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Multivariate physiological indices explain greengram yield response under conservation agriculture and nitrogen management practices in wheat-greengram-maize system

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Abstract

Residual productivity of summer greengram (*Vigna radiata* L. Wilczek) in cereal-pulse-cereal rotations depend strongly on nitrogen availability carried over from preceding cereal crops. A two-year field experiment (2021-22 to 2022-23) was conducted at ICAR-Indian Agricultural Research Institute, New Delhi, to evaluate whether synchronised nitrogen management in wheat and maize, combined with conservation agriculture (CA) practices, could sustain greengram performance without direct nitrogen application. Treatments comprised three crop establishment methods—conventional tillage, zero tillage with residue retention, and permanent raised beds with residue retention—and seven nitrogen management practices imposed only on wheat and maize, including graded basal nitrogen levels with foliar supplementation using prilled urea or nano urea. Greengram growth, physiology, nitrogen assimilation, biomass accumulation, and yield attributes were recorded, and multivariate relationships were analysed using principal component analysis and hierarchical clustering.

The first two principal components explained over 97% of the total variation, with canopy greenness indices (SPAD, NDVI), leaf nitrogen concentration, nitrogen uptake, and dry matter accumulation contributing most strongly to treatment discrimination. Nitrogen-efficient cereal treatments with synchronised basal and foliar nitrogen supply resulted in superior chlorophyll retention, biomass accrual, pod formation, and grain yield of greengram under residual conditions. In contrast, treatments lacking nitrogen synchronisation showed poor physiological expression despite CA-induced soil moderation. Cluster analysis clearly separated nitrogen-sufficient and nitrogen-deficient treatment groups. The study demonstrates that synchronised nitrogen management in cereals, combined with residue-retained CA practices, establishes physiological synchrony that sustains greengram productivity without direct fertiliser application, offering a viable strategy to improve nitrogen-use efficiency and sustainability of cereal-pulse rotations in semi-arid environments.

Keywords: Conservation agriculture, greengram, hierarchical clustering, nitrogen management, PCA, physiological synchrony

Introduction

Greengram (*Vigna radiata* L. Wilczek) is a key short-duration pulse crop in cereal-based cropping systems of the Indo-Gangetic Plains, contributing to dietary protein security, biological nitrogen fixation, and system diversification during the summer season (Giller 2001; Thiyagarajan *et al.* 2025) ^[9, 22]. Its inclusion between two nitrogen-intensive cereals such as wheat and maize improves system resilience, soil biological activity, and nutrient cycling; however, greengram productivity remains highly unstable because of its shallow root system, short growth duration, and strong dependence on residual soil fertility (Berg 1997; Kadam *et al.* 2022) ^[2, 14]. In intensive cereal-pulse-cereal rotations, inefficient nitrogen management in preceding crops frequently results in sub-optimal residual nitrogen availability, which constrains early canopy development, physiological vigour, and subsequent yield formation of greengram (Govaerts *et al.* 2009; Rashid *et al.* 2019) ^[10, 19].

Conservation agriculture (CA), founded on minimal soil disturbance, residue retention, and diversified crop rotations, has been widely promoted to improve soil structure, moisture

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conservation, and nutrient cycling in cereal-based systems (Hobbs *et al.* 2008; Kassam *et al.* 2019) ^[11, 15]. Residue-retained CA systems enhance soil organic matter turnover, microbial activity, and nitrogen mineralisation while reducing nitrogen losses through erosion and volatilisation, thereby improving nitrogen availability to succeeding crops (Bhattacharyya *et al.* 2021; Ladha *et al.* 2020) ^[3, 18]. Nevertheless, CA alone may not fully compensate for inadequate nitrogen supply in preceding cereals, particularly in short-duration pulses such as greengram, where early nitrogen availability governs rapid canopy establishment before effective nodulation and biological nitrogen fixation become functional (Berg 1997; Kadam *et al.* 2022) ^[2, 14].

Recent advances in nitrogen management practices, including reduced basal nitrogen rates combined with synchronised foliar applications of prilled urea or nano urea, have demonstrated significant improvements in nitrogen-use efficiency and nitrogen synchrony in cereals (Upadhyay *et al.* 2023a; Kumar *et al.* 2024) ^[16, 23]. Such strategies alter residual nitrogen dynamics by improving nitrogen uptake efficiency, reducing losses, and enhancing internal nitrogen recycling, thereby creating favourable physiological conditions for succeeding crops without direct fertiliser application (Devi *et al.* 2025) ^[5]. Greengram grown solely on residual fertility thus serves as an effective bio-indicator for assessing upstream nitrogen synchronisation within cereal-pulse-cereal cropping systems.

