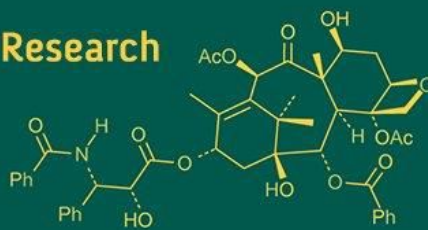


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Breeding for Fusarium wilt resistance in pigeonpea: From landraces to molecular techniques

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Abstract

Fusarium wilt, caused by *Fusarium udum* Butler, is a devastating disease of pigeonpea (*Cajanus cajan*) that causes 30-100% yield losses in affected fields (Kannaiyan *et al.*, 1984). Breeding for Fusarium wilt resistance in pigeonpea has evolved from simple field selection of landraces to advanced molecular breeding and biotechnology. Early efforts (1970s-1980s) focused on screening thousands of germplasm accessions in "wilt-sick" fields, yielding a handful of resistant lines such as ICP 8863 (released as 'Maruti' in 1986) which revolutionized pigeonpea cultivation in wilt-endemic regions (ICRISAT, 1986). Conventional breeding strategies including pure-line selection, hybridization, pedigree breeding, and induced mutation produced numerous wilt-resistant cultivars (e.g. 'Bahar', 'Pusa 9', 'Sharad', 'Durga', 'BDN-2004-1') adapted to different Indian agro-ecologies (Singh *et al.*, 1990; Choudhary *et al.*, 2013). Since the 1990s, breeding programs (notably at ICRISAT and Indian national institutes) achieved milestones such as the variety 'Asha' (ICPL 87119), the first pigeonpea combining high yield with dual resistance to Fusarium wilt and sterility mosaic disease (Saxena *et al.*, 2010). In the 2000s, attention turned to molecular approaches: genetic studies revealed both dominant and recessive genes for wilt resistance (Parupalli *et al.*, 2017) and molecular markers were developed to tag resistance loci (e.g. RAPD markers linked to a wilt-resistance gene by Kotresh *et al.* (2006)). The pigeonpea genome sequence was completed in 2012, enabling mapping of quantitative trait loci (QTLs) for wilt resistance and marker-assisted selection (MAS) in breeding (Varshney *et al.*, 2012; Saxena *et al.*, 2017). Advanced genomics tools high-density SNP arrays, QTL mapping via genotyping-by-sequencing and comparative transcriptomics have identified several genomic regions and candidate genes associated with wilt resistance (Singh *et al.*, 2016; Purohit *et al.*, 2021). However, breeding remains challenging due to the pathogen's high variability and the need to pyramid multiple disease resistances (Saxena *et al.*, 2020; Purohit *et al.*, 2021). Recent breakthroughs include diagnostic DNA markers for Fusarium wilt resistance (Saxena *et al.*, 2020) and transgenic pigeonpea lines expressing antifungal genes (e.g. rice chitinase, plant defensins, oxalate oxidase) that show enhanced wilt resistance in experimental trials (Kumar *et al.*, 2004; Choudhary *et al.*, 2013). This review traces the global progression of Fusarium wilt resistance breeding in pigeonpea with special emphasis on India from traditional methods to modern molecular techniques. It discusses historical milestones, key resistant varieties and breeding programs, and the latest advances in genomics-assisted breeding, gene discovery and biotechnology. Challenges and future prospects are analyzed, highlighting integrated strategies to develop durable Fusarium wilt resistance in pigeonpea.

Keywords: Wilt, genomics-assisted breeding, pigeonpea, molecular breeding

Introduction

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is an important grain legume grown on ~7 million hectares in tropical and subtropical regions (Nene & Sheila, 1990) ^[14, 9]. It is especially crucial in India, which accounts for about 75-80% of global pigeonpea area and production (Nene & Sheila, 1990; Purohit *et al.*, 2021) ^[14, 9, 10]. Pigeonpea seeds (split as dal) are a major protein source in South Asia and East Africa (Nene & Sheila, 1990) ^[14, 9]. However, pigeonpea yields are constrained by several diseases, among which Fusarium wilt is the most severe. Fusarium wilt is caused by the soil-borne fungus *Fusarium udum* Butler (formerly classified as *F. oxysporum* f. sp. *udum*), which infects the roots and blocks the vascular system, leading to wilting of the plant (Kannaiyan *et al.*, 1984) ^[6]. The disease can attack pigeonpea at any growth stage and cause 30 to 100% crop loss depending on infection timing (Kannaiyan *et al.*, 1984) ^[6].

