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Transgenic poplar for resistance against pest and pathogen attack in forests: an overview

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Forests are potential habitats for immense terrestrial ecosystems and aquatic biodiversity, performing an essential role in ecological preservation and regulation of climate. The anthropogenic pressures on the forests lead to forest loss, fragmentation and degradation. Requirements for sustainable methodologies for forest protection are of utmost priority under the climate change regime. Among forest trees, poplar trees (*Populus* L.) have attracted attention in global forestry as a promising material for improving the quality and quantity of urban landscapes. These plants provide wood, which can be utilized as raw resources for the paper industry and as a potential source of biofuel. However, several biotic stresses, such as attacks by pests and pathogens, severely affect poplar production and productivity. The improvement of *Populus* trees through conventional tree breeding methods is restricted due to their long-life cycles and the lack of suitable donors with resistance genes. *Populus* has been utilized as a model plant for studying gene functions due to its highly efficient genetic transformation capabilities. The present review will provide a comprehensive overview of pest and pathogen attacks on poplar, focusing on their infection mechanisms, transmission routes, and control strategies. Additionally, it will examine the most widely used genetic transformation methods (gene gun-mediated, *Agrobacterium tumefaciens*-mediated, protoplast transformation, micro-RNA mediated and micro-RNA clustered regularly interspaced short palindromic repeats (CRISPR)-associated (CRISPR-Cas) systems methods and RNA interference) for improving tolerance in poplar trees against pest and pathogens attack. Furthermore, it will delve into prospects, challenges, and recent advances in molecular biology tools and their safe application for genetic transformation to improve insect and pest resistance in poplar trees. Finally, the regeneration of transgenic poplar trees with enhanced resistance, developed through various genetic engineering techniques, is discussed.

KEYWORDS

forest protection, genetic transformation, protoplast transformation, *Agrobacterium*-mediated transformation, salicylic acid (SA), methyl jasmonate (MeJA)

1 Introduction

Forest trees have several pivotal roles, such as maintaining ecology, climate regulation, providing raw materials for the construction of buildings, greening roads, and being an energy source (Trumbore et al., 2015). Among these forest trees, poplar (*Populus* spp.) (known as 'the people's tree') is one of the most widespread trees in the world (Xi et al., 2021; Yevtushenko and Misra, 2019). The poplars are essential for maintaining the world's ecological balance and socio-economic wellbeing (Häggman et al., 2013). The first use of the poplar cultivar started in 1700–1720 when *Populus nigra* 'Italica' (*P. nigra, Lombardy nigra*) was used in Italy, Europe and North America. There was a rapidly increased demand for poplar plants after World War II when Europe was devastated by the lack of readily available wood for construction and fuel. Consequently, the domestication and cultivation of the genus poplar started in Europe by introducing eastern cottonwood (*Populous deltoides*) and followed by hybrids black poplar (*P. nigra*) (a hybrid of P. ×*canadensis*) for fulfilling the increased demands for woods (Stanton et al., 2009).

Poplar trees (family *Salicaceae*) are tall, deciduous, dioecious, paleopolyploids, or ancient polyploids, naturally diverse, fast-growing and widely distributed globally (Lubrano, 1992), especially in temperate, sub-temperate and sub-tropical regions of Northern hemisphere and in tropical Africa also (Tuskan et al., 2006; Guleria et al., 2022). These poplars, except aspens and Asian mountain balsam poplars, grow widely in several regions like hot-arid and desert-like regions of central Asia and Africa, alpine forests in Europe and North America, as well as riparian zones like river banks and flood plains (Guleria et al., 2022). Poplars are the dominant species in these habitats for tolerating and sustaining in the complete flood. China, Turkey, France, India and the Po River plain of Italy are the largest poplar farming areas for worldwide wood supply, whereas Italy, Spain, France and Hungary provide a landscape of 0.5 out of 0.61 million hectares for poplar farming only. Poplars shape global forests and woodlands in their natural habitats. Besides being domesticated as an agroforestry tree, they provide timber, fuel wood, plywood, industrial roundwood, sports materials, pallets, paper pulp for the paper industry and fodder (Kollert et al., 2014). In addition, *Populus* species are cultivated as energy crops/biofuel (ethanol) in Europe (England and Italy) for carbon sequestration and sustainable bioenergy production in USA because of its high biomass production in a relatively short time (Dou et al., 2017). They are also used in the phytoremediation of toxins, e.g., heavy metals (Cd, Pb, As, and Hg) from contaminated soils, indicating ozone pollution as a bio-indicator, rehabilitation of fragile ecosystems and restoration of forest landscapes (Alahabadi et al., 2017).

Poplar (Genus: *Populus*) comprises 32–40 species based on taxonomic and morphological traits (Cronk, 2005; Douglas, 2017). There is a record of a total 582 *Populus* species, with more than 100 species names recognized worldwide. The higher number of species is due to the presence of naturally occurring hybrids (The Plant List, 2013). According to Eckenwalder (1996), the genus *Populus* is classified into six groups including the cottonwoods (*Aigeiros*), aspens (*Populus*), balsam poplars (*Tacamahaca*), large-leaf or swamp poplars (*Leucoides*) and (*Abaso*) and Afro-Asian poplars (Turanga), and but the Flora of China recognized 71 species from five sections (except *Abaso*) (Park et al., 2004; Table 1). Species boundaries among poplars are sometimes variable as intrasectional and intersectional hybridization occurs among them. However, this has not been supported by molecular evidence. Hence relationships between these sections are reported to be controversial (Wang et al., 2014; Liu et al., 2017; Zhang et al., 2018). During 1950s, poplar was introduced in India from the United States of America. Since then, *P. deltioides,* have been cultivated in India and cover an area of 270,000 ha in India, according to the report of the Indian Council of Forestry Research and Education (ICFRE, 2016; Eqbal and Ansari, 2024). Several researches have proved that the genus *populus* is a rich source of active metabolites, like phenolic compounds, terpenoids, and flavonoids in different parts like stems, buds, leaves and bark (Guleria et al., 2022). These poplar trees have been used to cure various ailments and have several pharmacological properties such as antioxidants, antimicrobials, anticancer, and anti-inflammatory (Guleria et al., 2022).

Poplar was the first woody perennial tree used as a model/experimental tree species among forest trees worldwide for understanding several aspects such as taxonomy, genetics, evolution and the genomics of wood formation for decades because of their small genome size, clonal propagation, fast growth, easy transformation, and long-life cycle (Taylor, 2002). In addition, poplar genome has been completely sequenced after *Arabidopsis* and rice. Moreover, it is the most advanced genomics resource of any forest tree, having reference genomes for several tree species. The black cottonwood (*P. trichocarpa*) was the first forest tree whose genome was sequenced entirely, and currently, there are massive genomic resources available for other poplar species. Interestingly, *P. tomentosa* and *P. euphratica* genomes have been extensively studied, while others have been ignored. All *Populus* species contain 19 haploid genomes (Shi et al., 2024). However, massive rearrangement and diploidization of the whole genome of poplar have been reported. The arabidopsis-poplar genome comparative model approach has been used efficiently in many cases (Rottmann et al., 2000; Jansson and Douglas, 2007). The genus *Populus* is an excellent model for studying the molecular genetic mechanisms involved in pathogen defense responses in several forest trees. The availability of *Populus* genome sequences enhances the efficiency of the substantial molecular tool kit that already exists for *Populus* species, including expressed sequence tags (ESTs) collection and microarrays for transcriptome. In addition, these tools can be applied with valuable pedigrees and genetic maps developed for *Populus* breeding for decades (Sterky et al., 1998; Frewen et al., 2000; Hertzberg et al., 2001; Cervera et al., 2001; Bhalerao, 2003; Andersson Gunnerås et al., 2006). Such pedigrees have been proven useful and efficient in revealing *Populus* loci responsible for conferring resistance to fungal pathogens attacks (Goué-Mourier et al., 1996). Poplar cultivation is severely affected by several ranges of pest insects and pathogens such as fungi, bacteria, and viruses, resulting in reduced growth and quantity and quality of wood. These biotic stresses comprise the complex interactions between hosts, pests, pathogens, and environmental factors, negatively affecting the poplar population (Seserman, 2018). The efficiency of traditional methods for protecting poplar farming from attack of pests and pathogens is hindered by factors like climatic instability, global warming, flood, drought, high temperatures and humidity. Consequently, new biotechnological approaches like genetic transformation and genome editing are required to overcome these limitations of traditional breeding. These tools are utilized to increase the quality and yield of wood and improve pest and pathogen

TABLE 1 Taxonomic categorization of different species of the genus *Populus*.

