***Short Research Article***

**Resistance Evaluation of Mutagenized Sunnhemp (*Crotalaria juncea* L.) Lines to Phyllody (*Candidatus Phytoplasma*) Disease**

**ABSTRACT**

 **Sunhemp (*Crotalaria juncea* L.)** is a tropical leguminous crop valued for its multifaceted utility in fiber production, green manuring, and as a raw material in the manufacture of paper and rope. One of the major biotic stresses affecting sunhemp is **phyllody disease**, caused by a **phytoplasma**, specifically belonging to the “Candidatus Phytoplasma” spp. These are obligate intracellular pathogens classified under the class **Mollicutes**, and are primarily transmitted by sap-sucking insect vectors such as **leafhoppers,**  and **psyllids**. Phyllody-infected plants exhibit a range of morphological abnormalities, including **malformed floral structures**, **chlorosis**, **stunted growth**, and **proliferation of axillary shoots**, resulting in significant agronomic losses. In the present study, the sunhemp genotype **NTPSH 11-02** was subjected to **gamma irradiation** at doses of **20 kR, 30 kR, 40 kR, and 50 kR** to induce genetic variability. The M₂ generation lines derived from these treatments were evaluated under natural field conditions to assess their resistance to phyllody. Out of **44 mutant genotypes**, two genotypes, **NTPSH 63** and **NTPSH 32** exhibited **moderate resistance** to phyllody, while **nine genotypes**—**NTPSH 24, NTPSH 31, NTPSH 36, NTPSH 39, NTPSH 41, NTPSH 48, NTPSH 53, NTPSH 56**, and **NTPSH 60** demonstrated a **tolerant reaction** under natural infestation. Phyllody infection was found to **negatively impact key yield-contributing traits**, including **plant height, number of pods per plant, and 100-seed weight (test weight)**. A significant reduction in these parameters was observed in infected plants compared to healthy controls, underscoring the detrimental effect of phyllody on overall productivity and crop performance.

KEYWORDS: *Mutant lines, Phyllody, Phytoplasma, Sunhemp, Screening.*

**INTRODUCTION**

 **Sunhemp (*Crotalaria juncea* L.)** is a vital green manure crop renowned for its multifaceted agronomic benefits, including soil health improvement, nitrogen fixation, and suppression of weeds and soil-borne pests such as nematodes. As a fast-growing leguminous plant, sunhemp contributes substantial biomass and enriches the soil with organic matter and nutrients in a short span, making it an essential component of sustainable farming systems and crop rotation strategies. It is cultivated across a broad climatic range, from the semi-arid tropics and subtropics to temperate regions globally.

 In India, sunhemp is extensively cultivated for both green manure and fiber production, with major growing regions including Telangana, Maharashtra, Madhya Pradesh, Bihar, Rajasthan and Uttar Pradesh. These states serve as primary hubs for commercial cultivation due to favorable agro-climatic conditions and the crop’s adaptability.

Despite its benefits, sunhemp is vulnerable to several phytopathological threats. Phyllody disease is among the most severe threats, along with powdery mildew and Fusarium wilt, all of which significantly impair plant health, reduce biomass, and lower seed yields. Phyllody, a disease caused by phytoplasmas—obligate intracellular pathogens similar to mycoplasmas—is of particular concern. These pleomorphic, wall-less microorganisms (0.2–0.8 µm in diameter) are phloem-limited and transmitted primarily by sap-sucking insect vectors from the families Cicadellidae (leafhoppers) and Fulgoridae (planthoppers). Unlike viruses, phytoplasmas are not transmitted through mechanical inoculation of plant sap but may also be propagated vegetatively via grafting (Babitha choudary *et al* 2016).

 Phyllody-infected plants typically exhibit floral malformations, partial or complete sterility, and severe reductions in yield. The incidence of phyllody in sunhemp fields across India has been reported to range between 10% and 100%, posing a significant threat to crop productivity. Comparable diseases in related crops, such as sesame, have been shown to cause yield losses of up to 34%, and even complete crop failure under severe infestation (Thirumalaswamy *et al.,*.2023).

 Traditional control measures, including tetracycline treatments, weed eradication, and application of systemic insecticides targeting vectors, have yielded limited success. In contrast, **the deployment of resistant genotypes** remains the most promising, economical, and sustainable strategy for long-term disease management.

 In light of the economic and agronomic significance of this disease, the present investigation was undertaken to **evaluate the resistance of mutagenized sunhemp genotypes (M₂ generation) to phyllody disease under natural field conditions**.

