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SOYBEAN RESPONSE TO SIMULATED DEFOLIATION DURING VEGETATIVE AND REPRODUCTIVE STAGES

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Abstract

Defoliation is a well-studied stress factor; however, its stage-specific effects on seed yield and seed composition under field conditions remain insufficiently clarified. This study investigated soybean responses to simulated defoliation applied at two developmental stages (V1 and R1) and three intensities (33%, 67%, and 100%) under field conditions in Novi Sad, Serbia. Physiological, vegetative, and generative traits, as well as seed protein and oil content and their yields, were analyzed to evaluate relationships among stress intensity, developmental stage, and plant productivity.

The results showed a pronounced stage-dependent response. Defoliation at V1 resulted in high physiological stability and partial maintenance of biomass and yield components, indicating strong early-stage tolerance. In contrast, R1 defoliation had a stronger negative effect on yield formation, with complete defoliation (100%) significantly reducing photosynthetic activity, vegetative stability, and seed yield. Moderate defoliation at R1 (67%) resulted in the highest seed yield and consequently the highest protein and oil yields, despite reductions in yield components. This indicates a non-linear, threshold-dependent response of soybean to reproductive-stage defoliation. Importantly, protein and oil concentrations remained stable across all treatments, indicating that changes in protein and oil yields were driven primarily by variation in seed yield rather than changes in seed composition. Multivariate analysis revealed strong associations among yield-related traits, while vegetative traits contributed less to treatment differentiation.

The main contribution of this study lies in the integrated assessment of stage-specific soybean responses to defoliation, including seed yield, protein yield, and oil yield in cultivar 'Fortuna' under temperate continental field conditions. These findings indicate that developmental stage and defoliation intensity are key determinants of soybean stress tolerance and may support genotype selection and agronomic optimization under increasing climatic variability.

Key words: *Glycine max*, Regenerative potential, Seed yield, Simulated defoliation, Stress tolerance

Introduction

Soybean (*Glycine max* L.) is one of the most important leguminous crops worldwide, valued for its high nutritional quality. It is rich in proteins, essential amino acids, oils, and bioactive compounds, making it an important source of nutrition for both humans and livestock (Sebastião, 2024). In addition to its nutritional role, soybean contributes to sustainable agriculture through biological nitrogen fixation, which improves soil fertility and reduces the need for synthetic fertilizers (Ciampitti *et al.,* 2021). Its versatility enables wide industrial use, including production of plant-based milk, oil, flour, and protein concentrates, thereby supporting the growing demand for plant-based foods (Pandey *et al.,* 2024). Due to its broad adaptability to different agroecological conditions, soybean is cultivated globally and plays a key role in food security and rural economic development (Ray *et al.,* 2013).

During early developmental stages, soybean is highly sensitive to environmental stress, which can negatively affect growth, morphological development, and yield formation. This period includes seedling establishment, early root development, and the onset of photosynthetic activity (Kodadinne *et al.,* 2024). Among abiotic stress factors, mechanical damage caused by hailstorms is particularly severe in climatically unstable regions. Although short in duration, such events can cause substantial injury to young tissues, leading to structural damage and physiological dysfunction (Vollmer and Rahman, 2020; Zhang *et al.,* 2023). Consequences include reduced photosynthetic area, impaired assimilate transport, loss of turgor, and inhibition of meristematic activity, ultimately affecting pod formation, seed weight, and assimilate allocation to reproductive organs (Bukan *et al.,* 2024; Du *et al.,* 2020).

In addition to abiotic stress, soybean is exposed to biotic damage caused by phytophagous insects such as lepidopteran larvae, aphids (*Aphididae*), and thrips (*Thysanoptera*). These pests reduce leaf area through defoliation, tissue damage, and sap extraction, resulting in decreased photosynthetic efficiency and increased susceptibility to secondary infections (Hesler *et al.,* 2018). In addition, insect feeding can disrupt hormonal balance, particularly of auxins, cytokinins, and ethylene, thereby altering plant stress responses (Hesler & Beckendorf, 2021).

