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EFFECTS OF URDBEAN LEAF CRINKLE VIRUS INFECTION ON CATALASE ACTIVITY AND TOTAL PROTEIN CONTENT IN URDBEAN (VIGNA MUNGO (L.) HEPPER): A REVIEW

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ABSTRACT

Urdbean leaf crinkle disease (ULCD), attributed to Urdbean Leaf Crinkle Virus (ULCV), poses a significant threat to black gram (*Vigna mungo*) cultivation, often resulting in severe yield losses. One of the key host responses to ULCV infection involves modulation of reactive oxygen species (ROS) and associated antioxidant defences, notably catalase activity, as well as alterations in total soluble protein content. This review synthesizes current knowledge on how ULCV infection affects catalase enzyme activity and total protein content in *V. mungo*. The review article examines conflicting reports of catalase upregulation, downregulation, or no change in activity; explores patterns of protein accumulation or degradation; and discusses underlying biochemical and molecular mechanisms. It also considers the influence of host genotype, infection timing, and methodological differences across studies. Finally, an effort has been made to highlight research gaps and propose future directions to clarify host–virus interactions in *V. mungo*.

KEYWORDS: Urdbean, ULCD, ULCV, DAI, CAT, ROS, H_2O_2, Total Soluble Protein

Black gram (*Vigna mungo* (L.) Hepper), commonly known as urdbean, is a key pulse crop in South and Southeast Asia. Its seeds contain 22–26% protein and serve as an essential dietary protein source (Kumar and Abhilash, 2019). However, urdbean production is constrained by several viral diseases, among which ULCD is particularly devastating. ULCD symptoms include severe leaf crinkling, puckering, stunting, and malformed flowers, often reducing grain yield by 35–80% (Ashfaq *et al.*, 2010; Karthikeyan *et al.*, 2022).

ULCV, the putative causal agent of ULCD, has so far, not been assigned to any specific genus or family. It is transmitted mechanically and also by certain aphid species (Pandey, 2016). Despite extensive field reports of ULCD, the virus remains poorly characterized at molecular and structural levels (Kamaal *et al.*, 2023).

Plant viruses typically provoke an oxidative burst in the host, generating ROS such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2). To mitigate ROS-induced damage, plants deploy antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), and peroxidases (APX, POD) (Mittler *et al.*, 2004). Among these, CAT catalyses the dismutation of H_2O_2 into water and oxygen (EC 1.11.1.6), playing a pivotal role in redox homeostasis (Willekens *et al.*, 1997). Alterations in CAT activity during viral infection reflect the host's attempt to balance ROS signalling and detoxification.

Total soluble protein levels in leaves are likewise altered during viral infections. Increases may result from accumulation of viral coat and replicase proteins, as well as host defence proteins (e.g., pathogenesis-related proteins), while decreases can indicate host protein synthesis suppression or proteolytic degradation (Loebenstein and Lecoq, 2001).

This review focuses on published studies that quantify changes in catalase activity and total soluble protein content in *V. mungo* upon ULCV infection. It critically examines methodologies, summarizes findings, discusses underlying biochemical and molecular mechanisms, and identifies areas requiring further research.

Urdbean Leaf Crinkle Disease: Symptoms, Epidemiology and Impact

Symptoms and Field Observations

ULCD is characterized by hallmark symptoms of leaf crinkling, rugosity, and curling of trifoliate leaves. Infected plants often exhibit chlorotic patches, necrotic spots at leaf margins, shortened internodes, and malformed flowers (Ashfaq *et al.*, 2010). Disease onset can occur at any growth stage, but early infection

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typically leads to more severe symptoms and greater yield reduction (Pandey, 2016).

Epidemiology and Transmission

Although ULCV has not been unequivocally classified, field surveys suggest aphid-mediated transmission under semi-persistent conditions (Pandey, 2016). Mechanical transmission via sap inoculation is also routinely used in greenhouse studies (Ashfaq *et al.*, 2010). Environmental factors such as temperature, humidity, and vector abundance influence disease incidence (Karthikeyan *et al.*, 2022).