Divisions	Names	Occurrence	References
Turanga Bunge	Subtropical Asian poplars (*P. lasiocarpa* Oliv, Chinese necklace poplar), *P. ilicifolia* (Engl.) Rouleau (Kenyan poplar), *P. euphratica* Oliv (Euphrates poplar)	China, Northeast Africa, Southwest Asia, East Africa (subtropical and tropical)	Gai et al. (2021) and Du et al. (2024)
Populus or Leuce Duby	True white poplars and aspens *P. adenopoda* Maxim (Chinese aspen), *P. monticola Brandegee* (White poplar), *P. alba* L. (White poplar) *P. tremuloides* Michx (Quaking aspen) *P. tremula* (Japanese aspen)	China, Europe, North Africa, India, Mexico, North America, Northeast Asia	Chanda et al. (2010) and Du et al. (2024)
Aigeiros Duby	Black poplars, *P. trichocarpa* Torr. (Black cottonwood poplar), *P. fremontii S. Watson* (Fremont's cottonwood), *P. deltoides* Marshall (Eastern cottonwood)	Europe, Western Asia, Temperate region of North America, Central Asia	Gai et al. (2021) and Porth et al. (2024)
Abaso Eckenwalder	Endemic Mexican poplars (*P. pruinosa* Schrenk, Desert poplar), *P. Mexicana*	Mexico	Liu et al. (2017), Wang W. et al. (2022a), and Wang Y. et al. (2022b)
Leucoides Spach	Big leaf poplars (*P. heterophylla* L., *Swamp cottonwood poplar*), *P. jacquemontiana* Dode (Sichuan poplar)	Eastern North America (USA), Eastern Asia China, India (warm and temperate)	Wang W. et al. (2022a), Wang Y. et al. (2022b), and Zhang et al. (2019)
Tacamahaca Spach	Balsam poplars *P. ciliata* Wall. ex-Royle (Himalayan poplar), *P. angustifolia* E. James (Narrow leaf cottonwood poplar), *P. suaveolens Fisch.* ex-Loudon (Asian poplar), *P. trichocarpa* Torr. (Black cottonwood poplar)	North America, Asia [India, Pakistan, Bhutan, Nepal, Myanmar (Cool temperate), Northeast China, Japan]	Chanda et al. (2010) and Gai et al. (2021)

resistance in forest trees, including poplar (Li P. et al., 2024; Li Y. et al., 2024; Li Z. et al., 2024). This paper primarily summarized the details of pest and pathogen attacks that cause diseases in poplar trees. We also examined the mechanism of infection, transmission route, the role of lignin in protecting poplar against pests and pathogens and the mode of control of diseases in poplar. Here, we also emphasized conventional breeding and its limitation for poplar plantations on a large scale. The application of different tools of genetic transformation and genome editing for developing transgenic poplar resistant to pests and pathogens was discussed in detail. Finally, we summarize the regeneration of transgenic poplar trees that have been modified by incorporating defense genes, such as those conferring pest and pathogen resistance.

2 Poplar susceptibility to biotic stresses

Poplars are frequently impacted by infestations of insect pests such as mites, aphids, and caterpillars, as well as bacterial, viral, and fungal infections. These pests and pathogens target all parts of the tree, damaging buds and leaves, inducing gall formation, sucking sap, altering bark structure, and boring into shoots and roots, which facilitates the transmission of plant diseases. Over time, these attacks can lead to complete defoliation, reduced tree growth, and even tree death. As a result, affected poplars become unsuitable for various uses, including furniture production, biofuel generation, and veneering (Charles et al., 2018).

2.1 Pest attacks on poplar

Insect pests are a limiting factor affecting *Populus* productivity worldwide (Table 2). Globally, outbreaks of pests are boosted due to climate change (Fenning, 2013). Around 525 and 300 species of insects and mites feeding on *Populus* have been identified as serious threats for causing economic and ecological losses in poplar plantations in Europe and North America, respectively (Charles et al., 2018). Ahmad and Faisal (2012) documented that around 133 insect species feed on poplar plantations in India. These pests hinder plant growth and increase tree mortality (Dickmann, 2001; Coyle et al., 2005). In North America, the cottonwood leaf beetle (CLB) (*Chrysomela scripta*) is reported as the most widespread and severe defoliator of young *Populus* cultivation (Coyle et al., 2005). In the Mediterranean, *Saperda carcharias* (large poplar borer) is reported to be one of the most damaging insects for young poplar plantations (Biselli et al., 2022). In China, major pest species destroying poplar plantations are trunk borers and defoliators such insects of the *Lepidoptera* (*Hyphantria cunea* Drury), *Apocheima cinerarius* Ershoff, *Lymantria dispar* Linnaeus, *Malacosoma neustria* Motschulsky, and other moth species belonging to the *Notodontidae* and *Limacodidae* and *Coleoptera* (*Apriona germari* Hope, *Anoplophora glabripennis* Motschulsky, and *Plagiodera versicolora* Laicharting). In addition, up to 40% loss of hybrid *Populus* plantations is reported due to poplar looper (*Apochemia cinerarua*) and the spongy moth (*Lymantria dispar*) (Hu et al., 2001; Wang et al., 2018) in China. *Anaplophora glabripennis* also causes massive destruction of hectares of Chinese poplar (*P. simonii*) plantations. Biselli et al. (2022) observed that *Phloemyzus passerinii* [Wooly Poplar Aphid (WPA)] causes 10% of production losses of poplar, mainly in European and American countries. Other insects, for example, *Cossus cossus*, *Agrilus suvorovi*, *Megaplatypus mutatus*, *Paranthrene tabaniformis*, *Melanophila picta*, and *Gypsonoma aceriana*, also threaten poplar farming. Recently, transcriptomic and metabolomic analyses were conducted to investigate the species-specific defense responses of *Populus tremula* against herbivores such as spongy moths (*Lymantria dispar*) and aphids (*Chaitophorus populialbae*). The insights gained from these studies could

TABLE 2 List of pests/pathogens/bacteria/viruses affecting poplar plantations.

Plants	Pest/pathogens	Diseases	Affected Countries	References
Insects/pests				
Populus fremontii, P. tremula, P. nigra and P. angustifolia, P. alba	*Aceria parapopuli*	Soap sucker, galls, irregular, warty, cauliflower-like growth	North America, Iran	McIntyre and Whitham (2003) and Mehri et al. (2020)
P. tremula, P. deltoides, P. alba	*Agrilus suvorov*	Borer	Germany, Greece, Guernsey, Hungary, Ireland, Israel, Italy	Cavalcaselle (1972)
P. tacamahaca, P. tremuloides	*Altica populi*	Defoliation	North America	De Tillesse et al. (2007) and Ostry et al. (2014)
P. deltoides, P. alba P. nigra, P. x euramericana hybrids	*Leucoma wiltshieri*	Defoliation	Europe, Middle East, Japan, Iran, America, China	Sadeghi et al. (2009) and Charles et al. (2014)
P. × euroamericana, P. euphratica, P. alba, P. nigra L., P. deltoides Marsh.	*Melanophila picta*	Wood borer	Bulgaria, Southern France, Italy, Spain, Portugal, Turkey Pakistan	Mazhar and Sadeghi (2024)
P. tremuloides, P. purpurea, P. nigra	*Phratora laticollis*	Defoliation	North America, Belzium, Germany	Nagaraju et al. (2023)
P. deltoides × P. nigra [P. × euramericana (Dode) Guinier], P. alba	*Sesia apiformis*	Borer, root sucker	Europe, Canada, Asia Minor, Middle East, China, North America	Martín García et al. (2011) and Meert (2022)
P. deltoides, P. tremula	*Bycticus populi*	Defoliation	Europe	Urban (2013) and Schroeder and Fladung (2018)
P. tremuloides, P. deltoides, P. gradidentata	*Choristoneura conflictana*	Defoliation	Canada, Northeastern and Central USA, Alaska,	De Tillesse et al. (2007) and Charles et al. (2014)
P. deltoides	*Dasineura salicis*	Galls	Europe, North America	De Tillesse et al. (2007) and Charles et al. (2014)
Fungus				
P. davidiana × P. bollena, P. euphratica, P. deltoides.	*Alternaria alternata*	Leaf blight	India, China, Iran	Osdaghi et al. (2014), Uniyal et al. (2018), and Huang et al. (2022)
Populus× canescens 'Tower'	*Apioplagiostoma populi*	Bronze lea	North America	Wijekoon et al. (2021)
P. deltoides × P. nigra	*Botrydiplodia populea*	Canker	China, Poland	Kwaśna et al. (2021b)
P. deltoides, P. tremuloides, P. maximowiczii × P. x. berolinensis, P. serotina	*Ceratocystis fimbriata*	Black and target canker	USA, North America, Alaska, Poland, Quebec, India, Poland	Johnson et al. (2017)
P. tremuloides	*Ciborinia whetzelii*	Ink-spot disease	Northern USA, Canada	Zegler et al. (2012) and Kowalski (2013)
P. ×euramericana, P. yunnanensis, P. deltoides	*Corticium salmonicolor*	Pink disease	India	Saxena et al. (2017)
P. tremuloides, P. balsamifera, P. tremula	*Diplodia tumefaciens*	Bark alterations, woody gall	Canada, Europe, Northern USA	Kwaśna et al. (2021b)
P. trichocarpa ×P. deltoides, P. tremula, P. alba, P. grandidentata, P. tremuloides	*Linospora tetraspora*	Leaf blight	USA, Canada	Zobrist et al. (2023)
P. deltoides	*Melampsora medusae*	Leaf rust	Europe, New Zealand, Australia, South Africa, Argentina, North America, India, Canada, Japan	Zeng et al. (2023)
P. deltoides	*Septoria musiva*	Canker and leaf spot	Europe, North America	Feau et al. (2010) and Dunnell and LeBoldus (2017)
P. alba	*Venturia tremulae*	Spring leaf, shoot blight	North America, China, Africa	Martínez-Arias et al. (2019)

