**Original research article**

**Fusarium Wilt Resistance in Pigeonpea: A Combined Phenotypic and ISSR Marker Study**

**Abstract**

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is a vital leguminous crop with significant nutritional and economic value, particularly in tropical and subtropical regions. However, its productivity is severely constrained by Fusarium wilt, a soil-borne disease caused by *Fusarium udum*, which can lead to yield losses ranging from 30 to 100%. Traditional breeding for resistance faces challenges due to limited resistant germplasm and environmental variability in phenotypic screening. This study integrates morphological evaluation with Inter Simple Sequence Repeat (ISSR) marker analysis to identify resistant genotypes and assess genetic diversity. Eleven pigeonpea genotypes were screened under controlled greenhouse conditions using artificial inoculation, revealing four resistant (0-6.66% wilt incidence), three moderately resistant (13.33-26%), and four susceptible (46.66-100%) genotypes. Molecular characterization using 13 ISSR primers generated 761 scorable bands, with UBC-818 and UBC-850 showing 100% polymorphism and ISSR-843 exhibiting the highest polymorphism information content (PIC = 0.358). Cluster analysis based on Jaccard’s similarity coefficients (0.54-0.91) distinctly grouped resistant and susceptible genotypes, validating phenotypic results. The study highlights the effectiveness of ISSR markers in identifying resistance-linked genomic regions, providing a reliable tool for marker-assisted breeding. These findings contribute to the development of high-yielding, wilt-resistant pigeonpea varieties, offering a sustainable solution to enhance productivity in disease-prone regions.

**Keywords:** Pigeonpea, *Fusarium wilt*, ISSR markers, Genetic diversity, Disease resistance, Polymorphism, Cluster analysis

**1. INTRODUCTION**

Pigeonpea (*Cajanus cajan* (L.) Millsp.), known as red gram or "arhar" in India, is a crucial leguminous crop with high economic and nutritional value, especially in tropical and subtropical regions (Saxena *et al* , 2010). Pulses are staple protein-rich food for Indian vegetarians, and India is one of the largest producers in the world (Mishra *et al*.,2021; MoAFW. 2024). Pigeonpea serve as key protein source its seeds contain 20-22% protein, along with essential amino acids and minerals. The crop also provides fodder for livestock and fuel wood for households (Saxena *et al*., 2002; Shiferaw *et al*., 2007)

India dominates the global pigeonpea market, accounting for over 70% of production with major contributions from Karnataka, Maharashtra, and Madhya Pradesh (Gowda et al., 2012; Saxena,2007). However, pigeonpea productivity faces severe constraints from Fusarium wilt caused by *Fusarium udum* Butler, a soil-borne pathogen responsible for yield losses of 30-100% in susceptible varieties (Pande *et al*.,2013). The pathogen can persist in soil as chlamydospores for extended periods, with studies reporting survival times of 15-30 years (Haridasan & Sajeena, 2025).with recent surveys reporting 40-60% disease incidence in major growing regions (Saxena *et al*., 2010). While host-plant resistance remains the most sustainable solution, breeding programs face challenges including limited availability of resistant germplasm and environmental variability in phenotypic screening (Reddy.P *et al*., 2002).

To address these limitations, this study combines morphological screening with ISSR marker analysis to overcome environmental biases and identify resistance-linked DNA regions. The research aims to screen 11 pigeonpea genotypes for Fusarium wilt resistance, analyze their genetic diversity using 13 ISSR primers, and correlate molecular profiles with resistance levels to identify robust breeding material. The findings will provide breeders with molecular tools for accelerated cultivar development, reduce fungicide reliance in wilt-endemic regions.

**2. MATERIAL AND METHOD**

**2.1 Planting material and pot culture screening**

The present investigation was carried out in the Post Graduate Laboratory, Department of Plant Biotechnology, Vilasrao Deshmukh College of Agricultural Biotechnology, Latur [Vasantrao Naik Marathwada Krishi Vidyapeeth Parbhani (M. S.)] during 2023-2025 . A total of eleven pigeonpea *(Cajanus cajan* L.) genotypes collected from the Agriculture Research Station (ARS), Badnapur, Maharashtra were used for fusarium wilt disease screening in the present investigation.

