CRISPR-Cas9 system used in the animal pathogenic oomycete *Aphanomyces invadans*. The crispr RNA (crRNA) and trans-activating crRNA (tracrRNA) are synthetized *in vitro* and combined to form single guide RNA (sgRNA). sgRNA is then combined with Cas9 3NLS (nuclear localization signal) from *Streptococcus pyogenes* to form RNP complex. CRISPR/Cas9-RNP complex is then used for transfection of protoplast and zoospores to induce the mutation in the target gene.

for oxathiapiprolin resistance in *P. capsici* could confer the same resistance in *P. sojae*. Two sgRNA were used simultaneously to increase efficiency of HDR, which resulted in five transformants that had both the non-synonymous point mutations. The sgRNA target sequences were homozygous suggesting that after the sgRNA guided Cas9 cleavage in the genome, two alleles were repaired via HDR. Miao *et al.* (2020) suggested that the induction of two targeted DSBs resulting from the use of two sgRNAs could potentially trigger greater efficiency of HDR using DNA repair template. Similar results were reported from studies in mice (Han *et al.*, 2014). However, a profound study on the relationship between the number of sgRNA and effect on non-homologous end joining or HDR in oomycetes is still lacking.

Establishment of CRISPR/Cas9 in *P. palmivora* became a game changer as genetic studies on *P. palmivora* used to be very challenging due to its tetraploid and heterothallic nature (Ali *et al.*, 2017; Ko, 1988). Gumtow *et al.* (2018) studied the role of the extracellular cystatin-like cysteine protease inhibitor PpalEPIC8 in virulence in the host plant papaya using CRISPR/Cas9 via *Agrobacterium* mediated transformation (AMT). A homozygous PpalEPIC8 mutant exhibited increased papain sensitivity during *in vitro* growth and reduced pathogenicity during infection of papaya fruits compared to the wildtype. This was the first successful study combining CRISPR/Cas9 and AMT in oomycetes and the first study providing information that plant pathogenic oomycetes secretes cystatins which act as important weapons to invade plants. To study the potential emergence of fungicide resistance to the β-tubulin inhibitor ethaboxam in *P. sojae*, Peng *et al.* (2019) inserted three point mutations observed in β-tubulin of resistant isolates, into susceptible isolates through HDR mediated CRISPR/Cas9 method. One of the mutants developed high resistance, but this mutant was reduced in fitness. Such studies can in principle provide data for monitoring the emergence of pesticide resistance and management. Chen *et al.* (2019), generated CRISPR/Cas9 knockout mutants of the RXLR effector PcAvh1 in *P. capsici*, which all showed unaltered filamentous growth and zoospore production compared to the wildtype. However, virulence was significantly impaired in the mutants during infection of *Nicotiana*

Table 2 – CRISPR-Cas9 application in plant pathogenic oomycetes.

Species	Gene editing type	Transformation method[a]	Target gene/ repair template	sgRNA design tool[c]	No. of mutants obtained	Effect on growth/virulence of mutant	Reference
P. sojae	Single gene disruption	protoplast	*AVR4/6*	A	4 of 50 screened transformants were mutants	Non observed/Increased virulence in plants containing *Rps4* and *Rps6*	Fang and Tyler (2016)
		protoplast	*PsMAD1*	A	3 of 4 transformation reaction contained mutants	No zoospore production	Lin *et al.* (2018)
		protoplast	*PsORP2*	B	6 mutants obtained	No effect on growth and virulence	Miao *et al.* (2018a)
		protoplast	*PsSu(z)12*	A	3; no full-length deletion mutant obtained	Non observed/recognized by *Rps1b* plants	Wang *et al.* (2020)
	Singe gene replacement	protoplast	*AVR4/6-NPTII*[b]	A	9 of 68 transformants were mutants	Non observed/increase in virulence in plants containing *Rps4* and *Rps6*, but less virulence than in plants lacking *Rps4* and *Rps6*	Fang and Tyler (2016)
		protoplast	*β-tubulin-(Q8L, I258V, C165Y)*[b]	B	20 of 24 transformants were mutants	Effect on mycelial growth, zoospore production and lesions production in soybean seedlings	Peng *et al.* (2019)
		protoplast	*Mutant PsSu(z)12-PsSu(z)12*[b]	A	None	NA	Wang *et al.* (2020)
		protoplast	*PsORP1-(20-point mutations)*[b]	B	18 mutant lines with 11-point mutations	Non observed	Miao *et al.* (2020)
		protoplast	*Native promoter of PsAvr3b-(PsACT, PsXEG1, PsNLP1)*[b]	A	2 of 6 transformants were mutants	Non observed/Virulence of *PsXEG1* and *PsNLP1* mutants were enhanced against resistant (*Rps3b*), while *PsACT* mutant was unable to infect resistant soybean	Ochola *et al.* (2020)
P. palmivora	Single gene disruption	AMT	*PpalEPIC8*	A	4 of 16 transformants were homozygous mutants	Increased papain sensitivity during *in vitro* growth/reduced pathogenicity during infection of papaya fruits	Gumtow *et al.* (2018)
		AMT	*Ppal15kDa*	A	6 of 20 transformants were mutants	Compromised in virulence	Pettongkhao *et al.* (2020)
P. capsici	Singe gene replacement	protoplast	*PcORP1*	B	8 mutants obtained with G770V, G839W, ΔN837	No sporangia production in lines with G839W	Miao *et al.* (2018b)
		protoplast	*PcAvh1-NPTII*[b]; *Mutant PcAvh1 – PcAvh1*[b]	A	3 mutants	Non observed/impaired virulence	Chen *et al.* (2019)
		protoplast	*PcDHCR7 – G418*[b], *KD1-PcMuORP1*[b]	A	22 of 22 transformants were mutants	Non observed	Wang *et al.* (2019)