In India alone, wilt epidemics destroy an estimated 470,000 tons of pigeonpea grain each year, amounting to loss to an extent of about US\$71 million annually (Sharma *et al.*, 2013) [13]. *Fusarium* wilt was first reported in pigeonpea over a century ago, and by the late 20th century it had become widespread in South Asia, parts of Africa (e.g. Kenya, Malawi, Tanzania) and the Caribbean (Kannaiyan *et al.*, 1984; Singh *et al.*, 1990) [6, 14]. *F. udum* can survive in soil for 3-5 years as hardy spores or mycelia, making crop rotation only partially effective (Nene & Sheila, 1990) [14, 9]. Chemical control *via* soil fungicides has largely failed or is uneconomical for this disease (Nene & Sheila, 1990) [14, 9]. Hence, developing wilt-resistant pigeonpea varieties has long been recognized as the most sustainable and cost-effective solution (Singh *et al.*, 1990; Saxena *et al.*, 2020) [14, 17]. Indeed, breeding for disease resistance (including *Fusarium* wilt) became a core objective of pigeonpea improvement programs worldwide in the latter half of the 20th century (Singh *et al.*, 1990; Nene & Sheila, 1990) [14, 9]. This review provides an academic overview of breeding methods used to develop *Fusarium* wilt resistant pigeonpea, charting the path from early conventional breeding to current molecular approaches. We place special emphasis on India the epicenter of pigeonpea cultivation and research while also incorporating global perspectives (e.g. breeding efforts in Africa). The topics include: (1) historical breeding approaches such as germplasm screening, selection, hybridization and mutation breeding; (2) major milestones and varieties released with wilt resistance; (3) modern biotechnological interventions like marker-assisted selection (MAS), QTL mapping, prospects for genomic selection and transgenic strategies (4) recent advances in understanding the genetics of wilt resistance (gene identification, molecular markers, genomic resources) and (5) challenges and future prospects for breeding durable resistance. By compiling key studies and breeding achievements from the 1970s to the present, we aim to illustrate how breeding for *Fusarium* wilt resistance in pigeonpea has progressively become more precise and knowledge-driven.

Breeding Strategies: Conventional Approaches Germplasm Screening and Selection

The foundation of resistance breeding is the identification of genetic sources of resistance. Early pigeonpea breeders undertook massive screening of germplasm collections in wilt-sick plots (fields naturally infested with *F. udum*) from the 1970s onward (Upadhyaya *et al.*, 1986) [19]. These extensive field screenings revealed that only a tiny fraction (~0.3%) of accessions had high levels of wilt resistance (Upadhyaya *et al.*, 1986) [19]. For example, a multi-year screening of >11,000 pigeonpea entries in India identified just 33 lines with resistance to *F. udum* (Upadhyaya *et al.*, 1986) [19]. Such findings underscored the rarity of strong resistance alleles in the cultivated gene pool, but they provided valuable donor lines for breeding. One landmark success from direct selection was the variety 'Maruti' (ICP 8863). This line originated from a landrace (ICP 7626) collected in Uttar Pradesh, India (ICRISAT, 1986) [5]. Breeders at ICRISAT sowed the landrace population in a heavily infested wilt plot in 1977-78 and selected surviving plants. Through pedigree selection and rigorous multi-location testing in endemic areas (1978-1985), the line ICP 8863 consistently showed near-immunity to wilt (ICRISAT, 1986) [5]. It was released in 1986 as 'Maruti' in Karnataka,

where farmers' fields had been devastated by wilt (ICRISAT, 1986) [5]. Maruti quickly became popular across the Deccan Plateau; it was the only available pigeonpea variety at the time combining high yield with broad-based wilt resistance (ICRISAT, 1986) [5]. Farmers reported that Maruti's adoption revitalized pigeonpea production in wilt-prone districts, earning it nicknames like "the pigeonpea savior" (ICRISAT, 1986) [5]. This success demonstrated the power of landrace selection: Maruti's resistance came directly from natural variation present in the germplasm, without hybridization. Other examples of varieties derived by selection include 'Bahar' (a farmer variety selected in Bihar, India) and 'Durga', which were identified in the 1970s-80s for moderate wilt resistance (Singh *et al.*, 1990; Kannaiyan *et al.*, 1984) [14, 6]. Beyond cultivated germplasm, pigeonpea's wild relatives were also examined as potential resistance sources. Wild *Cajanus* species (e.g. *C. scarabaeoides*, *C. platycarpus*) are generally immune to many pigeonpea diseases. However, their use for *Fusarium* wilt resistance has been limited, since adequate resistance was found within *C. cajan* itself. Nonetheless, wild species have contributed genes for other stresses (insect pests, sterility mosaic disease) and breeders remain interested in wild introgressions to broaden the genetic base of wilt resistance in the future (Choudhary *et al.*, 2013; Singh *et al.*, 1990) [2, 14].