Despite its sensitivity during early growth, soybean possesses a notable capacity for recovery after defoliation. This recovery is achieved through activation of meristematic tissues, metabolic adjustments, and reorganization of photosynthetic processes (Tiwari *et al.,* 2023). The extent of recovery depends on the developmental stage and severity

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of damage. When the apical meristem remains intact, plants can initiate new leaf formation and lateral branching, enabling partial compensation of biomass loss (Parvej *et al.,* 2025). Recovery is further supported by antioxidant defense mechanisms, hormonal regulation, and adaptive changes in photosynthetic efficiency of remaining leaves (Li & Chen, 2023; Wang *et al.,* 2022).

In this context, early-maturing genotypes have a shorter vegetative period, which may limit the time available for regeneration and compensatory growth after defoliation compared with later-maturing genotypes.

In this study, simulated defoliation was used as a standardized approach to mimic mechanical injury and enable controlled evaluation of soybean responses to reduced leaf area. This method ensures experimental reproducibility and allows precise assessment of physiological and morphological responses at different developmental stages.

The objective of this study was to investigate soybean responses to simulated defoliation applied at two developmental stages: early vegetative stage (V1) and onset of flowering (R1). The study evaluates physiological, morphological, yield, and seed quality traits, with the aim of determining how defoliation intensity and developmental stage influence recovery capacity and productivity under early-season stress conditions.

The novelty of this study lies in the integrated assessment of physiological, morphological, yield, and seed quality responses of soybean cv. 'Fortuna' under simulated defoliation at two developmental stages (V1 and R1) and three defoliation intensities (33%, 67%, and 100%), conducted under temperate continental agroecological conditions of northern Serbia.

Material and Metods

Location and climatic characteristics: The experiment was conducted during the 2021 and 2022 growing seasons at the Rimski sancevi experimental field, part of the Institute of Field and Vegetable Crops in Novi Sad, Serbia. The site is located at 45°20′N latitude and 19°51′E longitude, in the northern part of the Republic of Serbia. The area is characterized by a temperate continental climate, with pronounced seasonal variations in temperature and precipitation. Summer periods at the site are marked by high temperatures and dry intervals, while spring often brings storms accompanied by hail, which can cause mechanical damage to above ground plant organs, particularly during early developmental stages. These climatic risks form the basis for designing experimental treatments that simulate leaf area loss, such as simulated defoliation, with the aim of assessing the plant's physiological response to stress under realistic agroecological conditions. Climatic data (average monthly temperatures and precipitation for the April–September period) were obtained from the database of the Republic Hydrometeorological Service of Serbia (RHMSS, 2025), for the Novi Sad station (Fig. 1). Comparison with the long-term average (1964–2020) enables evaluation of deviations from standard agroclimatic conditions during the years of experimentation, which is essential for interpreting the physiological response of the plant in the context of actual environmental conditions. The stability of agroecological characteristics at the site allows the results to be applicable in broader agronomic contexts.

The soil at the experimental site belongs to the chernozem type, formed on loess parent material, with a clay-loam texture. It is characterized by low carbonate content and a neutral pH reaction, making it highly suitable for soybean cultivation. These properties provide stable agroecological conditions for conducting field experiments, with minimal variation in the soil's physico-chemical characteristics throughout the growing season.