Conventional univariate approaches often fail to capture the complex interactions among physiological, biochemical, and reproductive traits governing yield formation under residual fertility conditions. Multivariate analytical tools such as principal component analysis (PCA) and hierarchical clustering on principal components (HCPC) enable integration of multiple traits and identification of dominant physiological drivers regulating productivity and nitrogen responsiveness (Sadras and Lemaire 2014; Kumar *et al.* 2025) ^[17, 20]. However, information remains limited on how multivariate physiological indices explain greengram yield response under combined CA and nitrogen management strategies in cereal-pulse-cereal systems.

The present study was therefore undertaken to evaluate the multivariate physiological and agronomic responses of summer greengram grown under conservation agriculture and nitrogen management practices applied to preceding wheat and maize. Specifically, the study aimed to determine whether nitrogen-regulated physiological synchrony established during cereal phases governs greengram performance and whether multivariate discrimination can identify nitrogen-efficient treatment combinations capable of sustaining greengram productivity without direct nitrogen inputs.

Materials and Methods

A two-year field experiment was conducted during Rabi 2021-22 to Kharif 2023 at the Research Farm of ICAR-Indian Agricultural Research Institute, New Delhi (28°38'N, 77°09'E; 228 m amsl). The region experiences a semi-arid subtropical climate with hot summers, cool winters, and mean annual rainfall of about 1,015 mm, predominantly received during July-August, typical of the western Indo-Gangetic Plains (Gathala *et al.* 2019) ^[8]. Pre-sowing soil analysis (0-15 cm) indicated sandy loam texture, alkaline reaction (pH 7.9), low organic carbon (0.43%), low available nitrogen (224.5 kg ha⁻¹), and medium available phosphorus (19.0 kg ha⁻¹) and potassium (246.2 kg ha⁻¹), determined following standard soil analytical procedures (Jackson 1973) ^[13].

The experiment was laid out in a split-plot design with three replications. Crop establishment methods—conventional tillage (CT), zero tillage with residue retention (ZT+R), and permanent raised beds with residue retention (PB+R)—were assigned to main plots, while nitrogen management practices were allotted to sub-plots. Crop residues (~3 t ha⁻¹) were retained in ZT+R and PB+R but removed in CT, consistent with the principles of conservation agriculture emphasizing minimal soil disturbance and residue retention (FAO 2016; Kassam *et al.* 2019) ^[15]. Crops were grown sequentially as wheat (Rabi) → greengram (summer) → maize (Kharif) in both years to assess residual nitrogen effects within a cereal-pulse-cereal rotation (Govaerts *et al.* 2009) ^[10].

Seven nitrogen management treatments were imposed only on wheat and maize: N₁ (0 N), N₂ (100% recommended dose of nitrogen, RDN), N₃ (75% RDN), N₄ (75% RDN + one foliar spray of 2% prilled urea), N₅ (75% RDN + one foliar spray of nano urea), N₆ (50% RDN + two foliar sprays of 2% prilled urea), and N₇ (50% RDN + two foliar sprays of nano urea). The RDN was 150 kg N ha⁻¹ for wheat and 180 kg N ha⁻¹ for maize, applied as per regional recommendations. Foliar nitrogen sprays were scheduled at critical growth stages to enhance nitrogen synchrony and improve nitrogen-use efficiency, as reported in earlier nitrogen management studies (Adhikari *et al.* 2020; Dabhi *et al.* 2025) ^[1, 4]. No nitrogen fertiliser was applied to greengram to enable evaluation of residual soil nitrogen availability generated through preceding cereal management (Berg 1997) ^[2]. Wheat variety HD 3226, greengram PUSA 1431, and maize hybrid PJHM-1 were sown using recommended agronomic practices.