Hybridization and Pedigree Breeding

Once initial resistant lines were identified, breeders moved to hybridization to combine resistance with high yield and adaptation. Pigeonpea is a partially outcrossing species, which facilitates cross-pollination, but traditional breeding still relies on manual crosses to create new variability. During the 1980s-1990s, several bilateral crosses were made between wilt-resistant donor lines and agronomically superior but susceptible cultivars. The segregating progenies were screened under wilt nursery conditions (often by inoculating soil with the pathogen or using naturally infested fields) to select resistant recombinants. A prime example is the development of ICPL 87119 (var. 'Asha'), which was bred at ICRISAT. 'Asha' was obtained from a cross between a susceptible cultivar ('ICP 1') and a wilt- and mosaic-resistant breeding line from a cross of 'ICP 8864' × 'C11' (Parupalli *et al.*, 2017) [11]. Progenies from this cross were advanced through a bulk-pedigree method under disease pressure at each generation, seeds from healthy (wilt-free) plants were bulked to form the next generation (Parupalli *et al.*, 2017) [11]. By the F₇ generation, a single plant selection showed stable resistance to both *Fusarium* wilt and sterility mosaic virus (another major disease) (Parupalli *et al.*, 2017) [11]. This line was evaluated in All-India trials (1987-1991) and consistently out-yielded the best checks by ~20% while maintaining dual disease resistance (Parupalli *et al.*, 2017) [11]. Released in 1993 as 'Asha', ICPL 87119 was the first medium-duration pigeonpea combining high-level resistance to *Fusarium* wilt and sterility mosaic disease (SMD) (Parupalli *et al.*, 2017) [11]. It became a popular variety in central and southern India and is still used as a key parent in breeding programs (Parupalli *et al.*, 2017) [11]. The success of 'Asha' illustrated how pedigree breeding and careful disease screening could pyramid multiple resistances into one cultivar. During the 1990s and 2000s, national agricultural research system (NARS) breeders in India also produced many wilt-resistant

varieties through crosses and selection. For instance, 'Pusa 9' (developed at IARI, New Delhi) and 'Narendra Arhar 1' (NDUAT, Faizabad) were medium-duration varieties with moderate wilt resistance (Choudhary *et al.*, 2013) [2]. 'Sharad' and 'Jawahar' were released in Madhya Pradesh, 'Malviya Arhar-2' in Uttar Pradesh, and 'Birsa Arhar-1' in Jharkhand all with improved wilt tolerance (Choudhary *et al.*, 2013) [2]. Each of these was bred by crossing local varieties with resistant donors, followed by multi-location testing in Fusarium "hotspots." The All India Coordinated Pulses Improvement Project (AICPIP) played a central role by organizing systematic disease screenings and trials, ensuring that only lines with stable wilt resistance across locations were advanced for release (Parupalli *et al.*, 2017) [11]. One challenge in conventional breeding was the genetic complexity of wilt resistance. Early inheritance studies were somewhat contradictory: some suggested a single recessive gene conferring resistance (e.g. in cultivars 'T-21' or 'Bahar') (Parupalli *et al.*, 2017) [11], whereas others found resistance to behave as a dominant trait or even polygenic. A recent study by Parupalli *et al.* (2017) [11] clarified that different resistance sources may have different inheritance in one recombinant inbred population resistance was monogenic dominant, while in another it was monogenic recessive (Parupalli *et al.*, 2017) [11]. This indicates multiple distinct resistance genes exist in pigeonpea germplasm. Conventional breeders had empirically noticed such patterns for instance, crossing two resistant lines sometimes yielded all susceptible progeny, implying non-allelic recessive genes were involved in the parents. Breeding strategies were adjusted accordingly using gene pyramiding (combining multiple resistance genes) to achieve broader and more durable resistance.