A total of 11 Healthy pigeonpea seeds were surface sterilized with sodium hypochlorite, rinsed three times with sterile water, and sown in sterilized soil-filled plastic bags maintained at 30°C in a greenhouse. After 15 days, seedlings were uprooted, roots washed, trimmed, and immersed in a *Fusarium udum* spore suspension (6×105 spores/ml) for 20 minutes(Pande *et al*.,2012). Inoculated seedlings were transplanted into autoclaved, moistened soil in plastic bags and watered as required. The experiment was conducted in three replications of five plants each.

**2.2 Disease scoring**

Wilt incidence was recorded seven days after transplanting as the percentage of infected plants, following Sharma *et al*. (2016)

|  |  |  |
| --- | --- | --- |
| Disease incidence (%) = | No. of plants infected  Total number of plants examined | X 100 |

The AICRP scale listed below was used to assess the genotypes in relation to the occurrence of wilt disease.

|  |  |  |
| --- | --- | --- |
| Wilt incidence (per cent) |  | Reaction |
| 0.00- 10.00 | : | Resistant |
| 10.10 - 30.00 | : | Moderately resistant |
| >30.00 | : | Susceptible |

This standardized classification enabled clear differentiation of host responses and insights into resistance levels among the tested germplasms.

**2.3 DNA extraction**

The extraction of genomic DNA from young leaves of eleven pigeonpea genotypes was done using an optimized CTAB protocol (Doyle & Doyle, 1987). Briefly, 2-3 g of fresh leaf tissue was flash-frozen in liquid nitrogen and homogenized before incubation in CTAB extraction buffer (2% CTAB, 100 mM Tris-HCl pH 8.0, 20 mM EDTA, 1.4 M NaCl, 1% β-mercaptoethanol). The lysate was purified through chloroform: isoamyl alcohol (24:1) extraction followed by isopropanol precipitation. DNA pellets were washed with 70% ethanol and resuspended in TE buffer. DNA quality was verified by 0.8% agarose gel electrophoresis, while concentration and purity were determined using a Nanodrop spectrophotometer (A260/A280 ratio of 1.7-1.9).

**2.4 ISSR marker-based DNA fingerprinting**

Genetic diversity among 11 pigeonpea genotypes was analyzed using Inter Simple Sequence Repeat (ISSR) markers. Eleven polymorphic primers were selected from preliminary screening for final analysis. PCR amplification was performed in 25 µL reactions containing 10X PCR buffer, 25 mM MgCl₂, 10 mM dNTPs, 10 pM primer, Taq DNA polymerase, and template DNA. Amplified products were electrophoresed on 1.5% agarose gels in 1X TAE buffer at 100V for 2.5 hours, stained with ethidium bromide (10 mg/ml), and visualized under UV light. Bands were scored dichotomously, with "1" indicating presence and "0" indicating absence of amplification products. The final analysis Included only distinct, reproducible bands. This approach followed established protocols for legume genetic studies (Pandey *et al*,. 2014).

**Table 1 : Primers selected for ISSR marker-based genetic diversity analysis**

|  |  |  |
| --- | --- | --- |
| **Sr no.** | **Primer code** | **Primer Sequence** |
|  | **ISD- 16** | AGAGAGAGAGAGAGAGC |
|  | **ISD- 20** | GAGAGAGAGAGAGAGACG |
|  | **ISD- 28** | AGAGAGAGAGAGAGAGTA |
|  | **ISD- 32** | AGAGAGAGAGAGAGAGAGAGT |
|  | **UBC-809** | AGAGAGAGAGAGAGAGG |
|  | **UBC-810** | GAGAGAGAGAGAGAGAT |
|  | **UBC-818** | CACACACACACACACAG |
|  | **UBC-825** | ACACACACACACACACAT |
|  | **UBC-841** | GAGAGAGAGAGAGAGACC |
|  | **UBC-860** | TGTGTGTGTGTGTGTGAA |
|  | **ISSR-843** | CTCTCTCTCTCTCTCTGA |
|  | **ISSR-857** | ACACACACACACACACYG |
|  | **UBC-850** | GTGTGTGTGTGTGTGTYC |