Greengram observations included leaf area index measured using a canopy analyser (LAI-2200C), chlorophyll index using a SPAD-502 Plus meter, canopy vigour using a GreenSeeker NDVI sensor, dry matter accumulation after oven drying, leaf nitrogen concentration determined by the Kjeldahl method, nitrogen uptake computed from biomass and nitrogen concentration, yield attributes, and seed yield, following standard physiological and analytical procedures (Jackson 1973; Evans 1989) ^[6, 13]. Data were analysed using analysis of variance appropriate for a split-plot design, with mean separation at $p < 0.05$. Multivariate analyses, including principal component analysis (PCA) and hierarchical clustering on principal components (HCPC), were performed using R software with FactoMineR and factoextra packages to integrate physiological, nitrogen, and yield-related traits (Husson *et al.* 2010) ^[12].

Results and Discussion

Nitrogen management practices imposed on the preceding wheat and maize crops exerted a pronounced influence on the physiological expression, nitrogen assimilation, and yield formation of residual summer greengram grown under conservation agriculture (CA). Despite the absence of direct nitrogen fertilisation to greengram, substantial variability was observed in canopy greenness, dry matter accumulation, and reproductive traits across nitrogen management practices, indicating strong carry-over effects of fertiliser nitrogen synchronisation and residue-mediated mineralisation under CA-based cropping systems. These responses confirm that greengram performance in cereal-pulse-cereal rotations are governed largely by upstream nitrogen availability and its temporal synchrony rather than by in-season nitrogen supply alone (Berg 1997; Kadam *et al.* 2022) ^[2, 14].

Table 1: Eigenvalues and contributions of first two principal components in greengram.

Principal component	Eigenvalue	Explained variance (%)	Cumulative variance (%)	Major contributing traits
PC1	10.12	92.03	92.03	SPAD, NDVI, chlorophyll content, leaf N concentration, N uptake, dry matter, LAI
PC2	0.55	4.99	97.02	Pods plant ⁻¹ , pod length, seeds pod ⁻¹ , test weight

Note: Values derived from multivariate trait matrix comprising physiological, nitrogen and reproductive attributes. Principal component analysis revealed that the first two principal components together accounted for more than 97% of the total variation among measured physiological, nitrogen, and yield attributes (Table 1; Fig. 1). The dominance of PC1, explaining 92.03% of the total variance, reflects a highly consolidated physiological axis regulated primarily by nitrogen availability. Traits with strong positive loadings on PC1 included SPAD values, chlorophyll concentration, NDVI, leaf nitrogen concentration, total nitrogen uptake, and dry matter accumulation, demonstrating a tight coupling between nitrogen status, photosynthetic capacity, and biomass production. This indicates that residual nitrogen availability governs canopy establishment and physiological efficiency in greengram, particularly during early vegetative stages when nodulation and biological nitrogen fixation are still developing (Giller 2001; Sadras and Lemaire 2014) [9, 20]. In contrast, PC2 explained only 4.99% of the total variation and was primarily associated with reproductive traits such as pods per plant, seeds per pod, pod length, and test weight, suggesting that yield differentiation along this axis was influenced by assimilate partitioning and sink strength during later growth stages rather than by nitrogen acquisition percentage.

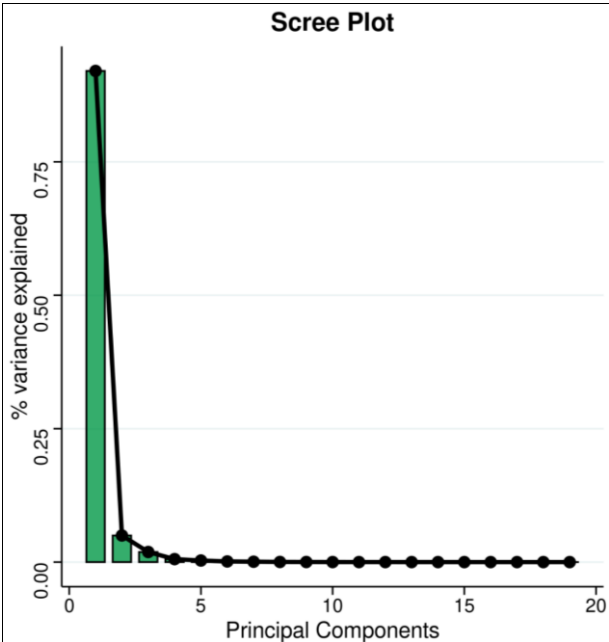


Fig 1: Scree plot showing percentage of total explained variance by principal components in greengram grown (mean of two years).