Mutation Breeding

In addition to hybridization, mutation breeding was occasionally employed to generate new variation for disease resistance. Induced mutations (via gamma rays or chemical mutagens) produced mutants like 'BDN-2004-1', a wilt-resistant pigeonpea variety released in Maharashtra. BDN-2004-1 was derived as a mutant of the popular cultivar BSMR-853 (Choudhary *et al.*, 2013) [2]. The mutant line exhibited enhanced resistance to Fusarium wilt (and also had a semi-dwarf plant type) (Choudhary *et al.*, 2013) [2]. Similarly, 'Mahavir (IPMD 1)' in Bihar was reportedly developed through mutation selection for wilt and sterility mosaic resistance (essentially a purified selection known in some sources as 'IPA 16F') (Choudhary *et al.*, 2013) [2]. While mutation breeding did not become a primary method for pigeonpea (due to low efficiency and long timelines), these examples show it contributed some useful alleles.

Hybrid Pigeonpea Breeding

A notable innovation in pigeonpea improvement was the development of commercial hybrids in the 2000s. Pigeonpea's partial outcrossing nature enabled the creation of cytoplasmic male sterility (CMS) systems for hybrid seed production. By 2010, ICRISAT scientists (led by Dr. K.B. Saxena) released the world's first pigeonpea hybrid, ICPH 2671, which offered a 30-40% yield advantage. Importantly, the parental lines of hybrids were bred to carry disease resistance. For example, the hybrid ICPH 2671's parents included ICPL 87119 ('Asha') as the female parent, thus conferring Fusarium wilt and SMD resistance to the hybrid

(Saxena *et al.*, 2010) [17]. Subsequent hybrids (e.g. ICPH 2740, ICPH 3762) have also incorporated wilt-resistant germplasm in their pedigree. Although hybrid breeding's main goal is yield, the success of hybrids depends on stable disease resistance in both the A-(male sterile) and R-(restorer) lines. Therefore, conventional resistance breeding by developing elite resistant lines has been foundational even for the hybrid program. India's national trials now include disease resistance as a mandatory criterion for pigeonpea hybrid release, just as for varieties (Saxena *et al.*, 2010) [17].

In summary, through the late 20th century, traditional breeding approaches yielded significant gains in Fusarium wilt management. By combining germplasm selection, strategic crossing, and rigorous field screening, breeders in India and elsewhere released multiple resistant cultivars that substantially reduced yield losses. The incidence of wilt in farmers' fields was dramatically curtailed wherever these varieties were adopted (Singh *et al.*, 1990; Choudhary *et al.*, 2013) [14, 2]. However, challenges remained notably, the pathogen's variability and the breakdown of resistance in some cultivars over time. For instance, the once-resistant Maruti (ICP 8863) eventually became susceptible in certain areas as more virulent pathogen variants emerged (Saxena *et al.*, 2020) [17]. This necessitated continuous breeding efforts and a move toward more advanced methods to achieve durable resistance.

Molecular Approaches: Genomics and Marker-Assisted Breeding

Genetic Mapping and QTL Identification

By the turn of the 21st century, plant breeders began leveraging molecular biology tools to supplement conventional pigeonpea breeding. Genomics-assisted breeding using DNA markers linked to traits promised to accelerate the development of wilt-resistant varieties. Pigeonpea was somewhat late in adopting these tools (due to its reputation as an "orphan crop"), but the past 15-20 years have seen rapid progress. Initial attempts to map Fusarium wilt resistance were made using low-density DNA markers in the 2000s. Kotresh *et al.* (2006) [8] were the first to report DNA markers linked to a Fusarium wilt resistance gene. They screened mapping populations and identified two RAPD markers tightly linked (within ~10 cM) to a recessive wilt resistance locus in a cultivar from Karnataka (Kotresh *et al.*, 2006) [8]. Although RAPD markers are not very reproducible, this study was a proof-of-concept that wilt resistance is tractable by molecular mapping. Soon after, sequence-tagged microsatellite (SSR) markers became available in pigeonpea. For example, Bohra *et al.* (2012) [1] developed comprehensive genetic maps using SSRs (Bohra *et al.*, 2012) [1]. By combining multiple mapping populations, Bohra *et al.* (2012) [1] constructed an intra-specific consensus map with 11 linkage groups corresponding to pigeonpea's chromosomes. These maps provided a framework to localize disease resistance QTLs. One target was sterility mosaic disease (SMD), whose resistance was mapped before wilt resistance for example, Gnanesh *et al.* (2012) [1] found a major SMD resistance QTL on linkage group LG7. For Fusarium wilt, early QTL studies encountered difficulties. *F. udum* resistance often showed quantitative inheritance, meaning the effects of individual loci were modest and inconsistent. Gnanesh *et al.* (2012) [1] found QTLs for SMD but could not get a clear major QTL