**MATERIALS AND METHODS**

**Screening of Sunhemp Genotypes for Resistance to Phyllody Disease Caused by Phytoplasma**

 A comprehensive screening of **44 sunhemp (*Crotalaria juncea* L.) genotypes** was undertaken to identify potential sources of resistance against **phyllody disease**, which is caused by phytoplasma infection. The lines are M2 lines, NTPSH-11-02 was exposed to gamma radiation at varying doses of 20 kR, 30 kR, 40 kR, and 50 kR to induce mutagenesis. The evaluation was conducted during the **Rabi season of 2024–25** under both **natural field conditions** and **protected conditions**. No chemical control measures or protective treatments were applied, thereby facilitating the natural buildup and transmission of the pathogen and its insect vectors to ensure maximum disease pressure. The assessment of disease severity was based on **percent disease incidence (PDI)**, a standard metric representing the proportion of infected plants within a given population. This was calculated using the formula provided by **Akhtar et al. (2013)**:

 Percent disease incidence = Number of plants infected in a row x 100

 Total number of plants in a row

 This approach allowed for an accurate and unbiased evaluation of the resistance levels exhibited by each genotype under high disease inoculum pressure. Observations recorded for field screeningof phyllody disease

**Symptomatological Assessment of Phyllody Disease**

 Symptom expression in sunhemp genotypes was meticulously recorded at key developmental stages, including the **vegetative**, **reproductive**, and **maturity** phases of crop growth. Observed disease manifestations included **plant dwarfing**, **leaf streaking**, **floral virescence** (green pigmentation of floral parts), **floral proliferation**, **bunchy top appearance**, and the characteristic **phyllody**, wherein floral structures are replaced by leafy structures.

**Temporal Monitoring of Disease Symptoms**

 Symptom expression on sunhemp plants was systematically monitored at distinct crop growth stages. Observations were recorded at **65, 75, 85, and 95 days after sowing (DAS)** to capture the progression and severity of phyllody disease over time.

**Disease Evaluation Methodology**

 Phyllody disease severity was assessed using a **standardized 0–6 disease scoring scale**, as proposed by **Akhtar et al. (2013)**. This scale enabled the systematic classification of symptom intensity across genotypes for reliable screening and comparison of resistance levels.

**List 1 :Disease scoring scale (0-6) for phyllody disease**

|  |  |  |
| --- | --- | --- |
| Rating | Percentage disease incidence (%) | Disease Reaction |
| 0 | No symptoms on any plant | Highly resistant |
| 1 | 0.1-10 | Resistant |
| 2 | 10.1-20 | Moderately resistant |
| 3 | 20.1-30 | Tolerant |
| 4 | 30.1-40 | Moderately susceptible |
| 5 | 40.1-50 | Susceptible |
| 6 | More than 50% | Highlysusceptible |

# RESULTS AND DISCUSSION

**Field Evaluation of Phyllody Disease Incidence in Sunhemp Genotypes (Rabi 2024–25)**

 During the **Rabi season of 2024–25**, the incidence of **phyllody disease** under natural field conditions was observed to range from **14.36% to 39.61%** across the evaluated genotypes. The occurrence of infection was scattered and the **disease progression remained relatively slow** throughout the cropping period. The **first symptomatic plant** was recorded at **65 days after sowing (DAS)**, with the **peak incidence observed at 95 DAS**.

 Among the screened genotypes, **NTPSH-63** and **NTPSH-32** exhibited **moderate resistance** to phyllody under natural field conditions (**Table 1**). These findings are in line with earlier reports by **Workishet T. et al. (2019)**, who identified **T-85** and **Argane** as moderately resistant genotypes in sesame. Furthermore, several genotypes—including **NTPSH-24, NTPSH-31, NTPSH-36, NTPSH-39, NTPSH-41, NTPSH-48, NTPSH-53, NTPSH-56**, and **NTPSH-60**—also demonstrated varying degrees of tolerance.

 In support of these observations, **Prasad *et al*. (2024)** reported **GTG-30**, **G-10-P1-P5-P3**, and **G-10-P1-P5-P6** as tolerant to phyllody in sesame. However, all other tested genotypes in the present study exhibited a **partial resistance to susceptible reaction** against sunhemp phyllody disease (**Table 1, Fig. 1**).