**2.5 Data Scoring and Genetic data analysis**

The binary (1/0) scoring data were analysed using Jaccard's similarity coefficients (Jaccard, 1908) to calculate pairwise genetic distances(Table 4). The resulting similarity matrix was subjected to hierarchical cluster analysis using the Unweighted Pair Group Method with Arithmetic Average (UPGMA) algorithm in NTSYS-pc software version 2.1 (Rohlf, 1990). Genetic relationships were visualized through dendrogram construction, while polymorphism percentage was determined as

|  |  |  |
| --- | --- | --- |
| Polymorphism percentage (%) = | Number of polymorphic bands  Total bands | X 100 |

This analytical pipeline enabled a comprehensive evaluation of genetic diversity patterns among the tested genotypes.

**3. RESULTS AND DISCUSSION**

**3.1 Morphological assessment of pigeonpea genotype’s resistance to fusarium wilt**

Globally, one of the main goals of breeding is to create pigeonpea types that are resistant to wilt. Greenhouse screening using artificial inoculation helps evaluate many plants efficiently under controlled disease conditions. This method ensures consistent testing by removing field variations, allowing reliable identification of resistant plants.(Bannihatti *et al*., 2022)

The phenotypic screening of eleven pigeonpea genotypes against *Fusarium udum* infection revealed three distinct response categories. Four genotypes (BDN-2004-1, Godavari, BSMR-853, and BDN-716) exhibited complete resistance, showing 0-6.66% wilt incidence with no visible symptoms of vascular browning or leaf wilting. These resistant lines maintained perfect physiological integrity throughout the evaluation period. Three genotypes (Vipula, BSMR-736, and BDN-711) demonstrated moderate resistance with 13.33-26% infection rates, displaying intermediate symptoms including mild leaf chlorosis and partial loss of turgidity. The remaining four genotypes (ICP-2376, Phule Rajeshwari, AKT-8811, and BDN-2) showed high susceptibility, with wilt incidence ranging from 46.66% to 100%. ICP-2376 was particularly vulnerable, showing complete (100%) susceptibility with rapid symptom development including severe wilting and plant death. The morphological findings corelates with (Ashitha *et al*., 2016).

The clear demarcation in disease responses (0-100% incidence) among the tested genotypes provides strong evidence for genetic variation in Fusarium wilt resistance.

**Table 2 . Reaction of pigeonpea host differentials to *F. udum***

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sr. No.** | **Variety** | **Plant**  **Stand**  **(Nos.)** | **Wilted**  **Plants** | **Percent wilt incidence (%)** | **Reaction** |
|  | BDN-2004-1 | 15 | 0 | 0.00 | R |
|  | VIPULA | 15 | 3 | 20.00 | MR |
|  | GODAVARI | 15 | 0 | 0.00 | R |
|  | ICP-2376 | 15 | 15 | 100.00 | S |
|  | BSMR-853 | 15 | 1 | 6.66 | R |
|  | BSMR-736 | 15 | 2 | 13.33 | MR |
|  | PHULE RAJESHWARI | 15 | 9 | 60.00 | S |
|  | BDN-711 | 15 | 4 | 26.00 | MR |
|  | AKT-8811 | 15 | 9 | 60.00 | S |
|  | BDN-2 | 15 | 7 | 46.66 | S |
|  | BDN-716 | 15 | 1 | 6.66 | R |

R:- Resistant ,MR:- Moderately resistant , S:-Susceptible.

**Fig 1 : Wilting symptoms on pigeonpea genotypes A) Resistance variety B) Susceptible variety**







CONTROL INFECTED







CONTROL INFECTED

**3.2 Molecular characterization of fusarium wilt resistance using ISSR markers**

**3.2.1 ISSR marker efficiency in genetic diversity analysis**

The molecular characterization using ISSR markers revealed substantial genetic variation among the 11 pigeonpea genotypes, with all 13 primers producing clear, reproducible amplification patterns. A total of 761 scorable bands were generated across all genotypes, demonstrating the effectiveness of ISSR markers for polymorphism detection in pigeonpea (Hemalatha & Shanmugasundaram (2010). The number of amplified bands per primer ranged from 16 (UBC-850) to 146 (UBC-809), with an average of 58.50 bands per primer. Notably, two primers (UBC-818 and UBC-850) showed 100% polymorphism, while ISSR-843 demonstrated the highest polymorphism information content (PIC value = 0.36), indicating its superior discriminative power for genetic studies (Joshi et al., 2000). The polymorphism percentage varied significantly among primers, ranging from 19.11% (ISSR-857) to 100% (UBC-818, UBC-850), with an average polymorphism of 56.2% across all primers. These results are consistent with previous ISSR studies in legumes that reported 45 to 90% polymorphism Swami *et al*. (2015).