for wilt in their populations. Likewise, initial mapping in India and Africa suggested multiple minor QTLs rather than a single dominant gene (e.g. one study in Kenya reported 2-3 recessive genes in a landrace, implying a polygenic basis). This polygenic nature made marker-assisted selection challenging, as markers explained only a portion of the phenotypic variance.

The breakthrough came after the pigeonpea genome was sequenced in 2012. An international team led by Varshney decoded the ~830 Mb genome of pigeonpea, identifying ~48,000 genes (Varshney *et al.*, 2012) [21]. Access to genomic sequences and abundant SNP markers allowed higher-resolution mapping. Researchers adopted approaches like “QTL-seq” and genotyping-by-sequencing (GBS) to identify loci in larger populations. For instance, Singh *et al.* (2016) [15] applied next-generation sequencing to contrasting DNA bulks (resistant vs. susceptible pools) and pinpointed candidate genome regions for *Fusarium* wilt resistance (Singh *et al.*, 2016) [15]. Around the same time, Saxena *et al.* (2017) [11] used GBS on several biparental populations, which led to mapping of at least three QTLs for wilt resistance on different chromosomes (Saxena *et al.*, 2017) [11]. One of these QTLs (on Chr. 11) co-located with a region from the elite parent ‘Asha’ and showed a significant effect in reducing wilt incidence (Saxena *et al.*, 2017) [11]. The percentage of phenotypic variance explained (PVE) by individual QTLs was moderate (often 10-20%), reinforcing that multiple genes contribute to resistance (Singh *et al.*, 2016; Saxena *et al.*, 2017) [15, 11]. Nonetheless, these studies yielded flanking SSR and SNP markers that breeders could use to track resistance alleles. A key outcome of recent genomics research has been the identification of stable QTL regions across studies. For example, QTLs on linkage groups LG4 and LG7 have been reported in more than one population, suggesting possibly conserved resistance loci (Saxena *et al.*, 2020) [17]. Moreover, resistance to *Fusarium* wilt often correlates with resistance to SMD in pigeonpea (because varieties like ‘Asha’ had both). Some mapping studies detected overlapping QTLs or closely linked loci for wilt and SMD resistance (Singh *et al.*, 2016; Saxena *et al.*, 2017) [15, 11]. This raises the prospect of simultaneously improving both diseases via marker-assisted breeding. Despite progress, a recurring issue was the difficulty of developing diagnostic markers i.e., markers so tightly linked (or causal) that they reliably predict resistance across diverse germplasm. Markers from one mapping population sometimes failed to work in others, due to genotype-specific resistance genes and the genetic heterogeneity of *F. udum* (many pathotypes). A 2020 analysis by Saxena *et al.* noted that earlier mapping efforts yielded markers “not very effective for selection in breeding” (Saxena *et al.*, 2020) [17]. The authors cited challenges like complex inheritance, variability in pathogen isolates, and low-resolution QTL mapping as limiting factors (Saxena *et al.*, 2020) [17]. To overcome this, Saxena *et al.* (2020) [17] adopted a multi-pronged strategy: integrating data from multiple studies and populations to find common “hotspots” for resistance. This led to a set of robust SNP markers that can distinguish resistant and susceptible genotypes. Dubbed a “diagnostic marker kit” for FW and SMD, it consisted of carefully chosen markers with high correlation to disease reaction (Saxena *et al.*, 2020) [17]. Such markers are now being validated in breeding programs for routine MAS.