**Table 1.Incidence of sunhemp phyllody in different genotype under natural field condition.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| S.No. |  Genotype | NaturalCondition | Protected conditions |  | S. No. |  Genotype | Naturalcondition | Protected conditions |
| 1 | NTPSH -20 | 30.28 | 0 |  | 24 | NTPSH-43 | 33.54 | 0 |
| 2 | NTPSH -21 | 33.61 | 0 |  | 25 | NTPSH-44 | 37.62 | 0 |
| 3 | NTPSH-22 | 37.62 | 0 |  | 26 | NTPSH-45 | 36.38 | 0 |
| 4 | NTPSH-23 | 34.59 | 0 |  | 27 | NTPSH-46 | 34.59 | 0 |
| 5 | NTPSH-24 | 23.51 | 0 |  | 28 | NTPSH-47 | 38.62 | 0 |
| 6 | NTPSH-25 | 39.61 | 0 |  | 29 | NTPSH-48 | 29.41 | 0 |
| 7 | NTPSH-26 | 38.53 | 0 |  | 30 | NTPSH-49 | 34.39 | 0 |
| 8 | NTPSH-27 | 36.48 | 0 |  | 31 | NTPSH-50 | 36.35 | 0 |
| 9 | NTPSH-28 | 37.41 | 0 |  | 32 | NTPSH-51 | 37.42 | 0 |
| 10 | NTPSH-29 | 36.59 | 0 |  | 33 | NTPSH-52 | 36.31 | 0 |
| 11 | NTPSH-30 | 34.21 | 0 |  | 34 | NTPSH-53 | 27.69 | 0 |
| 12 | NTPSH-31 | 26.38 | 0 |  | 35 | NTPSH-54 | 37.69 | 0 |
| 13 | NTPSH-32 | 17.36 | 0 |  | 36 | NTPSH-55 | 38.32 | 0 |
| 14 | NTPSH-33 | 31.26 | 0 |  | 37 | NTPSH-56 | 28.41 | 0 |
| 15 | NTPSH-34 | 33.64 | 0 |  | 38 | NTPSH-57 | 36.34 | 0 |
| 16 | NTPSH-35 | 38.62 | 0 |  | 39 | NTPSH-58 | 37.61 | 0 |
| 17 | NTPSH-36 | 24.68 | 0 |  | 40 | NTPSH-59 | 36.35 | 0 |
| 18 | NTPSH-37 | 34.68 | 0 |  | 41 | NTPSH-60 | 29.67 | 0 |
| 19 | NTPSH-38 | 37.62 | 0 |  | 42 | NTPSH-61 | 34.35 | 0 |
| 20 | NTPSH-39 | 27.28 | 0 |  | 43 | NTPSH-62 | 37.61 | 0 |
| 21 | NTPSH-40 | 38.32 | 0 |  | 44 | NTPSH-63 | 14.36 | 0 |
| 22 | NTPSH-41 | 26.92 | 0 |  |  | *CD (5%)* | *0.67* | *0* |
|  23 | NTPSH-42 | 31.26 | 0 |  |  | *SE(m)* | *0.23* | *0* |

**Fig 1. Incidence of sunhemp phyllody in different genotype under natural field condition.**

**Impact of Phyllody Disease on Agronomic and Yield Parameters in Sunhemp**

 Phyllody-affected sunhemp plants exhibited significant reductions in key agronomic and yield-contributing traits compared to healthy counterparts. A marked decline was recorded in the number of pods, plant height, and seed yield of infected plants, as presented in Table 2, Table 5, and Figure 2 & 3, respectively. The number of pods per plant in diseased samples ranged from 18.41 to 93.48, plant height ranged from 128.99 to 178.37 cm, and seed yield ranged from 4.57 to 21.43 g, in contrast to 93.48 pods, 128.37 cm height, and 21.43 g seed yield observed in healthy plants.

 Additionally, the 100-seed weight (test weight) in phyllody-infected plants ranged from 1.476 to 2.645 g, substantially lower than the 3.273 g recorded in healthy plants. This reduction translated to an overall yield loss of 21.38% to 78.64%, which is directly attributable to the detrimental effects of phyllody.

 These findings are consistent with earlier reports. Ahmed et al. (2022) demonstrated that phytoplasma-infected sesame plants exhibited severely reduced water content, chlorophyll concentration, growth, and yield components, resulting in 37.9% and 42.5% reductions in seed and oil yields, respectively. Similarly, Yashowardhan Singh et al. (2023) reported that phyllody adversely affected yield-determining parameters in sesame, including plant height, number of capsules per plant, and test weight.

 Historical studies by Verma and Daftari (1985) and Kolte (1985) further confirmed up to 18% reduction in oil content in phyllody-infected sesame plants. In addition, Krishnaswamy and Jayarajan (1983) found that when approximately 25% of productive plant growth was transformed by phyllody, seed yield losses reached as high as 39.73%.