P. litchii	Single gene replacement	protoplast	*PlPAE4* – *NPTII*[b]	B	3 of 70 transformants were mutants	Non observed	Kong *et al.* (2019)
			PlPAE5 – *NPTII*[b]		3 of 40 transformants were mutants	Reduced virulence	Kong *et al.* (2019)
P. infestans		protoplast	*Inf1*		–	–	Ah-Fong *et al.* (2021)

a Protoplast – PEG-mediated protoplast transformation; AMT – Agrobacterium mediated transformation.
b Repair template for HDR.
c A – http://grna.ctegd.uga.edu/; B – https://portals.broadinstitute.org/gpp/public/.

benthamiana and stems of bell pepper, while introducing a full-length copy of the *PcAvh1* gene in the knockout mutants restored the virulence. Kong *et al.* (2019) studied the role of pectin acetylesterases (PAEs) in the lychee pathogen *P. litchi* by creating knockout mutants of two predicted virulence genes (*PlPAE4* and *PlPAE5*). The pathogenicity tests of both mutants revealed that one of the mutants, the *PlPAE5*-knockout significantly reduced lesion diameter on lychee leaves. PlPAE5 contribution to virulence was further verified by overexpressing the gene in *N. benthamiana* followed by *P. capsici* inoculation (Kong *et al.*, 2019).

Wang *et al.* (2020) used CRISPR/Cas9 to elucidate the role of Histone H3 Lys27 tri-methylation (H3K27me3) in a naturally occurring *Avrb1*-silenced strain of *P. sojae* that makes the pathogen evade immunity mediated by the resistance gene *Rps1b*. The gene *PsSu(z)12* encoding the core subunit H3K27me3 was edited using CRISPR/Cas9. Despite the use of HDR approach, no homozygous full-length deletion mutants were obtained suggesting *PsSu(z)12* removal might be lethal. Ochola *et al.* (2020) reported the first use of the CRISPR/Cas9 technique to manipulate promoter sequences controlling expression of *Avr* effector genes. The native promoter of *P. sojae PsAvr3b was* replaced with three different promoters. The mutants with low PsAvr3b expression successfully colonized soybean plants carrying the cognate resistance gene Rps3b. However, all the selected mutants showed normal vegetative growth compared to the wildtype (Ochola *et al.*, 2020).

Based on the report of Gumtow *et al.* (2018) on successful deployment of CRISPR/Cas9 in *P. palmivora*, Pettongkhao *et al.* (2020) used the same method for functional study of a secreted glycoprotein of 15 kDa, Ppal15kDa, which when transiently expressed in *N. benthamiana* enhanced *P. palmivora* infection. Mutants obtained were found to be compromised in virulence in both in *N. benthamiana* leaves and papaya fruits.

Despite the successful applications of the CRISPR-Cas9 system in *P. sojae, P. palmivora, P. capsici and P. litchii,* Van den Hoogen and Govers (2018) reported unsuccessful attempts in *P. infestans* using multiple sgRNAs, different promoters, alternative expression vectors, different nuclear localization sequences (NLS) and alternative strategies for delivery of the ribonucleoprotein (RLP) complexes. The authors could not pinpoint the cause for failure of the system but speculated that it was an additive effect of several suboptimal conditions, such as Cas9 or gRNA expression levels, Cas9 localization, or the incubation temperature (van den Hoogen and Govers, 2018). Recently, Ah-Fong *et al.* (2021) discovered that it is difficult to express CRISPR/Cas9 in *P. infestans*, while a catalytically inactive variant of Cas9 could be expressed, suggesting that the active Cas9 is toxic. Toxicity of Cas9 has also been reported from other organisms (Foster *et al.*, 2018; Markus *et al.*, 2019). Ah-Fong *et al.* (2021) were able to achieve editing with CRISPR/Cas12a using vectors in which the nuclease and its guide RNA were expressed from a single transcript. Unlike Cas9, Cas12a has intrinsic RNase activity that enables Cas12a to form its own sgRNA by cleaving at direct repeats in the crRNA. Targeting the elicitor gene *Inf1,* which encodes a sterol-binding protein that induces defence responses in certain nonhost plants, they observed editing of one or both alleles in up to 13 % of transformants (Ah-Fong et al., 2021).