**3.2.2 Cluster analysis and genetic relationships**

The genetic relationships among pigeonpea genotypes were analysed using Jaccard's similarity coefficients, which ranged from 0.54 to 0.91, indicating substantial genetic variation. UPGMA cluster analysis clearly separated the genotypes into three distinct groups corresponding to their Fusarium wilt resistance categories (Swami et al., 2015). The resistant genotypes (BDN-2004-1, Godavari, BSMR-853, and BDN-716) formed a cluster with moderate genetic similarity (average 0.72), with the closest relationship observed between BSMR-853 and BDN-2004-1 (0.70 similarity). These genotypes showed complete (0%) to minimal (6.66%) wilt incidence, confirming their resistance status. The moderately resistant group (Vipula, BSMR-736, and BDN-711) exhibited exceptionally high genetic similarity (average 0.85), with BSMR-736 and Vipula being nearly identical (0.91 similarity). This group displayed 13.33-26% wilt incidence. In contrast, the susceptible genotypes (ICP-2376, Phule Rajeshwari, AKT-8811, and BDN-2) clustered together with intermediate similarity (average 0.74) and showed 46.66-100% wilt incidence, with the strongest association between ICP-2376 and BDN-2 (0.85 similarity). The minimum genetic similarity (0.54) was observed between the moderately resistant BDN-711 and resistant Godavari, confirming substantial genetic divergence in the germplasm. These molecular findings strongly correlate with phenotypic resistance, validating ISSR's effectiveness for resistance screening in pigeonpea, as previously reported (Swami *et al*. (2015).