(Continued)

TABLE 2 (Continued)

Plants	Pest/pathogens	Diseases	Affected Countries	References
Bacteria				
P. alba, *P. trichocarpa*, *P. deltoides*	*Erwinia herbicola*, *Erwinia carotovora*	Bacterial twig canker with gall like formations	Europe, North America	Fabi et al. (2008)
P. tremula L. 70 × (Populus × canescens)	*Phytophtora. cactorum and P. plurivora.*	Root rot	Asia, Europe, Africa, USA, Australia, New Zealand, Serbia	Cerny et al. (2022)
P. ×euramericana	*Lonsdalea populi*	Bark canker	China, Europe	Li and He (2019)
P. trichocarpa	*Pseudomonas syringae*	Bacterial blight	Worldwide	Saint-Vincent, et al. (2020)
P. tomentosa, *P* × *euramericana*	*Sphingomonas sanguinis*	Bark canker	Worldwide	Deng et al. (2023), Li P. et al. (2024), Li Y. et al., (2024), and Li Z. et al. (2024)
P. trichocarpa	*Xanthomonas populi*	Canker	Europe and America	Kwaśna et al. (2021b)
Virus				
P. nigra, *P. trichocarpa*, *P. deltoides*, *P. candicans*, *P. ×euramericana*	*Poplar mosaic virus*	Leaf mosaic	Worldwide	Smith and Campbell (2004)
P. tremuloides	*Tobacco necrosis virus*	Necrosis of leaf	Worldwide	Shen et al. (2015)
P. ×euramericana	*Arabis mosaic virus*	Leaf mosaic	Japan, New Zealand, America, Europe	von Bargen et al. (2020), Li P. et al. (2024), Li Y. et al., (2024), and Li Z. et al. (2024)
P. tremuloides	*Potato virus Y*	Mottling/yellowing of leaf, leaf drop leaf crinkling	Worldwide	Lawrence and Novak (2006)
P. euphratica and P. × *canescens*	*Tobacco rattle virus*	Mottling, chlorotic or necrotic local lesion, ringspots or line patterns, necrosis	Worldwide	Shen et al. (2015)
P. balsamifera	*Tomato black ring virus*	Mottling, deformation, leaf necrosis	Worldwide	Li P. et al. (2024), Li Y. et al., (2024), and Li Z. et al. (2024)

be valuable for developing transgenic poplar varieties with enhanced resistance to pest attacks (Pastierovič et al., 2024).

2.2 Pathogen attacks on poplar

Poplar trees are also constantly challenged by various pathogens like fungi, bacteria and viruses (Table 2). These pathogens inhibit the growth of poplar, impacting the quality and quantity of wood biomass. The different poplar culture practices and the introduction of exotic pathogens promote the widespread distribution of some specific pathogens (Newcombe et al., 1996).

2.2.1 Fungal attack on poplar

Fungi are usually considered as "primary parasites." They infect healthy plants, which can eventually affect poplar growth and hence decrease the quality and production of wood. The diseased poplars can exhibit reduced leaf photosynthetic areas. Leaf scars created allows entry of secondary pathogens. Repeated infections and premature poplar defoliation may weaken plants, making them susceptible to insect attack, high temperatures and drought (Kebert et al., 2022). Generally, plant pathogens are categorized into three groups: (a) biotrophs (feed on living plant tissue), (b) necrotrophs (feed on dead plant tissue), and (c) hemibiotrophs (first infect living plant tissue and make them dead and then feed on dead tissues) (McCombe et al., 2023). Examples of biotrophs infecting poplar are powdery mildews by *Phyllactinia* spp. or *Uncinula* spp., leaf rust by fungus *Melampsora* spp., while necrotrophs including leaf blight by *Septoria* spp. and leaf spot by *Coryneum* spp. and *Marssonin*a spp., canker (*Septoria* spp.) (Feau et al., 2010; Zeng et al., 2023). Among fungi, the genus *Melamspora* (biotrophic rust fungi), especially (*Melamspora. larici-populina*) is reported as the most severe and widespread fungi in poplar plantations (Polle et al., 2013). Infection with this genus is characterized by premature defoliation and reduced photosynthetic ability, resulting in loss of wood production (Polle et al., 2013). Moreover, *M. larici-populina* is also responsible for severe economic poplar losses in Europe and America (Duplessis et al., 2009), while *Melamspora medusae* caused leaf rust in *P. deltoides* in East-North America and the North-West USA (Newcombe et al., 1996). The other primary poplar diseases like stem canker and leaf spot in North America and Europe are caused by fungus *Septoria musiva* (also known as *Sphaerulina musiva*) (Zhao et al., 2023). *Venturia* spp. are found to cause shoot and leaf blight in poplar plantations in Asia, Europe and North America (Gennaro and Giorcelli, 2019). Other major fungal pathogens of *Populus* affecting leaf are *Apioplagiostoma populi* (causing bronze leaf disease) and *Taphrina* spp. (causing yellow blister of leaves), *Entoleuca mammata* (causing Hypoxylon canker), *Cytospora chrysosperma*, (causing canker) and *Phellinus tremulae* (causing aspen bracket) (Duplessis et al., 2009). The poplar blister canker disease develops upon infection with the Botryosphaeria pathogen during drought stress, commonly observed in southern China (Xing et al., 2022). Recently, black spot disease in poplar has

been reported to be one of the major diseases in China affected by fungi such as *Marssonina castagnei*, *Marssonina populi*, and *Marssonina brunnea* (Xiong et al., 2021).

2.2.2 Bacterial diseases in poplar

A few bacteria also negatively affect the growth of poplar plantations. The attack of bacteria (*Xanthomonas populi*, *Erwinia genus* and *Lonsdalea populi*) causes canker, resulting in reduced wood biomass yield of poplar (Li et al., 2019). *Xanthomonas populi* (Ridé) Ridé and Ridé and *Pseudomonas syringae* Van Hall are responsible for necrosis, wilting, injury, cankers, rots and tumors in poplar vegetations (Kalinichenko et al., 2017). The fluctuating temperatures cause *P. syringae* growth in poplar bark (Ramstedt et al., 1994). *Lonsdalea quercina* caused bark canker in *Populus×euramericana* (Tóth et al., 2013). Recently, *Pseudomonas aeruginosa* (Schröter) Migula was reported to cause disease in poplar plants. It causes rot, resulting in fast wilting, with trees dying within 48 h. *Agrobacterium radiobacter* Beijerinck and van Delden and *Agrobacterium tumefaciens* cause crown gall disease upon transfer and integration of the bacterial transfer DNA (T-DNA) into the plant genome (Kwaśna et al., 2021a). Currently, the large population of the hybrid poplar *Populus* × *euramericana* in Hungary and China is severely affected by *Lonsdalea populi* (Zlatković et al., 2020). Bacterial wetwood of poplar (*Populus alba* L.) by *Lelliottia nimipressuralis* has been common in the territory of Ukraine since 1974. The poplar wetwood disease was also reported in Bulgaria, USA, and other countries. The primary bacterial pathogens of poplar are *Xanthomonas populi, Pseudomonas syringae*, *Enterobacter cancerogenus* in the coastal zone of Western Europe, Eastern Europe and Central Europe, respectively (Goychuk et al., 2023).

2.2.3 Viral attack in poplar

Viral pathogens such as the poplar mosaic virus, poplar decline virus, tobacco necrosis virus, tobacco mosaic virus, rhabdoviruses, cucumber mosaic virus, tobacco rattle virus, arabis mosaic virus and tomato black ring virus are also severe threats to the poplar population other than fungus and bacteria (Table 2; Wang P. et al., 2023; Wang S. et al., 2023). Poplar mosaic virus (PopMV), with a single-stranded RNA, is the most common dangerous filamentous plant virus and is widespread worldwide (UK, "former Czechoslovakia and former Yugoslavia" Holland, France, Germany, Switzerland, Denmark, Italy, Bulgaria, USA and Canada) where poplar is grown at large scale. It attacks almost all the poplar plants in the *Aigeiros* section, including several clones of *P. x euramericana*. Members of the *Tacamahaca* section and crosses between these species and the *Aigeiros* section are also affected by viruses. The symptoms of a viral attack on poplar include stunted growth, leaf discoloration, necrosis, wilting and deformities in poplar. It causes severe losses in the quantity and quality of wood (Smith et al., 2004; Naylor et al., 2005; Smith et al., 2009). The virus is generally spread by cutting diseased parts (Berg, 1964).