Experimental design: The experiment was conducted under rainfed conditions, without artificial irrigation, with maize (*Zea mays* L.) as the preceding crop. Fertilization was applied in two clearly defined stages. The first stage was carried out in autumn, prior to primary soil tillage, using a compound NPK fertilizer (15:15:15) at a rate of 200 kg ha^{-1}, supplying 30 kg ha^{-1} of nitrogen. The second stage was implemented in spring, immediately before sowing, as part of pre-sowing soil preparation, with an additional 34 kg ha^{-1} of nitrogen applied via ammonium nitrate (34% N). No significant occurrence of pathogenic organisms or pest infestation was recorded during the growing season, allowing undisturbed plant development and ensuring the reliability of physiological and morphological assessments. The trial was arranged in a split-split plot design with three replications per treatment. Each experimental treatment consisted of three replications, while ten plants per replication were randomly selected from the central rows of each plot to avoid edge effects and ensure representative sampling. In total, 30 plants per treatment were analyzed, which is consistent with standard practices in soybean field experiments under a split-split plot design. This sampling structure provides sufficient statistical power to detect treatment effects and ensures the reliability of the results. Each plot covered an area of 5 × 3 meters (six rows), while the total experimental area was 27 × 50 meters. The soybean cultivar Fortuna (maturity group 00) was selected for its high lodging tolerance and stable yield performance under stress-prone conditions. A sowing density of 550,000 plants per hectare was applied, in accordance with recommended agronomic practices for this cultivar, enabling consistent evaluation of its response to simulated defoliation and other applied treatments.

Defoliation treatments: Simulated defoliation treatments were applied at two clearly defined developmental stages of soybean growth, based on the classification of Fehr & Caviness (1977): the vegetative stage V1 (formation of the first trifoliate leaf) and the reproductive stage R1 (beginning of flowering). At each stage, three levels of leaf removal were implemented: 33%, 67%, and 100%. Leaf removal was defined at the level of individual trifoliate leaves: at 33%, the central leaflet was removed; at 67%, the two lateral leaflets were removed; and at 100%, the entire trifoliate leaf (all three leaflets) was removed. The petiole was retained in all treatments to preserve nodal structure and prevent additional mechanical damage. Defoliation was performed manually using sterilized scissors, during morning hours under stable weather conditions, to minimize stress variability and ensure reproducibility of experimental conditions. Simple (unifoliate) leaves, which emerge shortly after cotyledons, were excluded from leaf area assessment and were not removed during treatment. According to recent studies, these leaves exhibit limited

photosynthetic activity and physiological relevance during the V1 and R1 stages, and their senescence occurs naturally in early vegetative development (Mun *et al.,* 2021; Zhang *et al.,* 2024). Therefore, simulated defoliation focused exclusively on trifoliate leaves, which constitute the dominant photosynthetic surface of the plant during the analyzed stages. This methodology enables standardized simulation of mechanical stress and precise assessment of physiological and morphological responses as influenced by developmental stage and defoliation intensity.

Measurement protocol for physiological parameter: Simulated defoliation was applied at two clearly defined developmental stages of soybean: V1 (formation of the first trifoliate leaf) and R1 (beginning of flowering). Physiological measurements were scheduled to capture key stage of plant development, with temporal spacing relative to treatment application, allowing for the assessment of leaf area loss effects on biomass regeneration and photosynthetic capacity. Physiological parameters were measured once, at the onset of the R2 stage (beginning of bloom formation), when all treatments had been completed and plants exhibited differentiated responses depending on developmental stage and defoliation intensity. The following indicators were analyzed at this stage: total fresh plant weight (TFW), residual trifoliate leaf weight (RTLW), and residual trifoliate leaf area (RTLA). TFW was determined after carefully uprooting the plants and mechanically removing soil residues. TFW and RTLW were measured using a high-precision analytical balance. RTLA was quantified using ImageJ software, which enables digital surface analysis based on leaf photographs. Leaves were photographed against a monochromatic background with a calibration marker of known dimensions. The software detects leaf contours based on contrast and calculates surface area in cm² using the calibration scale. This method provides precise, repeatable, and non-invasive leaf area estimation with high reliability in quantitative analysis. Leaf Area Index (LAI) was measured at two additional developmental stages to evaluate the dynamics of leaf area regeneration following defoliation. The first LAI measurement was conducted during the R3 stage (pod formation), when compensatory mechanisms are expected to activate in plants treated at R1. The second LAI measurement was performed during the R5 stage (beginning of seed filling), to assess the persistence of regeneration and its contribution to photosynthetic capacity prior to the final stages of development. LAI measurements were conducted using the LAI-2200C Plant Canopy Analyzer (LI-COR Biosciences, Lincoln, NE, USA), which operates on the principle of diffuse light transmission through the plant canopy. The device records the ratio of light above and below the canopy and calculates effective leaf area per unit ground surface based on detection angle and leaf geometry. Measurements were taken in the morning under diffuse light conditions to minimize interference from direct solar radiation. Each plot was measured at multiple points, and LAI values were expressed as the mean per treatment.