The correlation structure among traits further clarified these relationships (Fig. 2). Strong positive associations were observed among canopy greenness indices (SPAD and NDVI), leaf nitrogen concentration, nitrogen uptake, and dry matter accumulation, confirming that nitrogen-driven chlorophyll stability and photosynthetic efficiency underpin greengram growth under residual fertility conditions. These findings align with earlier reports that nitrogen availability stabilises

chloroplast structure, prolongs leaf functional duration, and enhances carbon assimilation in short-duration legumes (Evans 1989; Tegeder 2014) [6, 21]. Pod-related traits exhibited moderate correlations with physiological attributes, indicating that while nitrogen availability establishes the physiological ceiling for yield formation, reproductive efficiency and assimilate remobilisation ultimately determine the extent to which this potential is realised. Such decoupling between vegetative vigour and reproductive output is common in greengram under heat-prone summer environments, where sink limitation and flower shedding can constrain yield despite adequate early growth (Kumar *et al.* 2025) [17].

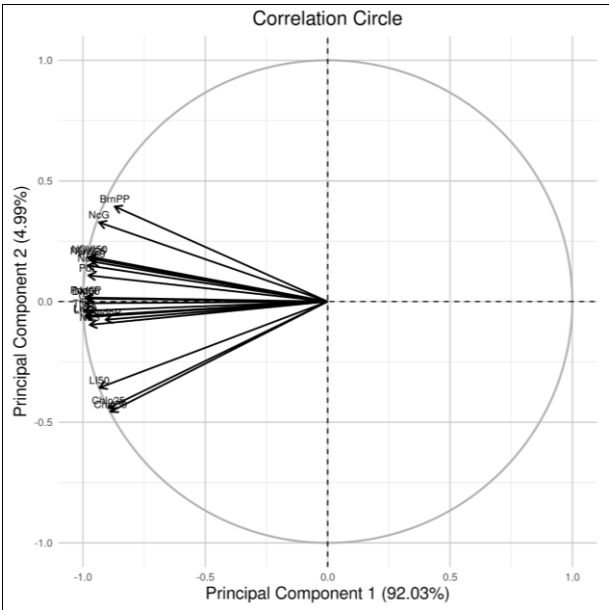


Fig 2: Correlation circle illustrating associations among physiological and yield-related traits contributing to treatment differentiation under residual greengram cultivation.

Clear discrimination among nitrogen management treatments was evident in the PCA biplot (Fig. 3). Nitrogen-adequate and synchronised treatments, namely N₂ (100% RDN), N₅ (75% RDN + nano urea), N₄ (75% RDN + prilled urea), and N₃ (75% RDN), clustered along the positive PC1 axis, corresponding to higher SPAD values, NDVI, leaf nitrogen concentration, nitrogen uptake, dry matter accumulation, and yield-attributing traits. These treatments benefitted from improved residual nitrogen availability and sustained nitrogen release through crop residue decomposition, which collectively enhanced canopy vigour and photosynthetic efficiency during the critical early growth stages of greengram. In contrast, treatments lacking adequate nitrogen supply or effective synchronisation (N₁, N₆, and N₇) were positioned along the negative PC1 axis, reflecting poor chlorophyll stability, reduced biomass accumulation, and inferior reproductive performance. These results confirm that synchronised nitrogen management in cereals exerts strong downstream effects on greengram physiology and yield formation, even in the absence of direct fertilisation (Govaerts *et al.* 2009; Rashid *et al.* 2019) [10, 19].

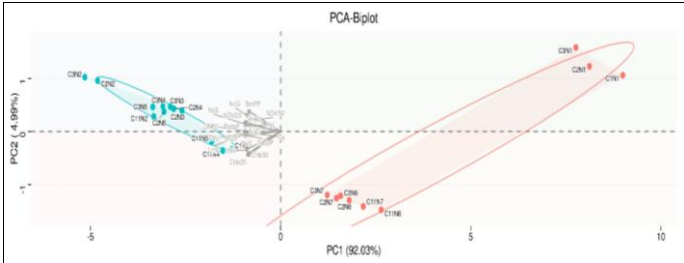


Fig 3: PCA biplot showing treatment discrimination based on multivariate trait expression of greengram.