3 Transmission route, infection, and defense mechanism in poplar attacked by pest and pathogens and their control

The vast diversity of insect pests and pathogens poses significant challenges to forest trees, severely impacting their health and productivity. These threats are particularly serious for poplar plantations worldwide. Climate change also plays a crucial role in altering the occurrence and spread of native and invasive insect outbreaks. Insects typically target susceptible trees for feeding or establishing habitats, further exacerbating the problem. These insects attack and affect all tree parts like shoot, xylem, phloem leaves, flowers, barks, and roots (Balla et al., 2021). In addition, most insects are generally introduced into a non-native area other than their native range and spread rapidly across the country. Imported alive plants and wood materials can act as carriers for introducing many pests (Dara et al., 2019). Fungi, the most common disease agent of poplar trees, have several invasion mechanisms and an array of virulent factors. In root rot disease, rhizomorphs (clusters of intertwining fungal hyphae) and secondary metabolites play a crucial role in infection. The rhizomorphs aggregate around the tree roots, feeding on the host tissues, and can persist in the dead tissues of infected plants for extended periods. This disease is marked by root decay, premature defoliation, wilting, and the production of dwarf fruits and leaves (Balla et al., 2021). Warmer winters, due to climate change, have increased the frequency of sporulation and the rate of fungal infections. Notably, poplar's defense mechanisms vary depending on the type of fungus involved. Rust diseases caused by the *Melampsora* spp. are the most common diseases in forest trees, such as poplar. Cankers are mainly caused by attacks of fungal pathogens which affect tree branches, shoots, and twigs. It has been noticed that canker-related diseases occur because of functional failure of the cambium and phloem, carbon starvation, and hydraulic failure. For instance, the fungus inoculations *Botryosphaeria* disease in poplar (*P. alba* var. *pyramidalis* = *Populus bolleana*) arrested the regeneration of callus and phloem and decreased the rate of photosynthesis and transpiration, as well as arrested the opening of the stomatal aperture and disrupted electron transport (Xing et al., 2022). Bacteria affect plants by forming colonies on their surface or within their tissues. Unlike fungi, they cannot penetrate host cells directly. Instead, they typically enter through natural openings like stomata or through wounded areas. Once inside, these bacteria secrete extracellular enzymes that break down host cells, allowing them to colonize plant tissues. Additionally, they produce polysaccharides that clog the plant's vascular system, reducing water transport through the xylem. Beetles and leafhoppers can also act as vectors, carrying pathogens and transmitting diseases to plants. Bacterial infections often manifest through symptoms such as spots, cankers, burns, tissue rot, and hormonal imbalances, which can lead to excessive root branching and leaf epinasty (Chatterjee et al., 2008). Certain bacteria, like *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes*, inject their plasmids into plant host cells through wounded areas, integrating them into the host genome. This results in tumor gall diseases and the production of hairy roots, respectively (Sharan et al., 2019). Viral pathogens are widespread in plant ecosystems, serving two roles: as agents of plant diseases and as natural enemies of pests and tree pathogens, offering indirect protection to trees. Viral infections often cause significant tissue damage and can lead to symptoms like yellowing, chlorotic lesions, necrotic spots, and ring spots on plant parts. Some stable viruses, such as tobacco mosaic virus, do not require vectors to spread, while other viruses rely on vectors, such as aphids, mites, leafhoppers, fungi, beetles and nematodes, soil, water, other plants and debris for transmission (Balla et al., 2021). Smith and Campbell (2004) reported that the poplar mosaic virus (PopMV) infection and spread depend on the poplar genotypes.

Insects are typically controlled by the release of toxic phytochemicals from plants, which either inhibit pest growth or kill the insects (Fernandez-Conradi et al., 2021). To defend against pathogen attacks, poplars utilize two types of defense mechanisms: induced and constitutive defenses. Induced defenses are activated in response to external stimuli and involve complex processes, while constitutive defenses, the first line of defense, involve non-host resistance through physical barriers and the accumulation of phytochemicals in the plant (Alkan and Fortes, 2015; Zeng et al., 2023). Induced resistance can be further classified into locally induced resistance and systemic induced resistance (SIR). SIR provides broad-spectrum, long-lasting protection against secondary infections. The exogenous application of signal molecules that trigger these defenses can enhance plant immunity and help manage pest populations (Balla et al., 2021). Some observations demonstrate that signal molecules such as salicylic acid (SA) and methyl jasmonate (MeJA) are involved in local and systemic defense responses. Recent research proved that both SA and MeJA pathways are induced in leaves of poplar upon infection with the fungus *M. larici-populina* proving that both hormone pathways are essential for defense response (Ullah et al., 2019; Chen et al., 2021). Upon fungus attack, the poplar trees activates constitutive defenses involving several processes such as recognition of the fungus by receptor proteins and resistance (R) proteins (PR) resulting into pattern-triggered immunity (PTI) (receptors of plant membranes recognize molecular patterns (PAMPs) of pathogens) and effector-triggered immunity (ETI) (intracellular receptors, (a nucleotide-binding leucine-rich repeat (NLR) class recognize effectors released by pests and pathogens) as well as noncoding RNA (ncRNA)-mediated defense (non-coding RNA having more than 200 nucleotides in length with having role in plant growth and development, and stress responses), initiation of hormone signaling network pathways (mitogen-activated protein kinase (MAPK) cascades and calcium-dependent protein kinase (CDPK) involved in plant growth and development and stress response), activation of defense-related genes and transcription factors (TFs) involved in controlling gene expression by binding DNA elements at 5′ non-coding regions (promoters) of desired genes and modulating transcription rate) and accumulation of phytoconstituents (De Kesel et al., 2021; Zeng et al., 2023). In addition, the pathogen-associated protein 1 (PR1) gets activated as a plant response to abiotic and biotic stresses. Total 17 PtPR1 genes were found in *Populus trichocarpa* (Wang P. et al., 2023; Wang S. et al., 2023). A total of 1888 lncRNAs and 52,810 mRNAs were recognized in poplar coma (Song et al., 2024). The 30 CDPK genes and 20 closely related kinase genes were identified in *Populous* spp. (Zuo et al., 2013), The 11 MAPKKs (PtMKKs) and 21 MAPKs (PtMPKs) were identified in the *Populus trichocarpa* (Hamel, 2006). A total of 104 WRKYs (TFs) have been identified in poplar (He et al., 2012). Recently, the integrated transcriptomic and transgenic analyses were applied to understand mechanisms of poplar resistance against *Alternaria alternata* attack (Wang W. et al., 2022; Wang Y. et al., 2022).

The most effective method for preventing leaf diseases caused by pests, fungi, bacteria, and viruses is selecting and planting pathogen-resistant poplar clones. Another approach involves using fungicides, such as copper- and carbamide-based treatments, to prevent infections. Fungal diseases can also be managed by maintaining proper spacing between poplars, reducing weed competition, and optimizing plant density, as high relative humidity contributes to disease development. Infected leaves, roots, stems, and branches should be pruned, particularly during the dormant season, to minimize pest and pathogen attacks. Additionally, poplars should be planted in appropriate soil conditions within nurseries to promote healthy growth. Additionally, the soil from infected areas must not be used and moved with equipment (Kebert et al., 2022). Proteomic and genomic technologies offer valuable tools for precisely identifying and characterizing bacterial infections by analyzing their genetic and protein markers (Zubair et al., 2022). Recent studies have shown that lactic acid bacteria (LAB) can effectively combat plant pathogens due to their high biosecurity and ability to promote plant growth (Jaffar et al., 2023). Quorum sensing (QS) molecules, such as 3-OH PAME, regulate the virulence genes in bacteria and fungi, making the identification and development of QS-quenching genes and enzymes promising for disease control (Wang P. et al., 2023; Wang S. et al., 2023). Additionally, eucalyptus oil, known for its antibacterial properties and ability to stimulate plant defense mechanisms, has been shown to reduce plant diseases and could be used to protect poplar in the future (Montesinos et al., 2023). A few genes have been reported whose expression can impart disease resistance in poplar trees. For example, the overexpression of PdbLOX2 was able to induce the resistance in *P. davidiana* × *P. bollena* against *A. alternata* attack, while silencing this gene increased the susceptibility of the poplar tree to *A. alternata* infection (Huang et al., 2022). Furthermore, the study reported that PtoMYB142 can regulate transcription of wax biosynthesis genes [fatty acid hydroxylase (CER4) and 3-ketoacyl CoA synthase (KCS6)] mediating adaption of poplars against drought conditions were highly expressed upon infection with fungal pathogens (Song et al., 2022). In addition, lignin has a vital role in protecting poplar from pest and pathogen attacks. It is a primary three-dimensional phenolic biopolymer of the secondary cell wall in vascular plants (Ma et al., 2024). It imparts strength and imperviousness to cell walls, mediating long-distance water transport in vascular tissues. In addition, it acts as a barrier to the spread of invading pathogens as it is non-degradable to pathogens, thereby preventing their penetration into the plant cell wall and the supply of water and nutrients from plant cells to pathogens. It is noticed that the gene expression of lignin increased with higher lignin content upon pathogen infection. The genes (phenylalanine ammonia lyase (PAL), HCT4-Coumarate: coenzyme A ligase (4CL), cinnamate 4-hydroxylase (C4H), cinnamoyl-CoA reductase (CCR), cinnamyl alcohol dehydrogenase (CAD) and hydroxycinnamoyl transferase) are involved in lignin biosynthesis and highly expressed during fungal infection leading to increase in lignin content (Lee et al., 2019; Zeng et al., 2023; Ma et al., 2024; Riseh et al., 2024). Hence, regulating the lignin biosynthesis pathway may be critical for improving poplar resistance against pathogen attacks (Polle et al., 2013). It is reported that the higher expression of Pto4CL1 of *P. tomentosa* increased the lignin content from 33.11 to 46.65%, leading to the decreased formation of cellulose, hemicellulose, and pectin (Hu et al., 2019). RNAi technology was used to down-regulate the expression of 4CL gene to modify lignin biosynthesis in *P. tremula* (Kovalitskaya et al., 2016). A significant 30% reduction in lignin content has been observed in poplars due to the downregulation of cinnamate 4-hydroxylase (C4H) genes (Bjurhager et al., 2010). Similarly, the downregulation of CAD genes in *Populus tremula* × *Populus alba* led to reduced lignin levels (Özparpucu et al., 2017). Dirigent (DIR) proteins have also been identified as crucial players in lignin biosynthesis. Li et al. (2022) reported that the overexpression of PtDIR11 in poplars enhanced lignin biosynthesis, thereby increasing the trees' resistance to *Septotis populiperda*. Hence, gene editing can be utilized to regulate the

expression of these genes to enhance lignin biosynthesis, which confers pest and pathogen resistance in poplar trees.