Marker-Assisted Selection (MAS)

Marker-assisted selection has been gradually implemented in pigeonpea breeding, albeit with caution. In India, breeders traditionally relied on field screening, which is laborious but directly reflects resistance. With markers becoming available, a few marker-assisted backcrossing (MABC) projects were initiated. For example, ICRISAT undertook a program to introgress wilt-resistance QTLs into the elite landrace ‘ICP 8863’ (Maruti) to broaden its resistance (Saxena *et al.*, 2020) [17]. However, until recently, no pigeonpea variety had been released through MAS for wilt partly due to the aforementioned marker limitations. This contrasts with other legumes like chickpea, where MAS produced wilt-resistant lines (e.g. the variety ‘Super Annigeri-1’ in chickpea). Encouragingly, with the new diagnostic markers, MAS is expected to accelerate. Indian breeders now routinely screen breeding lines with markers linked to major QTLs before field testing. A case in point is the ICPL 332 series: ICPL 332 is a popular landrace susceptible to wilt; an improved line ICPL 332-WR (wilt resistant) was developed by transferring resistance from a donor via MAS (reported at a conference in 2019). Similarly, the AICPIP coordinated trials in 2021 included several MAS-derived hybrids/varieties that carry marker-tracked resistance alleles. Another modern strategy is genomic selection (GS). Given the polygenic nature of *Fusarium* resistance, GS which uses genome-wide markers to predict breeding values could capture small-effect loci better than QTL-based MAS. The development of high-density SNP arrays (such as a 62K SNP chip for pigeonpea) now permits genomic prediction models. While GS in pigeonpea is still in experimental stages, pilot studies are underway to test its utility for complex traits including disease resistance. The relatively long generation time of pigeonpea (and thus long breeding cycles) means GS could greatly accelerate gains if prediction accuracy is high.

Gene Discovery and Functional Genomics

Beyond markers and QTLs, researchers have sought to identify specific genes underlying wilt resistance. Transcriptome and proteome analyses have been valuable here. For example, Purohit *et al.* (2021) [10] performed comparative transcriptomic profiling of a susceptible variety (ICP 2376) vs. resistant ‘ICP 8863’ (Maruti) during *Fusarium* infection (Purohit *et al.*, 2021) [10]. They found that resistant plants showed early up-regulation of defense pathways notably the jasmonic acid (JA) and salicylic acid (SA) signaling pathways, cell wall strengthening, pathogenesis-related (PR) proteins, and reactive oxygen species detoxification (Purohit *et al.*, 2021) [10]. Several defense-responsive genes were identified, including those encoding chitinases, glucanases, PR proteins, and transcription factors. These are likely contributors to resistance mechanisms. Similarly, proteomic studies have indicated that resistant pigeonpea accumulates higher levels of enzymes like β -1,3-glucanase and chitinase in roots after infection (these enzymes can degrade fungal cell walls). A significant leap was the use of “Indel-seq” (Insertion-Deletion mapping) by Singh *et al.* (2017) [16]. This approach identified short genomic insertion/deletion polymorphisms associated with wilt resistance. The study narrowed down two candidate genomic regions containing a handful of genes (including receptor-like kinases and NBS-LRR resistance genes) which were strong candidates for

controlling wilt resistance. Although functional validation (e.g. via gene knock-out or overexpression) has not yet been fully achieved in pigeonpea, these candidate genes provide targets for future gene editing or transgenic approaches. It is noteworthy that unlike some crops (e.g. tomato or melon where single *R*-genes for *Fusarium* wilt have been identified), pigeonpea's resistance does not appear to be based on a classic single dominant "R gene" with a known race-specific interaction. Instead, resistance is quantitative and broad-spectrum, likely involving multiple genes each contributing partial resistance. This may be advantageous, as it can mean more durable resistance (polygenic control is harder for the pathogen to break). However, it complicates efforts to clone a single "Fusarium wilt resistance gene." As of 2025, no single gene (for example, a NBS-LRR class gene) has been officially cloned as an Fw (Fusarium wilt) resistance gene in pigeonpea. Research is ongoing to validate candidates through advanced genetic transformation and gene editing experiments.