Hierarchical clustering on principal components grouped the treatments into two statistically distinct clusters (Table 2; Fig. 4). Cluster I, comprising N₂, N₅, N₄, and N₃, recorded significantly higher cluster means for chlorophyll concentration, NDVI, nitrogen uptake, dry matter accumulation, pods per plant, and grain yield. This cluster represents nitrogen-efficient treatment combinations where moderate reductions in basal nitrogen application to cereals were effectively compensated through synchronised foliar nitrogen interventions, resulting in favourable residual fertility conditions for greengram. Notably, the inclusion of reduced basal nitrogen treatments within this cluster demonstrates the potential for nitrogen savings in cereal phases without compromising pulse productivity, provided nitrogen synchrony is maintained (Upadhyay *et al.* 2023a; Nayak *et al.* 2025) [23]. In contrast, Cluster II, consisting of N₁, N₆, and N₇, consistently exhibited inferior physiological and yield performance, highlighting that CA alone cannot overcome nitrogen deficiency in short-duration greengram when residual nitrogen availability is inadequate.

Table 2: Cluster-wise mean values of key physiological and yield attributes of residual greengram derived from hierarchical clustering on principal components (HCPC)

Trait	Cluster I (N ₂ , N ₅ , N ₄ , N ₃)	Cluster II (N ₁ , N ₆ , N ₇)
SPAD value	45.8 ^a	38.6 ^b
NDVI (0-1)	0.78 ^a	0.63 ^b
Chlorophyll content (mg g ⁻¹ FW)	3.46 ^a	2.71 ^b
Leaf nitrogen (%)	3.12 ^a	2.36 ^b
N uptake (kg ha ⁻¹)	72.5 ^a	54.8 ^b
Dry matter (g plant ⁻¹)	39.7 ^a	31.2 ^b
Pods plant ⁻¹	29.4 ^a	21.7 ^b
Seed yield (kg ha ⁻¹)	978 ^a	746 ^b

*Means followed by different superscript letters within a row differ significantly at *p*<0.05 (LSD). Cluster I represents nitrogen-efficient combinations; Cluster II represents nitrogen-limited combinations. *

The combined multivariate evidence indicates that residual nitrogen availability, regulated by synchronised nitrogen management in cereals, is the dominant driver of greengram productivity under CA-based wheat-greengram-maize systems. While conservation agriculture enhances soil moisture retention, moderates soil temperature, and promotes gradual nitrogen mineralisation through residue decomposition, its beneficial effects are amplified only when combined with appropriate nitrogen management strategies (Bhattacharyya *et al.* 2021; Ladha *et al.* 2020) [3, 18]. Physiological traits such as SPAD, NDVI, and leaf nitrogen concentration emerged as reliable indicators of residual nitrogen sufficiency and were closely linked to biomass accumulation and yield formation, supporting their use as diagnostic tools for evaluating nitrogen synchrony in CA systems.

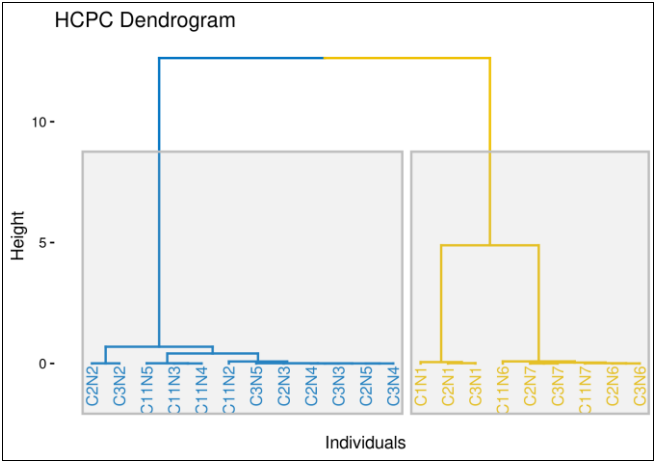


Fig 4: Hierarchical clustering dendrogram of nitrogen management treatments derived from PCA scores indicating performance grouping under residual greengram conditions.

Residual greengram productivity in wheat-greengram-maize rotations is governed primarily by nitrogen-regulated physiological synchrony established during preceding cereal crops, while conservation agriculture provides complementary moderation of soil and microclimatic conditions. Multivariate analysis confirmed that synchronised nitrogen management in cereals sustains canopy greenness, nitrogen assimilation, biomass accumulation, and pod development in greengram under residual fertility. These findings indicate that combining residue-retained conservation agriculture with synchronised nitrogen strategies in cereals offers a practical pathway to reduce fertiliser inputs without compromising greengram productivity, thereby improving nitrogen-use efficiency and sustainability of cereal-pulse systems in semi-arid environments.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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