4 Conventional breeding in poplar

The recent global temperature, drought, humidity and climate instability render poplar plants vulnerable to pests and pathogens, severely affecting wood quality and quantity (Gullino et al., 2022). Plants have physical and physiological barriers against microbial pathogens, preventing their access to plants (Kumudini et al., 2018). Plants produce antimicrobial peptides and other molecules that cause detoxification and the inhibition of virulence factors (Silva et al., 2016). Moreover, plants also apply RNA interference (RNAi), which detects invading viruses and cleaves the RNAs of viruses (Bocos-Asenjo et al., 2022). However, these pathogens have evolved to cope with the defense systems of their plant cells by secreting cell-wall degrading enzymes, which gain access for molecules into plant cytoplasm, inhibiting host defenses and promoting susceptibility of plants toward pathogens (Kaur et al., 2022). Some viral pathogens are also reported to attack and silence the host RNAi system, promoting viral pathogenicity (Leonetti et al., 2021). The increased demand for wood and its sustainability requires approaches to improve efficient production even under environmental constraints and minimize the threats due to pests affecting wood properties for industrial purposes (Polle et al., 2013). However, controlling pests and pathogens with chemicals is increasingly considered unsafe due to their high toxicity, environmental accumulation, and harmful impacts on beneficial insects, non-target organisms, and humans (Ahmad et al., 2024). The commonly adopted traditional methods for the protection of plants from attack of poplar pests and pathogens are the use of resistant clones that have good adaptability to different soil (salinity, calcium level and pH), drought and climatic conditions coupled with the adoption of proper cultivation practices (i.e., fertilization, low plant density and irrigation) (Biselli et al., 2022). Enormous progress has been made in enhancing traits such as plant growth rate, pest and pathogen resistance, and environmental adaptations in poplar plantations by applying conventional breeding practices (Ye et al., 2011). However, these traditional methods are time-consuming because of their long-life span, costly land demand, and high labor costs. In addition, because of the heterozygosity of most *Populus* genotypes and inbreeding depression, it is difficult to estimate the genetic control of particular traits (Ye et al., 2011). Genetic engineering and molecular breeding methods for developing transgenic poplar plants can address the limitations of traditional breeding, such as the challenges of distant hybridisation, the complexity of cultivation, and issues with interspecific hybridisation (Begna, 2021).

These tools have enormous potential to improve two or more traits simultaneously by introducing desired exogenous genes of donor plant or non-plant origin into a particular plant genome, enabling the improvement of poplar against pests and pathogens, herbicide resistance, abiotic stress, wood properties, flowering regulation and phytoremediation (Thomas, 2022).

5 Genetic engineering and different transformation methods in poplar

Genetic transformation has been widely employed in research on various forest trees. This process involves introducing exogenous genes into tree cells, thereby altering their genetic traits (Li et al., 2023). Poplar trees were among the first forest trees used successfully for genetic engineering for gene research (Ye et al., 2011). For more than 20 years, several progress has been made in *Populus* transformation. The most widely used transgenic tools involve vector-mediated transformation, such as *Agrobacterium tumefaciens*-mediated and *A. rhizogenes* mediated and non-vector mediated transformation (Gene gun-mediated, pollen tube pathway, and protoplast transformation methods). The Genome editing method is an advanced approach for adding, deleting, or modifying genes within the specific genome (Li et al., 2023). Mobile genome editing techniques, such as clustered regularly interspaced short palindromic repeats (CRISPR)-associated (CRISPR-Cas) systems, RNA interference (RNAi), and nanoparticle-meditated gene transformation have been recently applied to improve poplar tree (Yin et al., 2021; Figure 1). Among these methods, *Agrobacterium*-mediated and gene gun-mediated transformations are the most widely used techniques for forest trees (Lv et al., 2020).

5.1 *Agrobacterium tumefaciens*-mediated transformations: basic mechanisms

Agrobacterium tumefaciens-mediated transformation is the most preferred method for the genetic transformation of forest trees. *A. tumefaciens* (a gram-negative soil bacteria) infects the wounded sites in many dicotyledons, gymnosperms and a few angiosperms. It delivers its transfer DNA (T-DNA) molecules into plant cells and then integrates them into the plant genome (Chilton et al., 1980; Sekine and Shinmyo, 2020). The *Agrobacterium*-mediated transformation method involves removing oncogenes causing tumorigenesis, inserting exogenous genes in disarmed T-DNA, and delivering and integrating foreign genes into the plant genome (Pratiwi and Surya, 2020). The success of *Agrobacterium*-mediated transformation depends on different parameters such as the virulence of *Agrobacterium* cells, explant types and plant genotypes and regeneration of transgenic populations. *Agrobacterium rhizogenes* is also a relative of *A. tumefaciens*, which develops hairy root at the wounded site of plant cells (also known as "hairy root disease") and can be used to transfer the T-DNA into a binary vector into developing root cells (Limpens et al., 2004; Sharan et al., 2019). Various wild-type strains of *A. tumefaciens* and *A. rhizogenes* have transformed various trees. *A. tumefaciens*-mediated genetic transformation system has been widely applied in various poplars, such as *Populus alba* × *Populus glandulosa*, *Populus simonii* × *Populus nigra*, and *Populus tomentosa*. Several attempts were made to improve *A. tumefaciens*-mediated transformation in poplar by optimizing several parameters such as types of explants, different strains of *Agrobacterium* and culture densities, incubation time and concentration of acetosyringone and sucrose (Movahedi et al., 2014, Sharan et al., 2019). Pest infestation and bacterial, fungal and viral diseases are limiting factors which affect the healthy growth of poplar trees (Li P. et al., 2024; Li Y. et al., 2024; Li Z. et al., 2024). By introducing insect and disease-resistance genes into poplar trees using *A. tumefaciens*, these trees can protect themselves from invading pests and diseases, enhancing their survival rate and disease-resistance capabilities. However, *Agrobacterium*-mediated transformation has been done in several poplars, but many other poplars remain recalcitrant to *Agrobacterium*-mediated transformation (Song et al., 2019).

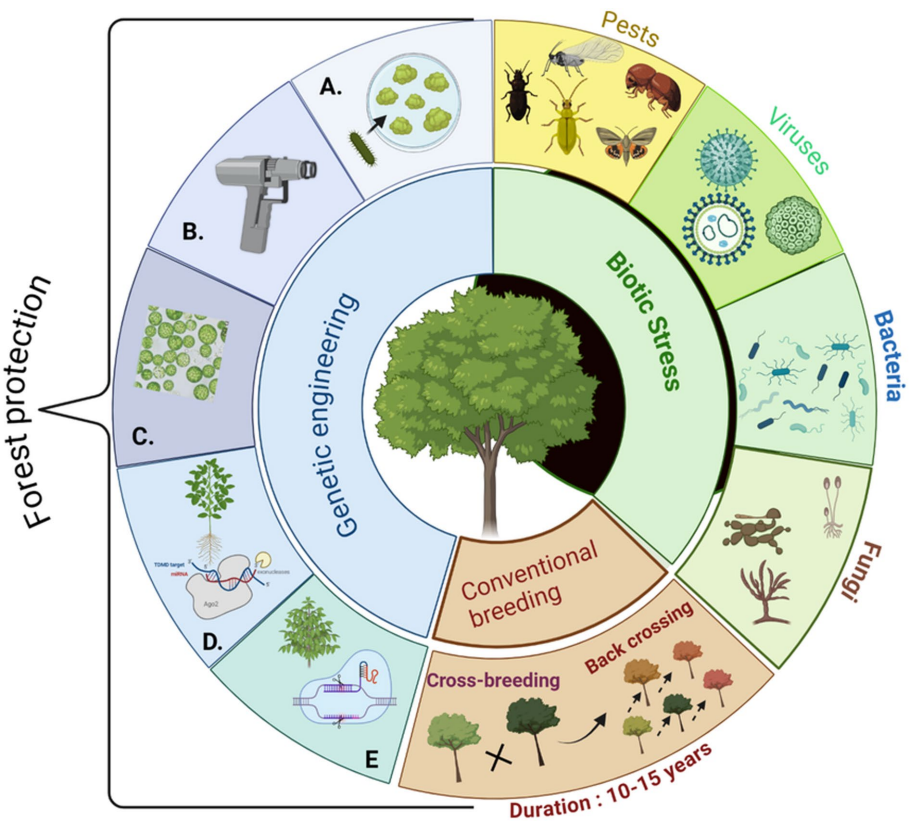

FIGURE 1
Summarizing forest protection using genetic engineering. Increasing biotic stressors (i.e., pests and pathogens) cause dramatic tree mortality in the forests worldwide. The conventional breeding method is time-consuming and fails to cope with the demands. Transgenic pest and disease resistance trees (i.e., Poplar) can potentially mitigate the challenges. Transgenic trees can be generated using various transformation techniques such as (A) agrobacterium-mediated transformation, (B) gene-gun mediated transformation, (C) protoplast-mediated transformation, (D) mi-RNA or RNAi-mediated transformation, and (E) CRISPR-mediated transformation techniques. (The figure is prepared using biorender.com).