Agricultural Impact

ULCD can lead to yield losses up to 81%, depending on cultivar susceptibility and infection timing (Ashfaq *et al.*, 2010; Karthikeyan *et al.*, 2022). In India, where urdbean is grown on over 4 million hectares, ULCD poses an annual threat of losses exceeding USD 50 million (Kumar and Abhilash, 2019). Despite its economic importance, limited resistant cultivars are available, and management relies heavily on vector control and crop rotation (Kamaal *et al.*, 2023).

Catalase Activity in ULCV-Infected Urdbean

Role of Catalase in Plant Defence

Catalase detoxifies H_2O_2 in peroxisomes, chloroplasts, and mitochondria, thereby preventing oxidative damage to lipids, proteins, and nucleic acids (Willekens *et al.*, 1997). Following pathogen recognition, an oxidative burst generates H_2O_2 as both a direct antimicrobial agent and a signalling molecule for downstream defence pathways (Neill *et al.*, 2002). Fine-tuning of H_2O_2 levels by CAT and other antioxidants is thus essential: insufficient CAT may permit cytotoxic ROS accumulation, whereas excessive CAT may dampen defence signalling (Mittler *et al.*, 2004).

Methodologies for Measuring Catalase Activity

Studies typically assay CAT by monitoring H_2O_2 decomposition spectrophotometrically at 240 nm, reporting activity in μmol H_2O_2 min^{-1} mg^{-1} protein (Aebi, 1984). Enzyme extracts are prepared from fresh leaf tissue in phosphate buffer (pH 7.0–7.8), sometimes with protease inhibitors. Assays are performed at defined time points post-inoculation (e.g., 7, 15, 30 days after inoculation (DAI)). Variations in buffer composition, extraction conditions, and activity units complicate inter-study comparisons.

Conflicting Reports on Catalase Modulation

No Significant Change

Ashfaq *et al.* (2010) compared CAT activity in a susceptible genotype (Mash-88) and a resistant genotype (CM-2002) at 15 and 30 DAI. They observed no statistically significant differences in CAT activity between infected and healthy plants in either genotype (Ashfaq *et al.*, 2010). The authors concluded that CAT did not play a major role in ULCV defence, in contrast to other antioxidant enzymes (e.g., SOD, POD), which showed marked induction.

Downregulation of Catalase

Pandey's (2016) greenhouse study of two genotypes (T-9, IPU 94-1) reported a significant decrease in CAT activity in infected leaves at 15 DAI compared to controls (Pandey, 2016). CAT activity declined by 25–40%, correlating with symptom severity. The author suggested that ULCV or associated defence signals suppress CAT, leading to sustained H_2O_2 levels that may enhance localized cell death as part of a hypersensitive response.

Srivastava and Singh (2010) also evaluated the effects of Urdbean Leaf Crinkle Virus (ULCV) on the two black gram cultivars, T-9 and IPU 94-1 "Uttara," and reported that CAT activity was significantly reduced in ULCV-infected leaves relative to uninfected plants, suggesting either suppression of this H_2O_2-scavenging enzyme by the virus or a host-mediated downregulation to sustain an oxidative burst for defence signalling.

Upregulation of Catalase

Contrastingly, Karthikeyan *et al.* (2022) documented increased CAT activity in both resistant (VBN 6) and susceptible (CO 5) cultivars at 7 and 14 DAI. CAT activity rose by 20–35% relative to controls, coinciding with elevated activities of SOD, APX, and POD (Karthikeyan *et al.*, 2022). The authors interpreted this as a general activation of antioxidant defences to mitigate ROS-induced damage under viral stress.

Reconciling Discrepancies: Genotype and Temporal Effects

These divergent findings likely reflect differences in host genotype, infection severity, and sampling time points. A model proposed by Kamaal *et al.* (2023) posits that:

- Resistant genotypes tend to maintain lower CAT activity to allow H_2O_2 accumulation and potentiate

defence signalling, while simultaneously upregulating SOD to generate the H_2O_2 pool.