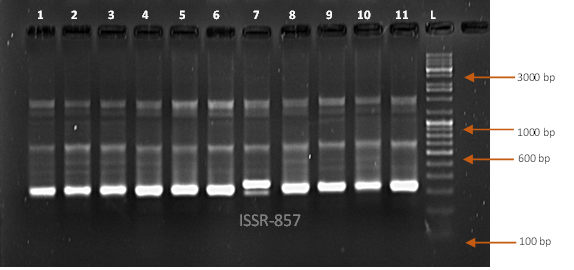
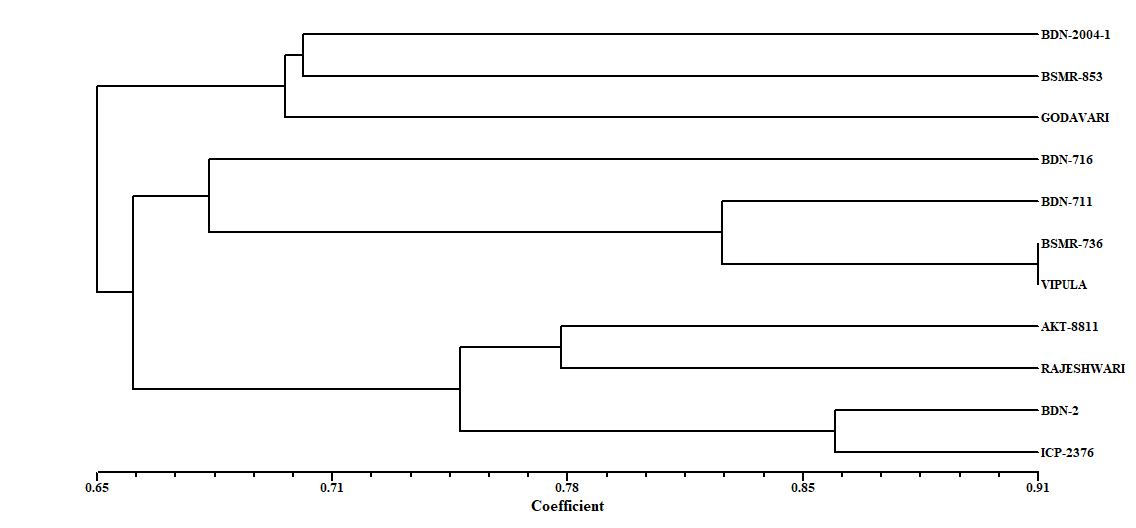
**Table 3 . Analysis of Molecular data generated using ISSR markers**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Sr No.** | **Primer** | **Total No. of Alleles** | **Total No. of Bands** | **Total No. of polymorphic band** | **Polymorphism %** | **PIC** | **Band size(bp)** |
| 1 | ISD- 16 | 8 | 40 | 29 | 72.5 | 0.35 | 250-1815 |
| 2 | ISD- 20 | 7 | 64 | 42 | 65.6 | 0.22 | 220-2400 |
| 3 | ISD-28 | 5 | 33 | 11 | 33.3 | 0.26 | 200-750 |
| 4 | ISD-32 | 10 | 81 | 26 | 32 | 0.20 | 150-1185 |
| 5 | UBC-825 | 6 | 50 | 33 | 66 | 0.29 | 350-1400 |
| 6 | UBC-860 | 6 | 41 | 19 | 46.34 | 0.31 | 500-2100 |
| 7 | UBC-841 | 8 | 53 | 31 | 58.49 | 0.28 | 500-3500 |
| 8 | ISSR-843 | 6 | 40 | 29 | 72.5 | 0.36 | 220-1185 |
| 9 | ISSR-857 | 8 | 68 | 13 | 19.11 | 0.13 | 220-1520 |
| 10 | UBC-809 | 16 | 146 | 47 | 32.19 | 0.18 | 150-1815 |
| 11 | UBC-810 | 9 | 73 | 18 | 24.65 | 0.17 | 300-2300 |
| 12 | UBC-818 | 7 | 56 | 56 | 100 | 0.32 | 500-2400 |
| 13 | UBC-850 | 2 | 16 | 16 | 100 | 0.40 | 400-800 |

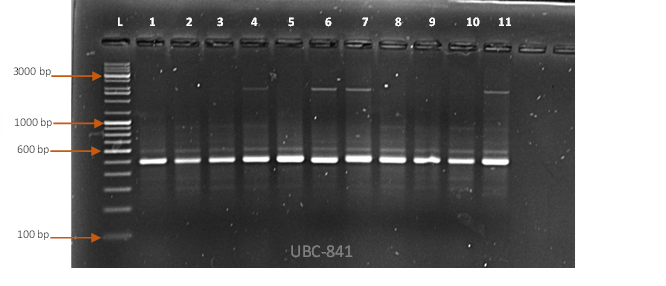
**Table 4 . Similarity matrix of pigeonpea genotypes generated using ISSR marker data through UPGMA clustering method in NTSYS-pc software.**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **BDN-2004** | **BSMR-853** | **GODAVARI** | **BDN-716** | **BDN-711** | **BSMR-736** | **VIPULA** | **AKT-8811** | **BDN-2** | **ICP-2376** | **RAJESHWARI** |
| **BDN-2004-1** | 1.0000 |  |  |  |  |  |  |  |  |  |  |
| **BSMR-853** | 0.7051 | 1.0000 |  |  |  |  |  |  |  |  |  |
| **GODAVARI** | 0.7013 | 0.6986 | 1.0000 |  |  |  |  |  |  |  |  |
| **BDN-716** | 0.6548 | 0.6923 | 0.6667 | 1.0000 |  |  |  |  |  |  |  |
| **BDN-711** | 0.6628 | 0.6190 | **0.5402** | 0.6905 | 1.0000 |  |  |  |  |  |  |
| **BSMR-736** | 0.6782 | 0.6163 | 0.5930 | 0.6860 | 0.8395 | 1.0000 |  |  |  |  |  |
| **VIPULA** | 0.7093 | 0.6471 | 0.6047 | 0.6591 | 0.8072 | **0.9125** | 1.0000 |  |  |  |  |
| **AKT-8811** | 0.7195 | 0.6543 | 0.7368 | 0.6867 | 0.6552 | 0.7093 | 0.7619 | 1.0000 |  |  |  |
| **BDN-2** | 0.6420 | 0.5949 | 0.6316 | 0.5904 | 0.6190 | 0.6353 | 0.6471 | 0.7403 | 1.0000 |  |  |
| **ICP-2376** | 0.7089 | 0.6000 | 0.6579 | 0.5765 | 0.5682 | 0.6023 | 0.6322 | 0.7436 | 0.8551 | 1.0000 |  |
| **RAJESHWARI** | 0.6824 | 0.6000 | 0.6145 | 0.6706 | 0.6404 | 0.7326 | 0.7857 | 0.7778 | 0.7215 | 0.7922 | 1.0000 |
|  | Maximum : 0.9125 | | | Average: 0.7264 | | | | Minimum: 0.5402 | | | |