5.2 *Agrobacterium*-mediated transformation for pest resistance in poplar

Several transgenic poplars have been developed that overexpress genes encoding different serine proteinase inhibitor proteins (Heuchelin et al., 1997; Confalonieri et al., 1998) and *Bacillus thuringiensis*-derived genes (Cry/Bt genes) (McCown et al., 1991; Wang et al., 1996), *Androctonus australis* hector insect toxin, Kunitz trypsin inhibitor (KTI) and chitinase gene for conferring pests resistance (Clemente et al., 2019; Ren et al., 2021; Table 3). However, Bt gene is the most widely used for generating pest-resistant poplar trees. The first stable transfer of Bt was reported in *Populus nigra* (McCown et al., 1991). Recently, the simultaneous introduction of two Bt genes into the trees' genomes expanded the scope of insect resistance in transgenic forest trees (Dong et al., 2015; Wang et al., 2018). China has been the first nation to generate and commercialize two transgenic lepidopteran-resistant poplar lines since 2002 (Thakur et al., 2021). The plant *P. alba*×*P. glandulosa* was transformed with a Bt Gene (CRY3A) using *Agrobacterium*-mediated transformation method, which resulted in the development of transgenic line BGA-5 and toxic to the larvae of *Anoplophora glabripennis* with a growth inhibition rate of 78.6% (Zhang et al., 2006). *P.* × *euramericana* was transformed with Cry1AC and Cry3A genes to confer resistance to the poplar plants against *H. cunea* exhibiting mortality rate of 42.2–66.1 and 100% of *Plagiodera versicolora* larvae of L1 and L2 stages, respectively (Yang et al., 2016). Transgenic poplar lines 'Shanxin' (*Populus davidiana*×*Populus bolleana*) were developed through *Agrobacterium*-mediated transformation method carrying Cry1Ac + SCK, Cry1Ah3, and Cry9Aa3, respectively against fall webworm (*Hyphantria cunea*) and gypsy moth (*Lymantria dispar*) as these genes Cry1Ac + SCK, Cry1Ah3, and Cry9Aa3 were toxic to the larvae of both insects (Ding et al., 2017). Two Bt toxin genes, Cry1Ac and Cry3A, were simultaneously integrated into the genome of *Populus* × *euramericana* 'Neva' with the help of *Agrobacterium tumefaciens,* to develop transgenic poplar, which was highly resistance to *Lepidopteran and Coleopteran* pests (Satish et al., 2021). Other than Bt, many other genes, such as cowpea trypsin inhibitor (CPTI), cysteine proteinase inhibitor (Atcys) gene, *glycine max* trypsin proteinase inhibitor (KTi3 and PtdPP01 genes, etc.) were inserted into *Populus* species, which conferred some degree of resistance against insect pests (Table 3). La Mantia et al. (2018) observed that the overexpression of *Arabidopsis* AlgolS3 (AtGolS3) and *Cucumber sativus* Raffinose synthase (CsRFS) in *Populus alba* × *P. grandidentata* antagonizes leaf rust defense mechanism by inhibiting reactive oxygen species (ROS) and attenuating phosphatidic acid and calcium signaling pathways leading to salicylic acid (SA) defense. Lin et al. (2006) generated transgenic *P. simonii*×*P. nigra* plants by inserting the spider neurotoxin gene along with C-terminal of CryIA(B) gene resistance against *Lymantria dispar*. Moreover, the scorpion neurotoxin AaIT expression in hybrid *Populus* was responsible for developing resistance against the spongy moth (Lin et al., 2006).

TABLE 3 *Agrobacterium*-mediated transformation for imparting pest resistance in poplar species.

Poplar species	Gene	Targets	Percentage of transformation	Percentage of tolerance	References
P. alba ×*P. grandidentat*	Maize Ac transposable element and *Bt*	–	67–100% with Ac gene 67–75% with Bt gene	–	Howe et al. (1994)
P. alba×*P. grandidentata*	Cry1A	Spongy moth	–	91.9%	Kleiner et al. (1995)
P. deltoides × *P. simonii*	Bt	*Lymantria dispar* and *Clostera anchoreta*	17.8%	45%	Rao et al. (2001)
P. tremula × *P. tremuloides*	Cry3Aa	*Chrysomela tremulae*	–	100%	Génissel et al. (2003)
P. simonii × *P. nigra*	Spider insecticidal peptide and Bt	*Lymantria dispar*	–	92%	Cao et al. (2010)
P. tomentosa Carr	Cry1Ac; API	*L. dispar* and *C. anachoreta* larvae	39.3%	80%	Yang et al. (2006)
P. euramericana	cry1AC-cry3A-NTHK1	*Hyphantria cunea and Plagiodera versicolora*	–	60% (*Hyphantria cunea*) 100% (*Plagiodera versicolora*)	Liu et al. (2016)
P. euramericana	cry1Ac, cry3A,	*Hyphantria cunea* and *Plagiodera versicolora*	–	42.2–66.7% (for *Hyphantria cunea 100%*) (for *Plagiodera Versicolora*)	Yang et al. (2016)
P. davidiana × *P. bolleana*	cry1Ac + SCK, cry1Ah3, cry9Aa3	*Lymantria dispar* and *Hyphantria cunea*	–	97% (for *Lymantria dispar*) 91% (for *Hyphantria cunea*)	Ding et al. (2017)
P. deltoides×*P. euramericana*	Cry1Ah1	*Hyphantria cunea*	–	90%	Xu et al. (2019)
Populus × *euramericana 'Neva'*	Cry1Ac Cry3A	*Lepidopteran* and *Coleopteran pests*	–	100%	Ren et al. (2021)
P. nigra L. *mtlD*	Cry3A, Cry1Ac	*Hyphantria cunea* larvae and *Plagiodera versicolora* larvae	–	More than 80% (for *Hyphantria cunea*) larvae 100% for *Plagiodera versicolora*	Zhou et al. (2020)
P. simonii × *P. nigra*	spider neurotoxin gene fused with C-terminal of cryIA(B) gene	*Lymantria dispar*	–	37 and 92%	Lin et al. (2006)
P. alba	ATCYS	*Chrysomela populi*	11%	77–100%	Delledonne et al. (2001)
P. alba × *P. grandidentata*	Arabidopsis AlgolS3 (AtGolS3) and Cucumber sativus Raffinose synthase (CsRFS)	Leaf rust	–	100%	La Mantia et al. (2018)
P. tremula × *P. alba*	PtdPPO1	*Malacosoma disstria*	–	50-fold greater PPO activity relative to untransformed controls	Wang and Constabel (2004)
P. tomentosa × *P. bolleana* × *P. tomentosa*	CPTI	*Malacosoma disstria* and *Stilpnotia candida*	–	40–55%	Zhang et al. (2005)

5.3 *Agrobacterium*-mediated transformation for disease resistance in poplar

A diverse array of bacterial and fungal attacks and viral infestation causes significant losses in the poplar yield. Transgenic poplars have various antibacterial and antifungal genes encoding proteins capable of breaking down mycotoxins and inhibiting cell-wall-degrading enzymes such as rabbit defensin (NP-1), osmotin, glucanases, chitinases (CH5B), lysozyme and thaumatin were able to combat pathogens mentioned in Table 4 (Zhao et al., 1999; Juge, 2006; Karlovsky, 2011; Thakur et al., 2021). Noël et al. (2005) generated

TABLE 4 *Agrobacterium*-mediated transformation for imparting disease resistance in poplar species.