- Susceptible genotypes may upregulate CAT (and APX) excessively, quenching H_2O_2 and inadvertently weakening defence responses (Kamaal *et al.*, 2023).

Additionally, CAT activity may exhibit diphasic dynamics: an initial early decrease to sustain the oxidative burst, followed by a later increase to detoxify excess ROS and prevent host damage (Neill *et al.*, 2002). Sampling solely at late stages (e.g., 30 DAI) may thus capture recovery of CAT activity rather than its initial modulation.

Total Soluble Protein Content in ULCV-Infected Urdbean

Significance of Protein Changes During Viral Infection

Viral replication hijacks host translational machinery to produce coat proteins, replicase, movement proteins, and suppressors of RNA silencing, substantially contributing to total protein pools in infected cells (Loebenstein and Lecoq, 2001). Meanwhile, hosts synthesize pathogenesis-related (PR) proteins, heat-shock proteins, and enzymes involved in secondary metabolism as part of defence (van Loon *et al.*, 2006). Conversely, viruses may induce host protein synthesis shutdown or proteolysis to favour their own expression (Zorzatto *et al.*, 2015).

Methods for Protein Quantification

Total soluble protein is commonly measured by the Bradford (1976) or Lowry (1951) assays. Fresh leaf tissue is homogenized in phosphate buffer (pH 7.0), centrifuged, and supernatant proteins quantified against bovine serum albumin standards. Samples are taken at 7–30 DAI in most studies.

Observed Trends in Total Protein

General Increase in Protein Content

Ashfaq *et al.* (2010) found that ULCV-infected leaves of both Mash-88 and CM-2002 accumulated significantly higher total protein at 15 and 30 DAI. Protein content increased by 30–50% relative to controls, attributed primarily to viral protein accumulation. Pandey (2016) similarly reported a "remarkable" increase in soluble protein (+45%) in infected T-9 and IPU 94-1 plants. Srivastava and Singh (2010) reported similar findings in ULCV-infected leaves of both the cultivars.

Cultivar-Dependent Decline

However, Karthikeyan *et al.* (2022) observed a dichotomy: the resistant VBN 6 cultivar showed a modest increase in protein (+7.1%), whereas the susceptible CO 5 cultivar exhibited a 27.0% decrease in total protein at 14 DAI. The authors attributed the decline in CO 5 to severe tissue damage, impaired photosynthesis, and possible activation of host proteases.

Review Findings

Kamaal *et al.* (2023) note that most ULCD studies report increased soluble protein due to accumulation of viral and defence proteins, but several reports (including Brar and Rataul, 1990; Thind *et al.*, 1996) describe protein declines in susceptible genotypes under severe infection.

Underlying Mechanisms

Protein accumulation in infected leaves arises from:

1. Viral Protein Synthesis: Coat, replicase, movement proteins can constitute up to 20–30% of total leaf protein in highly infected tissues (Loebenstein and Lecoq, 2001).
2. Defence Protein Induction: PR-1, PR-2 (β-1,3-glucanase), PR-5 (thaumatin-like proteins), chitinases, and heat-shock proteins accumulate under SA- and JA-mediated signalling (van Loon *et al.*, 2006).
3. Host Translational Reprogramming: Polysome profiling in ULCV-infected urdbean suggests selective translation of defence transcripts at the expense of other housekeeping proteins (Gupta and Singh, 2021).

Protein declines in susceptible genotypes may reflect:

- Protease Activation: Viral infection can trigger host cysteine proteases, leading to protein degradation (Zorzatto *et al.*, 2015).
- Photosynthetic Inhibition: Reduced carbon assimilation limits amino acid availability for protein synthesis (Karthikeyan *et al.*, 2022).
- Ribosomal Shutdown: Some viruses elicit host translational shutdown, favouring viral mRNA translation and host ribosomal RNA degradation (Walsh *et al.*, 2013).