 These findings collectively underscore the severe impact of phyllody disease on plant development and yield, reinforcing the need for effective resistance breeding and integrated disease management strategies.

**Table 2. Effectof phyllody appearance at different growth stages on number of pods per plant (Mean of data collected from susceptible genotypes).**

|  |  |  |  |
| --- | --- | --- | --- |
| S.No | Age at symptoms appear (days) | Numberof pods (\*) | Reduction in no.of pods *over* check *(%)* |
| 1 | 65 days | 18.41 | 80.3 |
| 2 | 75 days | 36.52 | 60.94 |
| 3 | 85 days | 52.57 | 43.76 |
| 4 | 95 days | 72.47 | 22.47 |
| 5 | Healthy plant | 93.48 | 0 |

(\*)Average of five plants

**Fig 2. Effectofphyllody appearanceat different growth stages on number of pods per plant (Mean of data collected from susceptible genotypes).**

**Table 3. Effectof phyllody appearanceat different growth stages on seed yield per plant (Mean of data collected from susceptible genotypes).**

|  |  |  |  |
| --- | --- | --- | --- |
| S.No | Age at symptoms appear (days) | Seed yield (g) (\*) | Reduction in seed yield *over* check *(%)* |
| 1 | 65 days | 4.57 | *78.64* |
| 2 | 75 days | 8.5 | *60.32* |
| 3 | 85 days | 12.22 | *42.96* |
| 4 | 95 days | 16.84 | *21.38* |
| 5 | Healthy plant | 21.43 | 0 |

**Fig 3. Effectof phyllody appearanceat different growth stages on seed yield per plant (Mean of data collected from susceptible genotypes).**

**Table 4. Effectof phyllody appearanceat different growth stages on plant height (Mean of data collected from susceptible genotypes).**

|  |  |  |  |
| --- | --- | --- | --- |
| S.No | Age at symptoms appear (days) | Plant Height (\*)(Cm) | Reduction in height over check (%) |
| 1 | 65 days | 128.99 | 27.68 |
| 2 | 75 days | 150.3 | 15.73 |
| 3 | 85 days | 164.12 | 7.98 |
| 4 | 95 days | 173.14 | 2.93 |
| 5 | Healthy plant | 178.37 | 0 |

 (\*)Averageoffiveplants

**Fig 4. Effectof phyllody appearanceat different growth stages on plant height (Mean of data collected from susceptible genotypes).**

**Table 5. Effectof phyllody appearanceat different growth stages on test weight of seed (Mean of data collected from susceptible genotypes).**

|  |  |  |  |
| --- | --- | --- | --- |
| S.No | Age at symptoms appear (days) | Test weight (g)(\*) | Reductionin test weight over check (%) |
| 1 | 65 days | 1.476 | 54.900 |
| 2 | 75 days | 1.670 | 48.970 |
| 3 | 85 days | 2.175 | 33.540 |
| 4 | 95 days | 2.645 | 19.180 |
| 5 | Healthy plant | 3.273 | 0.000 |

 (\*)Averageoffiveplants

**Fig 5. Effectof phyllody appearanceat different growth stages on test weight of seed (Mean of data collected from susceptible genotypes).**

**CONCLUSION**

 Among the 44 sunhemp genotypes screened for resistance to phyllody disease under natural field conditions, disease incidence varied between 11.32% and 20.50%. No genotype exhibited complete resistance to the disease. However, two genotypes demonstrated a moderately resistant response, while nine genotypes were classified as tolerant. In contrast, all genotypes evaluated under protected conditions remained completely disease-free, confirming the effectiveness of vector exclusion in preventing infection.

 Phyllody disease had a pronounced negative impact on key yield-contributing traits in affected sunhemp plants. Critical parameters such as plant height, number of capsules per plant, and test weight (100-seed weight) were significantly reduced, underscoring the destructive nature of the disease and its influence on overall productivity.

**Future scope**

 **Further investigations into the epidemiological dynamics and mechanisms of disease transmission in sunhemp phyllody should be intensified to facilitate the development of effective and sustainable management strategies.**

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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|  **Fig 6a. Phyllody affected Sunhemp plant** | **Fig 6b. Healthy Sunhemp Plant** |

fect of Phytoplasma Associated with Sesame Phyllody on

Ultrastructural Modiﬁcation, Physio-Biochemical Traits,

Productivity and Oil Quality

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