Morphological trait assessment prior to harvest: Ten soybean plants per replication were selected from the central area of each plot to minimize edge effects and ensure sample representativeness. Morphological measurements were performed manually using standard protocols immediately prior to harvest.

The following vegetative traits were recorded: total plant weight (PW), plant height (PH), stem diameter (SD), first pod height (FPH), and number of lateral branches (NLB). Reproductive traits included: number of nodes (NN), number of fertile nodes (NNP), number of pods (NP), pod weight (WP), number of seeds (NS), and seed yield per plant (SY).

Harvesting was conducted using a small-plot combine harvester (Wintersteiger), and seed yield was standardized to 14% moisture content, in accordance with agronomic norms for soybean.

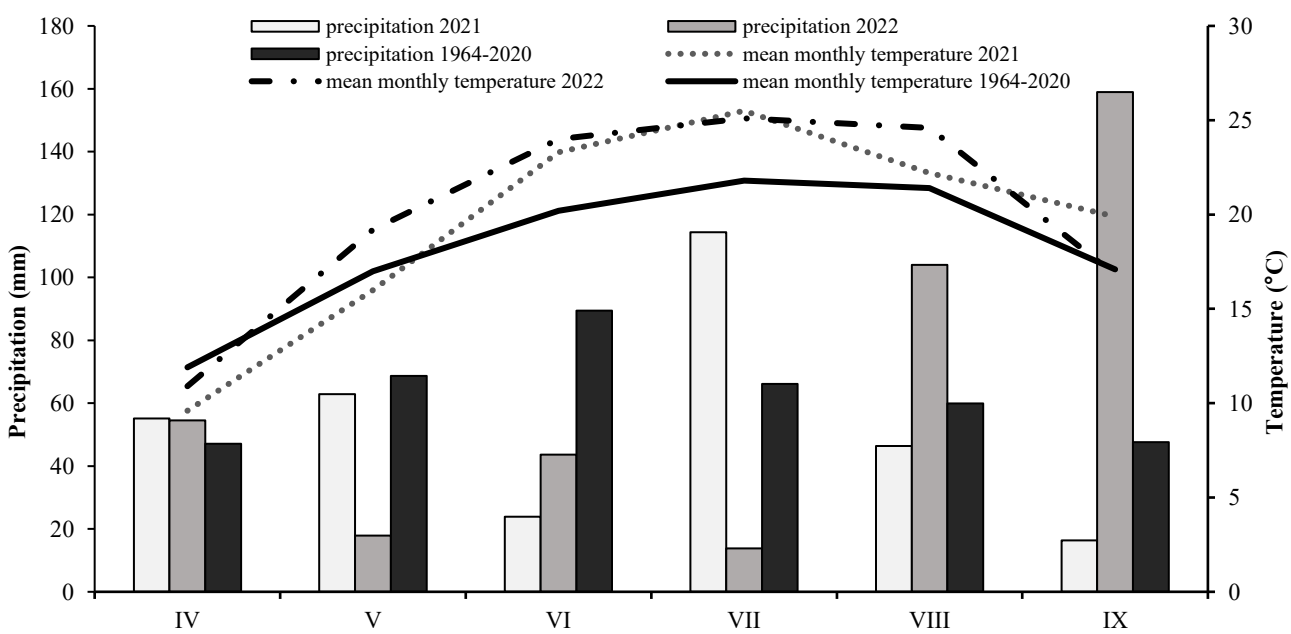

Fig. 1. Average monthly temperatures and precipitation during the growing season in the period from 2021 to 2022 (RHMSS, 2025).