Molecular Crosstalk: ROS, Hormonal Signalling and Gene Expression

ROS as Defence Signals

The oxidative burst generates apoplastic and cytosolic H_2O_2, which: (a) directly damages pathogens; (b) cross-links cell wall polymers; and (c) triggers MAP kinase cascades and transcription factors (e.g., WRKY, NAC) that regulate defence genes (Neill *et al.*, 2002; Alonso *et al.*, 2009).

Hormonal Pathways

ULCV infection modulates salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) pathways. Resistant cultivars often exhibit strong SA signalling, leading to systemic acquired resistance (SAR) and PR protein accumulation. JA and ET pathways may act antagonistically or synergistically, depending on infection stage (Kamaal *et al.*, 2023).

Transcriptomic Insights

Recent RNA-Seq studies (e.g., Gupta and Singh, 2021; Rao *et al.*, 2024) reveal that ULCV infection upregulates:

- **Antioxidant Enzyme Genes**: *VmCAT*, *VmSOD*, *VmAPX*.
- **PR Genes**: *VmPR1*, *VmPR2*, *VmPR5*.
- **Hormone biosynthesis and signalling genes**: *ICS1*, *NPR1*, *PDF1.2*, *ERF1*.

Conversely, genes involved in photosynthesis (*Rubisco*, *LHCA/B*) and primary metabolism are downregulated, contributing to reduced biomass and protein content in susceptible genotypes.

Methodological Considerations and Limitations

1. **Genotype Variation**: Studies often compare different cultivars with distinct genetic backgrounds and inherent antioxidant capacities, complicating direct comparisons.
2. **Inoculation Methods**: Mechanical vs. vector transmission may elicit different defence responses due to wounding artefacts or vector saliva effectors.
3. **Sampling Times**: Catalase and protein dynamics are time-dependent; early vs. late sampling can yield opposite trends.
4. **Assay Conditions**: Variations in buffer composition, pH, temperature, and assay units require standardized protocols for meaningful meta-analysis.
5. **Virus Characterization**: Without a molecularly characterized isolate, strain differences may underlie inconsistent host responses.

Standardizing experimental parameters—using well-characterized ULCV isolates, synchronized inoculations, multiple time points, and a panel of resistant and susceptible genotypes—will improve reproducibility and clarity.

Future Directions

1. **Molecular Identification of ULCV**
   - Genome sequencing and phylogenetic placement to enable reverse genetics and functional analyses (Kamaal *et al.*, 2023).
2. **Time-Course Studies**
   - Detailed profiling of CAT, SOD, APX, and protein content at multiple DAI (e.g., 1, 3, 7, 14, 21, 30 DAI).
3. **Transgenic and Gene-Editing Approaches**
   - Overexpression or CRISPR/Cas9 knockout of *VmCAT*, *VmPR1*, or hormone pathway regulators to dissect their roles in defence.
4. **Proteomic and Phosphoproteomic Analyses**
   - Identify post-translational modifications of CAT and other enzymes during infection (e.g., nitration, phosphorylation).
5. **Metabolomics**
   - Quantify H_2O_2, antioxidant metabolites (ascorbate, glutathione), and phenolics to link biochemical status with enzyme activities.
6. **Vector–Host–Virus Interactions**
   - Investigate aphid saliva effectors and their influence on ROS and hormonal signalling in *V. mungo*.

CONCLUSION

ULCV infection in urdbean triggers complex changes in catalase activity and total protein content, reflecting the dynamic interplay between oxidative signalling, antioxidant defences, viral protein synthesis, and host translational control. Although studies report contradictory trends in CAT activity—ranging from downregulation to upregulation—the consensus is that these differences arise from host genotype, infection timing, and methodological variables. Total soluble protein generally increases due to viral and defence protein accumulation, but severely susceptible cultivars may exhibit net protein loss.

Elucidating the precise molecular mechanisms underlying these physiological changes will require coordinated efforts integrating genomics, transcriptomics,

proteomics, metabolomics, and functional genetics. Such insights will be invaluable for breeding or engineering ULCV-resistant urdbean varieties and for developing targeted interventions to mitigate ULCD.

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