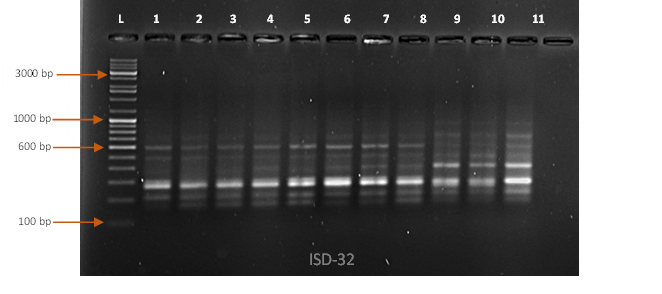
**Fig 2: Phylogenetic tree depicting the genetic associations between various pigeonpea genotypes**



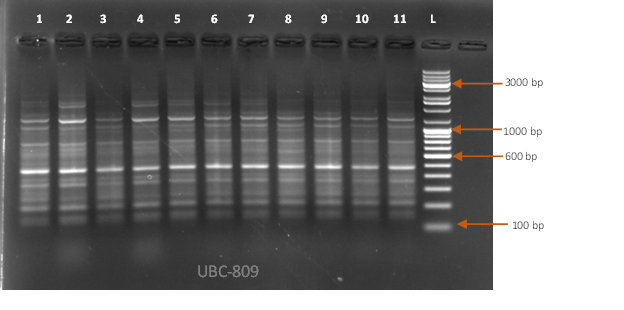
**Plate 2 : ISSR-857 marker showing polymorphism of 11 genotypes**



**Plate 3 : UBC-841 marker showing polymorphism of 11 genotypes**



**Plate 1: ISD-32 marker showing polymorphism of 11 genotypes**



**Plate 4: UBC-809 marker showing polymorphism of 11 genotypes**

**Fig 3 : Plate (1, 2, 3, 4): Amplification profile of 11 Pigeonpea genotypes with (1) ISD-32 (2) ISSR-857 (3) UBC-841 (4) UBC-809 primers. L: Ladder (5 kb) , 1) BDN-2004-1, 2) BSMR-853 , 3) GODAVARI , 4) BDN-716 , 5)BDN-711 , 6) BSMR-736 , 7) VIPULA , 8) AKT-8811, 9) BDN-2 , 10) ICP-2376 , 11) PHULE RAJESHWARI.**

**CONCLUSION**

This study successfully characterized pigeonpea genotypes for Fusarium wilt resistance using integrated morphological and molecular approaches. ISSR markers revealed substantial genetic diversity among the germplasm, with UBC-818 and UBC-850 showing 100% polymorphism and high discriminatory power (PIC up to 0.396). Cluster analysis distinctly separated resistant (BDN-2004-1, Godavari) and susceptible genotypes, confirming the reliability of these markers for resistance screening. The identified polymorphic markers demonstrate significant potential for marker-assisted breeding programs. These findings provide valuable genetic resources for development of high-yielding, disease-resistant pigeonpea varieties, particularly in wilt-endemic regions. The study establishes ISSR markers as an effective tool for genetic diversity assessment and resistance breeding in pigeonpea.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**  
The authors affirm that no generative artificial intelligence tools including Large Language Models (e.g., ChatGPT, Copilot) or text-to-image generators were utilized in the preparation or revision of this manuscript.

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