Recent Advances and Biotechnological Interventions

Genomic Resources and Big-Data Breeding

The publication of the pigeonpea draft genome by Varshney *et al.* (2012) ^[21] was a watershed moment. It paved the way for whole-genome resequencing of diverse pigeonpea lines, uncovering many novel alleles. Thousands of SNP markers were mined from the genome data, leading to high-throughput genotyping platforms (for example, a 56K SNP "Axiom_Cajanus" array and a subsequent 62K SNP chip). These tools have dramatically improved the resolution of mapping studies and the ability to perform genome-wide association studies (GWAS) for traits like disease resistance. Indeed, a recent association mapping by Yadav *et al.* (2019) identified several SSR markers significantly associated with wilt resistance across a panel of pigeonpea germplasm. Another advance is the use of genotyping-by-sequencing (GBS) to generate ultra-dense linkage maps. Saxena *et al.* (2017) ^[11] constructed a GBS-based map with >1,000 markers and used it to identify QTLs for *Fusarium* wilt with high confidence (Saxena *et al.*, 2017) ^[11]. The improved mapping resolution helps pinpoint resistance loci within narrower genomic intervals (<1 Mb), making it easier to identify candidate genes. Breeders have also started using "breeder-friendly" markers such as Kompetitive Allele Specific PCR (KASP) assays for routine screening. Markers linked to major QTLs from ICRISAT's research (e.g., on LG4 and LG7) were converted to KASP assays, which are now used in marker-assisted backcrossing schemes. This exemplifies the bridging of upstream genomics to applied breeding.

Transgenic Approaches

While conventional breeding remains the primary strategy for pigeonpea improvement, transgenic technology has been explored to introduce novel resistance genes that are absent in the pigeonpea gene pool. One of the first efforts was by Kumar *et al.* (2004) ^[7], who genetically engineered pigeonpea with a rice chitinase gene. Chitinase is an antifungal enzyme; the idea was that constitutive expression of rice chitinase in pigeonpea might enhance its ability to inhibit *Fusarium*. Kumar *et al.* (2004) ^[7] successfully produced transgenic pigeonpea plants (cv. 'LRG 30') via *Agrobacterium*-mediated transformation and confirmed stable integration of the chitinase gene. Subsequent

greenhouse tests by Hussain *et al.* (2005) ^[4] (unpublished results) indicated that these transgenics had increased resistance the transgenic plants showed significantly lower wilt incidence compared to non-transgenic controls when challenged with *F. udum* (the first evidence that a single gene could confer partial resistance in pigeonpea). Encouraged by this, researchers tried other antifungal genes. One approach was to use plant defensins and other antifungal proteins from various species. Defensins are small cysteine-rich proteins that can inhibit fungal growth. In a recent study, Indian scientists transformed pigeonpea with two genes: Tfgd2, a defensin from fenugreek (*Trigonella foenum-graecum*), and RsAFP2, an antifungal protein from radish (*Raphanus sativus*) (Choudhary *et al.*, 2013) ^[2]. Transgenic pigeonpea lines overexpressing these genes have shown enhanced resistance to fungal pathogens in laboratory assays. There is a report by Choudhary *et al.* (2013) ^[2] that such transgenic lines not only resisted *Fusarium* better but also exhibited some resistance to the pod borer insect (*Helicoverpa*), possibly due to general anti-pathogen effects (this cross-protection is still being studied) (Choudhary *et al.*, 2013) ^[2]. Another breakthrough came from deploying an oxalate oxidase (OXO) gene. *Fusarium udum* is thought to produce oxalic acid in infected tissue, which can aid its virulence. Oxalate oxidase (found in cereals like wheat and rice) breaks down oxalic acid. In Purohit *et al.* 2021 ^[10] (a team in India) developed transgenic pigeonpea expressing the rice *OsOXO4* gene. The transgenics showed markedly higher tolerance to wilt; proteomic and metabolomic analyses confirmed that the OXO gene enhanced the activation of multiple defense pathways and reduced oxidative stress in infected plants (Tripathi *et al.*, 2024) ^[18]. These *OXO4*-transgenic lines suffered much lower wilting than wild-type plants under pathogen challenge, representing a promising new strategy. It should be noted that all these transgenic approaches are at the experimental stage. No transgenic pigeonpea has yet been released for farmers, due to regulatory hurdles and the long testing pipeline. However, the research shows that transgenes can significantly boost resistance. In the future, gene editing (CRISPR/Cas) could also be employed-for instance, to knock out pigeonpea susceptibility genes or to activate native defense genes. Gene editing would bypass some regulatory issues of transgenics and could accelerate the introduction of durable resistance traits.