Plant	Genes	Target	Percentage transformation	Percentage resistance	References
P. trichocarpa×*P. deltoides* and *P. trichocarpa*×*P. nigra*	Bacterio-opsin resistance	*Melampsora occidentalis and Septoria populicola*	–	Ineffective	Mohamed et al. (2001)
P. euramericana × *P. canadensis* and *P. nigra*× *P. maximowiczii*	AcAMP1,2 and ESF12	*Septoria musiva*	–	40%	Liang et al. (2002)
P. tremula×*P. alba against*	D4E1 resistance	*Xanthomonas populi*	–	57%	Mentag et al. (2003)
P. alba	BS	*Melampsora pulcherrima*	2.5%	40–63%	Giorcelli et al. (2004)
P. nigra × *P. maximowiczii*	ECH42	*Melampsora medusae*	–		Noël et al. (2005)
P. tomentosa × *P. alba*	Antisense and sense PtWRKY23	*Melampsora* species	–	Expression level was of 10-fold after infection	Boyle et al. (2010)
P. tomentosa	LJAMP2	*Alternaria alternata* and *Colletotrichum gloeosporioides (Penz.)*	–	–	Jia et al. (2010)
P. trichocarpa Torr. and *P. tomentosa Carr*	PtrWRKY18 and PtrWRKY35 transcription factors	*Melampsora* rust	–	Enhanced expression level of these genes	Jiang et al. (2017)
P. nigra L. × *P. maximowiczii* A. Henry (NM6)	MsrA2, N-terminally modified amphibian host defense peptide (HDPs)	*S. musiva*	–	95%	Yevtushenko and Misra (2019)

transgenic hybrid poplar plants harboring the ECH42 (*Trichoderma harzianum* endochitinase) gene responsible for imparting an enhanced level of resistance against *Melampsora medusa*, a leaf rust pathogen of poplar. Levée et al. (2009) functionally identified and characterized the transcription factor PtWRKY23 gene in *P. tomentosa* × *P. alba* whose silencing is responsible for enhanced susceptibility of transgenic poplars toward *Melampsora* infection. In addition, the overexpression of a transcription factor PtoWRKY60 in *P. tomentosa* clone 741 was noticed for conferring resistance to the fungal pathogen *Dothiorella gregaria* (Ye et al., 2014). Jiang et al. (2017) observed that over-expression of PtrWRKY18 and PtrWRKY35 transcription factors increased resistance in poplar transgenics against *Melampsora* rust. Hybrid *Populus* having over-expressed a wheat (*Triticum aestivum*) germin-like oxalate oxidase gene encoding enzyme responsible for metabolizing the oxalic acid molecules secreted by fungal pathogen *Septoria musiva*, showed delayed infection by the fungal pathogen (Liang et al., 2001). Interestingly, developing genetically engineered transgenic poplar resistant to bacterial pathogens is less common as bacterial damage is rare in poplar plantations. However, severe infections by *Xanthomonas* spp. on poplar plantations are reported (Ye et al., 2011). Mentag et al. (2003) generated transgenic *P. tremula* × *P. alba* having a gene encoding a synthetic antimicrobial peptide D4E1 imparting resistance to several fungal and bacterial pathogens. The nucleotide MsrA2 [N-terminally modified amphibian host defense peptide (HDPs) from *the skin secretion of arboreal frogs*] was inserted into the hybrid poplar *Populus nigra L.* × *P. maximowiczii A* through *Agrobacterium-mediated* transformation method. The peptide was reported to inhibit *S. musiva* conidia germination but is non-toxic to poplar (Yevtushenko and Misra, 2019). Certain viruses, such as the poplar decline virus, poplar mosaic virus, and arabis mosaic virus, pose significant threats to the poplar population by stunting plant growth and severely impacting wood biomass and quality (Pinon and Frey, 2005). To date, there have been no reports of developing transgenic poplar plants with improved viral resistance using the *Agrobacterium tumefaciens*-mediated transformation method. Therefore, this method holds potential for future use in enhancing viral resistance in poplar.

5.4 Gene gun-mediated transformations

The gene gum method (biolistic particle delivery system) has excellent potential in forest tree research. This physical method is commonly applied for genetic transformations of several plants. This method was first developed by Sanford and colleagues in 1982. The process involves the transfer of gold or tungsten microparticles (or microcarriers) coated with exogenous donor genes into receptor cells or tissues or organs with the help of accelerators like pressurized helium (He) gas and integration of genes into receptor genome and expression of the genes (Zhang et al., 2014; Cunningham et al., 2018). The efficiency of gene gun-mediated transformation depends on the factors, for example, types of receptors, culture and transformation conditions (Wang et al., 2018). In addition, this method is independent of plant genotypes compared to *A. tumefaciens*-mediated

transformation. This method is commonly applied to generate transgenic poplars, as mentioned in Table 5 (Ozyigit and Yucebilgili Kurtoglu, 2020). The insect resistance Bt gene was co-transformed into *P. nigra* through gene gun mediated transformation protocol (Li et al., 2000). The Bt gene (cry3Bb) gene was successfully incorporated into the genome of poplar plastid through biolistic bombardment, generating transformed poplar with a mortality rate of 100% to *Plagiodera versicolora* (Xu et al., 2020). Wang et al. (2007) inserted three foreign *Bacillus subtilis* genes vitreoscilla hemoglobin (vgb), fructan sucrase (SacB), and bivalent stem borer resistance (BtCry3A + OC-I), and the regulatory gene (JERF3) into *Populus* × *euramericacana* 'Guariento' through particle bombardment method. No incorporation of pathogen and disease-resistant genes in poplar trees with the help of gene gun mediated transformation tools has been reported. The disadvantages of this gene gun-mediated transformation are low efficiency, silencing the transformed genes, inserting multiple gene copies and unstable expression of exogenous genes (Yin et al., 2021).

5.5 Protoplast transformation

The use of protoplasts for genetic transformation in plants has grown significantly in recent years. This technique involves introducing and incorporating exogenous genes into plant protoplasts, leading to the generation of transgenic plants with stable gene expression. The protoplast method has proven to be easy, fast, and efficient, with minimal or no interference from surrounding cells or the microenvironment (Yin et al., 2021; Adjei et al., 2023). Because of their versatility and efficiency, protoplast transformation systems have been optimized, established and applied to many recalcitrant non-model plants, along with the efficient delivery of several genes (Rehman et al., 2016; Naing et al., 2021; Ojuederie et al., 2022). This method is affected by several parameters such as explant types, tissue types, the composition of the digestion solutions, the pH of the digestion solution, the digestion time, the concentration of polyethene glycol (PEG) and the transformation time (Rezazadeh et al., 2011, Biswas et al., 2022). The protoplast transformation method is easy and efficient in annual herbaceous plants such as *Oryza sativa, Arabidopsis thaliana* and *Nicotiana tabaccum* (Jiang et al., 2013; Sun et al., 2018). The separation and transformation of protoplasts and regeneration from transformed protoplasts are difficult in forest trees. Advances have been made in PEG-mediated transformation method by applying liposome-mediated shock perforation and *A. tumefaciens* co-culture transformation method (Wu et al., 2014). The PEG-mediated method is the widely used protoplast transformation system in plants (Lenaghan and Neal Stewart, 2019). In addition, protoplasts can be transformed directly by imbibing DNA followed by PEG pre-treatment, microinjection, and electroporation. However, protoplast isolation and its transformation are complex and challenging for woody trees like poplar and have not been fully optimized and developed. Xu et al. (2020) used the leaf protoplast of poplar (*P. davidiana* *P. *bollaena*) to introduce cry3Bb genes for developing insect-resistant transgenic poplar. This method has not yet been utilized to generate transgenic poplar with pathogen-resistant genes.

5.6 Micro RNA mediated transformation

MicroRNAs (miRNAs) are endogenous, short, single-stranded, non-coding RNAs of 20–24 nucleotides, processed from hairpin RNA precursors by Dicer-like (DCL) enzymes. These are found in all eukaryotic cells and negatively regulate gene expression. After their discovery in plants, several miRNAs have been recognized with the help of high-throughput sequencing technology and bioinformatics and for there essential roles in regulating critical genes involved in plant-pathogen interactions at the transcriptional or post-transcriptional levels (Islam et al., 2022; Nizamani et al., 2023). According to the host and the specific pathogen, miRNAs can be up- or down-regulated, thereby promoting plant disease resistance by participating in hormone signaling and regulating and moderating resistance (R) genes (Yang et al., 2021). The first report of plant microRNAs was reported in *Arabidopsis* by Llave et al. (2002). Several studies established the pivotal roles of microRNAs in regulating biotic and abiotic stresses in several plants (Kar and Raichaudhuri, 2021). Transgenic poplar overexpressing miR159a (OX-159) showed enhanced resistance to necrotrophic fungi *C. chrysosperma* while enhanced susceptibility to infection by *L. populi (bacterial canker)* and hemi-biotrophic fungi *C. gloeosporioides* (Yang et al., 2023). Furthermore, in transgenic poplar (*P. trichocarpa*), miR472a positively regulated resistance to *Colletotrichum gloeosporioides* by targeting nucleotide-binding site and leucine-rich repeat domains (largest R proteins, NBS-LRR) and regulated negatively resistance to *Cytospora*

TABLE 5 Gene gun mediated transformation in poplar species.