Chemical analysis of seeds and calculation of protein and oil yield: The chemical composition of soybean seeds was analyzed to determine protein and oil content using nuclear magnetic resonance (NMR), following the standardized protocol described by Granlund & Zimmerman (1975). Analyses were conducted on homogenized seed samples using a calibrated NMR analyzer, which enables rapid, non-destructive quantification of seed constituents. Results were expressed as a percentage of dry matter.

To calculate protein yield (PY) and oil yield (OY) per hectare, the following formulas were applied:

$$PY\ (kg\ ha^{-1}) = Seed\ yield\ (kg\ ha^{-1}) \times Protein\ content\ (\%) / 100$$

$$OY\ (kg\ ha^{-1}) = Seed\ yield\ (kg\ ha^{-1}) \times Oil\ content\ (\%) / 100.$$

Statistical data analysis

All data were analyzed using analysis of variance (ANOVA) to evaluate the effects of simulated defoliation across different developmental stages. Mean comparisons were performed using Tukey's HSD test at a significance level of $p \leq 0.05$.

Multivariate analysis was applied to vegetative and generative traits to identify parameters with the highest contribution to overall variability. Results were visualized using biplot diagrams to enable interpretation of relationships between treatments and examined traits.

To assess the interaction between years and defoliation treatments, the AMMI model was used. AMMI analysis of variance and AMMI1 biplot diagrams were generated using GenStat software (VSN International, Hemel Hempstead, UK).

Pearson's correlation coefficients were calculated for all examined traits using Microsoft Excel, based on the standard formula for linear relationships between quantitative parameters. Only the most significant correlations are presented in the manuscript, highlighting key physiological and generative responses to simulated defoliation.

Results

Physiological response of soybean to simulated defoliation: The physiological response of soybean to simulated defoliation was assessed using four quantitative indicators: total fresh plant weight (TFW), residual trifoliate leaf weight (RTLW), residual trifoliate leaf area (RTLA), and leaf area index (LAI). Data are presented for two consecutive growing seasons, with comparisons across treatments applied at vegetative (V1) and reproductive (R1) developmental stages. Table 1 summarizes the effects of each treatment on biomass retention and leaf area regeneration following defoliation.

Total fresh plant weight (TFW): In 2021, the TFW of soybean under the control treatment was 70.7 g. Treatments involving simulated defoliation at the V1 maintained relatively high TFW, particularly V1 67% (66.3 g) and V1 100% (67.1 g), while V1 33% showed a slightly lower value (60.6 g). Among treatments applied at the R1, R1 33% exhibited a comparable TFW (60.05 g), whereas R1 67% (23.7 g) and R1 100% (13.2 g) resulted in a pronounced reduction. In 2022, the control treatment recorded a TFW of 65.2 g. V1 treatments with simulated defoliation retained moderate values: V1 33% (56.4 g), V1 67% (61.1 g), and V1 100% (62.0 g). R1 33% showed a similar TFW (54.3 g), while R1 67% (21.4 g) and R1 100% (11.8 g) again exhibited substantial reductions.

Residual trifoliate leaf weight (RTLW): In 2021, the highest RTLW was recorded in the control treatment (34.6 g) and in the V1 100% simulated defoliation treatment (34.1 g). Moderate values were observed in V1 33% (30.7 g) and V1 67% (29.9 g). Treatments applied at the R1 showed a progressive decline: R1 33% (19.9 g), R1 67% (6.1 g), while R1 100% resulted in complete removal of trifoliate leaves (0 g). In 2022, the highest RTLW was again recorded in the control (31.8 g) and V1 100% (30.7 g) treatments. V1 33% (27.6 g) and V1 67% (26.9 g) maintained moderate values. R1 treatments exhibited a similar pattern of reduction: R1 33% (17.6 g), R1 67% (5.4 g), and complete leaf removal in R1 100% (0 g).