Integrated Disease Management and Other Innovations

While breeding is the focus of this review, it's worth mentioning that integrated disease management (IDM) practices complement the deployment of resistant varieties. Biocontrol agents (like *Trichoderma* and *Pseudomonas* strains) and cultural practices (crop rotation, soil amendments) can further reduce wilt incidence (Kannaiyan *et al.*, 1984) ^[6]. Nonetheless, resistant cultivars remain the cornerstone; farmers are advised to use wilt-resistant varieties as the first line of defense (Kannaiyan *et al.*, 1984) ^[6]. Modern breeding programs thus often aim for multi-disease resistance (wilt + SMD + *Phytophthora* blight, etc.), ensuring that a variety can withstand the disease complex. For example, lines like 'ICPL 87' and 'C 11' were noted to combine resistance to wilt, SMD, and *Phytophthora* blight (Saxena *et al.*, 2020) ^[17]. The future lies in creating such broad-spectrum resistance through both conventional and molecular stacking of genes.

Challenges and Future Prospects

Despite considerable advancements, breeding pigeonpea for *Fusarium* wilt resistance faces ongoing challenges. A primary issue is the pathogen's pathogenic variability. *Fusarium udum* exhibits a high degree of diversity with multiple strains (often termed races or variants) identified across regions (Ravikumara *et al.*, 2022) [12]. In India, at least five distinct pathogenic variants were reported by the 2010s (designated as Variant-1, 2, 3, etc.) (Ravikumara *et al.*, 2022) [12]. Some aggressive variants (e.g. Variant-7 and 8 in southern India) can overcome the resistance of previously durable cultivars like Maruti (Ravikumara *et al.*, 2022) [12]. Similarly, in East Africa, isolates differ such that a line resistant in Kenya might succumb in Malawi. This “boom and bust” cycle where a resistant variety is widely grown and then a new virulent pathogen strain emerges is a constant threat (Ravikumara *et al.*, 2022) [12]. To address this, breeders must continuously scout for new resistance sources and pyramid genes to achieve durable, broad-based resistance. The identification of cultivars like ICP 9174 and BDN 2, which remained resistant across diverse Indian locations (Ravikumara *et al.*, 2022) [12], is encouraging. These could serve as future parents to introgress geographically broad resistance.

Another challenge is the lengthy breeding cycle of pigeonpea. It is a long-duration crop (4-6 months to maturity for many varieties) and often only one generation per year is possible in the field. This slows down conventional breeding progress. Techniques like off-season nurseries, single-seed descent in greenhouses, or even speed breeding under controlled conditions could be more widely adopted to hasten generational turnover. Combining speed breeding with marker-assisted selection could in theory produce a new resistant variety in 5-6 years instead of 10-12. The integration of modern genomics into routine breeding is still at a nascent stage for pigeonpea. Many breeders in national programs require training and resources to implement MAS and GS effectively. Capacity building and collaborations (such as those led by ICRISAT with national institutes) will be crucial so that the advances in marker technology translate to on-ground variety development. The establishment of “precision breeding” pipelines where genotypic selection (via markers) is done alongside field phenotyping is a future goal in India's pulses breeding network. On the biotechnology front, while transgenic and CRISPR technologies are powerful, their deployment will depend on regulatory approvals and public acceptance. If regulatory environments become favorable, we may see biotech pigeonpea varieties in the coming decade, especially those stacked with insect resistance (e.g. a Bt gene for pod borer) and disease resistance genes for a comprehensive solution. Until then, conventional breeding enriched by genomic tools will remain the mainstay. Looking forward, breeders are also exploring novel breeding methods like heterosis breeding (developing more disease-resistant hybrids) and introgression from the secondary gene pool (wild species) for novel resistance genes. The wild relative *Cajanus platycarpus*, for instance, is immune to wilt successful crossing with pigeonpea is difficult but advances in embryo rescue and bridge crosses might bring such alien genes in.

In conclusion, the trajectory of *Fusarium* wilt resistance breeding in pigeonpea demonstrates a clear evolution from arduous field selection to today's data-driven, gene-centric

approaches. India's focus on dual resistance (wilt + SMD) as a release prerequisite (Saxena *et al.*, 2020) [17] has set a benchmark that new cultivars must meet, ensuring farmers get holistic disease protection. While challenges like pathogen evolution persist, the toolkit available to breeders is richer than ever encompassing classical breeding wisdom and cutting-edge genomics. With continued research and breeding investments, the vision of *Fusarium* wilt-proof pigeonpea is increasingly attainable. This will significantly contribute to stable pulse production and the livelihoods of millions of farmers who rely on this hardy “orphan crop” as a lifeline.

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