Plant	Gene	Target	Percentage of transformation	Percentage of tolerance	References
P. alba× *P. grandidentata and P. nigra*× *P. trichocarpa*	Bt	*Malacosoma disstria* and *Lymantria dispar*	–	60% (for *Malacosoma disstria*) 24% (for *Lymantria dispar*)	McCown et al. (1991)
P. nigra	Chimaeric TA29-barnase gene	Insect	16.1	–	Li et al. (2000)
P. euramericana 'Guariento	SacB/vgb/BtCry3A, OC-I/JERF36/NPT II	*Coleopterus* insect	–	–	Wang et al. (2007)
P. davidiana ×*P. bollaena*	(Bt) cry3Bb	*Plagiodera versicolora*	–	100%	Xu et al. (2020)

chrysosperma (Su et al., 2018). miR156a was found to be the most stable miRNA examined as a reference gene in *P. tomentosa* under canker pathogen stress (Zhang et al., 2021). Several evidences proved that miRNAs can regulate and mediate biological processes during plant-insect and plant-viral interactions, ultimately conferring pest/viral resistance in plants (Zhang et al., 2022; Satish et al., 2021). To date, miRNA molecules have not yet been used to generate transgenic poplar with pest and viral resistance genes. We believe that with the growing recognition of miRNA molecules, as highlighted by the 2024 Nobel Prize, artificial mi-RNA holds the potential to be used for manipulating tree traits in the future.

5.7 RNA interference-mediated transformation

RNA interference (RNAi) is a naturally occurring cellular defense system in most eucaryotic cells. It is mediated by double-stranded RNA (dsRNA) as either a source of virus infection or because of transposon activity, both seeking need to be suppressed (Obbard et al., 2009). RNAi pathway involves the formation of several interfering molecules, i.e., small interfering RNAs (siRNAs) and microRNAs (miRNAs), generated through the activity of a dicer enzyme. These interfering molecules are then loaded on an RNA-induced silencing complex (RISC) containing argonaute protein (AGO). RISC directs the interfering molecules to their target gene, and homology-based cleavage of target mRNA occurs in the cells (Mamta and Rajam, 2017). RNAi has an important role in functional genomics research and is also a promising species-specific pest/pathogen management strategy in agroforestry (Mamta and Rajam, 2017; Joga et al., 2021; Mogilicherla et al., 2023; Sandal et al., 2023; Sellamuthu et al., 2024). RNAi tool is a sequence-based method that suppresses target gene expression for pest growth, development, and reproduction without affecting non-targeting other pest species (Whyard et al., 2009; Christiaens et al., 2020). Transgenic poplar plants harboring dsRNA targeting CYP6B53 from *Lymantria dispar* via *A. tumefaciens*-mediated transformation exhibited inhibited larval feeding and delayed growth (Sun et al., 2022). Such studies endorse the potential for using trees expressing dsRNA against target forest pests. RNAi-mediated lignin modification has also been successfully applied in poplar. The overexpression of microRNA, such as miR6443 reduces S lignin biosynthesis during shoot development in *Populus tomentosa,* making the plant susceptible to pathogens (Fan et al., 2020). Thus, RNAi can be utilized to modulate gene expression miR6443 to produce more lignin in poplar to confer resistance against pathogens.

5.8 Microparticle-mediated CRISPR DNA delivery for genome editing in poplar

The clustered regularly interspaced palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) system is the most promising technique used for precise genetic engineering in plants, including poplar (Bewg et al., 2018; Anders et al., 2023; Sulis et al., 2023). This method is harnessed to improve sustainable production and introduce precise alterations at target sites, thereby altering plant architecture and floral development and developing biotic/abiotic resistance in trees (Borthakur et al., 2022). This method does not introduce foreign genes into the forest trees, making it safer than other genetic engineering methods. In this process, CRISPR gene-editing reagents, i.e., Cas9 protein and the guide RNA (gRNA), are generally delivered through *A. tumefaciens,* resulting in the stable genome integration and expression of the transfer DNA (T-DNA) in the plant genome (Hoengenaert et al., 2023). Alternative strategies other than *Agrobacterium*-mediated method for the delivery of gene-editing reagents into plant genomes are either through the expression of a gRNA- and Cas9-coding DNA/RNA or ribonucleoproteins (RNPs) into callus or protoplasts (Lin et al., 2018). However, this approach has drawbacks, such as inducing somaclonal variation and large genome rearrangements resulting in altered phenotype of plants (Serres et al., 1991; Fossi et al., 2019). Another commonly used method applies mechanical force like a gene gun to deliver gene-editing reagents coated with microparticles into plant tissue. Several researchers applied CRISPR-mediated gene editing for wood quality improvement and drought/pest/disease resistance in forest trees (Dort et al., 2020). In addition, microparticle-mediated DNA delivery technology has previously been used to deliver the CRISPR gene in poplar trees (Devantier et al., 1993; Nowak et al., 2004; Canto, 2016). Jang et al. (2021) and Huang et al. (2022) utilized this method for knocking out caffeoyl shikimate esterase (CSE) to improve lignocellulose biomass and root growth transcription factor PDNF-YB21 for repression of root and inducing drought resistance in transgenic poplar, respectively. However, it has not yet been applied to develop pest and pathogen resistance in poplar trees.

6 Regeneration methods used in *Populus* species

An efficient regeneration system is crucial for successful genetic transformation, as it enables the development of transgenic plants from a single cell carrying the desired genes. However, genetic transformation and regeneration remain significant challenges in many forest trees, including poplar. Various plant regeneration methods have been developed for poplars (Thakur et al., 2005; Li et al., 2017), which can be employed to produce transgenic trees with resistance to pests and diseases. In recent decades, significant research efforts have focused on creating transgenic poplars with enhanced resistance to abiotic stress and improved wood quantity and quality. There have been a few reports on regenerating transgenic poplar trees with biotic stress resistance. The established suspension cultures of *P. alba x P. grandidentata* cv. 'Crandon' were transformed with vectors *A. tumefaciens* carrying the maize Ac transposable element and an insect toxin gene isolated from *Bacillus thuringiensis* (Bt). These transgenic plants were regenerated by subculturing the transformed callus on the medium, supplemented with a growth regulator Thidiazuron (TDZ) of 0.11–27.0 μM (Howe et al., 1994). *A. tumefaciens* mediated genetic transformation and regeneration of hybrid poplar (*P. alba x P. grandidentata*) and transgenic quaking aspen from cuttings from young leaves were also readily achieved (Tsai et al., 1994). *A. tumefaciens*-mediated transformation of leaf explants of *P. nigra* L. was done with a Kunitz trypsin proteinase inhibitor (KTi3) gene for pest resistance, and regeneration of this transgenic leaf explants was successfully achieved (Confalonieri et al., 1998). The stems and petioles of transformed hybrid aspen (*Populus tremula* × *P. alba*) clones containing PtdPPO1 genes (conferring pest

and pathogens resistant in plants) of *in vitro* plantlets were used for regeneration (Wang and Constabel, 2004). Further research is needed to establish a protocol for regenerating transgenic poplars with enhanced resistance to pests and pathogens from modified cells.

7 Conclusion and future perspectives

Poplars play a crucial role in supporting global ecological and socioeconomic wellbeing. The growing demand for poplar products has driven genetic engineering efforts to enhance various traits, particularly pest and disease resistance, as these trees are highly susceptible to numerous pests, fungi, and viruses. Considering the long growth cycle with low transformation tendency in forest trees, including poplar trees, it is necessary to establish a stable and efficient transformation system. Adopting pest and disease-resistant transgenic poplar plants to minimize yield loss and pesticide consumption has been successful. Many researchers have employed genetic transformation methods, including *Agrobacterium tumefaciens*, protoplast, gene gun, RNA interference, and miRNA-mediated transformations, to improve poplar resistance to pests and pathogens. These techniques, along with genome editing to introduce pest resistance genes and modulate lignin biosynthesis, offer promising avenues for developing transgenic poplar trees capable of withstanding pest and disease attacks, thus improving their survival rates. However, no research has been conducted on the pollen tube method, *A. rhizogenes* mediated and nanoparticle-mediated transformation to enhance pest/pathogen or virus resistance in *Populus* species. Further efforts are required to establish transgenic poplars with single/multiple genes for increasing biotic stress tolerance limits (pest/pathogens infestations) using nanoparticles, *A. rhizogenes*, and the pollen tube method. It will be optimal if the methods developed in poplar can be used in other forest trees to make them resistant to biotic and abiotic stresses. The use of omics technologies (i.e., genomics, transcriptomics, and proteomics), along with high-throughput screening and selection methods, accelerates the identification of successful transgenic poplar lines. Integrating big data, machine learning, and artificial intelligence (AI) into poplar breeding programs (i.e., data-driven breeding) can enhance the accuracy of predicting genetic alteration outcomes (Farooq et al., 2024), enabling more targeted and efficient transgenic strategies for trees. A key factor for success is the competence to regenerate transgenic plants from modified cells. Addressing the genotype dependency in poplar transformation is crucial for expanding the applicability of transgenic approaches. Developing "transgene-free" or non-GMO techniques, such as transient CRISPR expression, could alleviate regulatory and public concerns, facilitating the adoption of genetically improved poplar.

Author contributions

AS: Conceptualization, Formal analysis, Funding acquisition, Investigation, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing. SS: Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft. AC: Investigation, Validation, Visualization, Writing – original draft, Writing – review & editing. AR: Formal analysis, Funding acquisition, Investigation, Resources, Software, Visualization, Writing – review & editing. IS: Data curation, Formal analysis, Investigation, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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