4.2. *Animal pathogenic oomycetes*

Aphanomyces invadans is the only animal pathogenic oomycete in which the CRISPR/Cas9 has been established successfully thus far (Majeed *et al.*, 2018). *A. invadans* is a devastating pathogen of fish, in particular carps in Asia are very susceptible (Iberahim *et al.*, 2018). Majeed *et al.* (2018) developed an *in vitro* CRISPR/Cas9 transfection system where zoospores and protoplasts of *A. invadans* were treated with a guide-RNA combined with Cas9 that forms a ribonucleoprotein (RNP) complex to mutate a target gene in *A. invadans* (Fig. 3D). To test the gene mutation system, a serine protease gene was targeted in the genome of *A. invadans*. Serine proteases have long been considered as virulence factors in both bacteria and eukaryotes (Minor *et al.*, 2014; Jiang *et al.*, 2013). Majeed *et al.* (2017) suggested that at least one of the abundantly secreted extracellular proteases belonging to the peptidase_S8 domain superfamily plays a role in virulence of *A. invadans* and this was subsequently tested with CRISPR/Cas9 (Majeed *et al.*, 2018). In this study, three guide-RNAs were designed, checked for secondary RNA structures and off target sites using online prediction tools. The guide RNAs were individually combined with the Cas9 to form a ribonucleoprotein (RNP) complex. Protoplasts or zoospores were then transfected with the individual RNP complexes using the polyethylene glycol mediated RNP delivery method described by Malnoy *et al.* (2016). Sequencing of the target gene after amplification showed the incidence of a point mutation in the target gene from one of the three RNP complexes used. *In vitro* results showed slow growth pattern of the transfected protoplasts which was attributed to the point mutation in the serine protease gene, as these enzymes are also known to be essential for growth and development of many microorganisms (Di Cera, 2009). *In vivo* infection experiments with the RNP complex were performed in dwarf gourami (*Trichogaster lalius*), which is known to be highly susceptible to *A. invadans* (Hatai *et al.*, 1994). The CRISPR/Cas9-treated *A. invadans* zoospores were not able to produce clinical signs in the fish (i.e., reddening, swelling, deep ulcers in skin and muscles, and visible hyphae at the site of infection) whereas fish treated with wildtype zoospores did. The virulence and infectivity of CRISPR-treated zoospores of *A. invadans* were likely affected by the mutation in the serine protease gene, which confirmed the potential role of this serine protease in the development and virulence of this animal pathogenic oomycete.

The developed CRIPR/Cas9 system for *A. invadans* by Majeed *et al.* (2018) was not used to create stable edited lines. However, in theory this should be possible and would be a great step forward in functional characterisation of genes in this and other animal pathogenic oomycetes such as *Saprolegnia*.

5. Conclusion/Future perspectives

Studies on oomycetes with the use of molecular techniques like transformation, gene silencing, gene editing, genomics, and transcriptomics have resulted in a profound understanding of host–oomycete interactions. However, there are certain limitations of each technique in the study of individual oomycete pathogens because of their high heterogeneity. Many but far from all economically important oomycetes have been successfully transformed using the PEG-mediated, electroporation, AMT, or micro-projectile bombardment method, mostly with the purpose of studying their virulence mechanisms. As the transformation is a crucial step, continued effort and optimalisations for the different oomycete species and strains are necessary. Developing viral vectors that can deliver foreign DNA molecules inside oomycete cells and amplify delivery upon viral multiplication might offer a solution for more efficient functional gene studies (Mascia *et al.*, 2014; Zaidi and Mansoor, 2017). However, large nucleotides insertion into viral genome may incur fitness cost and can reduce viral infectivity (Li *et al.*, 2019; Willemsen and Zwart, 2019), so this needs careful consideration during vector development.

Only two oomycetes, *P. infestans* and *P. parasitica*, have been successfully used in host-induced gene silencing. Nevertheless, RNAi techniques have proven powerful in many oomycetes to knockdown target genes for functional studies.

CRISPR-cas9 is also a promising tool for gene functional analysis and elaborating virulence mechanisms. In comparison with other molecular editing techniques like zinc finger nuclease, TALENS, and RNAi, CRISPR-cas9 is relatively easier to use, more efficient, and highly precise (Gaj *et al.*, 2016; Hruscha *et al.*, 2013; Khan, 2019). Even though CRISPR-cas9 is regarded as the most powerful technique in modern biology, this gene-editing technique has still not been established in many important oomycete pathogens. However, the establishment of this system in five plant pathogenic oomycetes and one animal pathogenic oomycete in recent years has paved the way for starting similar studies in other economically important oomycete pathogens. The recent success of CRISPR/Cas12a in *P. infestans* has opened up for the use of Cas12a to further explore this technology in other oomycetes thus avoiding the potential toxicity of the active Cas9 nuclease. Since homologous recombination or homology-directed repair seems rare in oomycetes, refinement of the CRISPR/Cas technique has the potential to revolutionize our knowledge in oomycete biology and pathogenicity, creating knowledge and tools to limit the detrimental effects of oomycetes in plants, fish, and other aquatic animals.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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