Residual trifoliate leaf area (RTLA): In 2021, the control treatment exhibited the highest RTLA, reaching 2191.35 cm^2. Treatments involving simulated defoliation at the V1 retained comparable leaf areas: V1 67% (1810.33 cm^2) and V1 100% (1832.33 cm^2), while V1 33% showed a reduced value (1479.01 cm^2). Treatments applied at the R1 demonstrated a progressive decline: R1 33% (1325.07 cm^2), R1 67% (509.54 cm^2), and complete elimination of trifoliate leaf area in R1 100% (0 cm^2). In 2022, the control treatment recorded an RTLA of 1985.20 cm^2. V1 67% (1642.50 cm^2) and V1 100% (1652.90 cm^2) maintained similar values, while V1 33% showed a reduced area (1341.10 cm^2). R1 treatments again exhibited a declining trend: R1 33% (1187.40 cm^2), R1 67% (456.30 cm^2), and complete leaf area removal in R1 100% (0 cm^2).

Leaf area index (LAI): In 2021, LAI measured at the R3 stage (pod formation) was highest in the control treatment (4.58) and in R1 treatments with simulated defoliation: V1 67% (4.33) and V1 100% (4.53). V1 33% showed a slightly lower value (4.18). R1 treatments exhibited a declining trend: R1 33% (3.94), R1 67% (3.41), and R1 100% (3.19). LAI measured at the R5 stage (beginning of seed filling) remained relatively stable in V1 treatments: V1 33% (3.64), V1 67% (3.84), while V1 100% showed a significant reduction (2.13). R1 treatments recorded lower values: R1 33% (2.33), R1 67% (2.23), and R1 100% (2.82), likely due to partial redistribution of residual leaf area. In 2022, LAI at the R3 stage was again highest in the control (4.42) and V1 treatments: V1 33% (4.18), V1 67% (4.38), and V1 100% (4.21). R1 treatments showed a similar declining pattern: R1 33% (3.79), R1 67% (3.21), and R1 100% (3.02). At the R5 stage, LAI remained stable in V1 33% (3.21), V1 67% (3.43), while V1 100% showed a marked reduction (1.92). R1 treatments recorded lower values: R1 33% (2.11), R1 67% (2.01), and R1 100% (2.61), again reflecting partial redistribution of leaf area.

Vegetative response of soybean to simulated defoliation: The vegetative response of soybean to simulated defoliation was assessed using five vegetative indicators: plant weight (PW), plant height (PH), stem diameter (SD), first pod height (FPH), and number of lateral branches (NLB). Data are presented for two consecutive growing seasons, with comparisons across treatments applied at vegetative (V1) and reproductive (R1) developmental stages. Table 2 summarizes the effects of each treatment on structural traits relevant to biomass allocation and canopy architecture following defoliation.

Table 1. Physiological response of soybean to simulated defoliation showing strong stage-dependent tolerance at V1 and severe reduction under high-intensity R1 defoliation.

Year	Treatment	TFW (g)	RTLW (cm^2)	RTLA (cm^2)	LAI at R3	LAI at R5
2021	Control	70.7a	34.6a	2191.35a	4.58a	3.81a
	V1 33%	60.6ab	30.7ab	1479.01b	4.53a	3.84a
	V1 67%	66.3a	29.9ab	1810.33a	4.48ab	3.64ab
	V1 100%	67.1a	34.1a	1832.33ab	4.33ab	2.13c
	R1 33%	60.05ab	19.9bc	1325.07bc	3.94b	2.33bc
	R1 67%	23.7c	6.1cd	509.54cd	3.51c	2.23bc
	R1 100%	13.2d	0.0d	0.0d	3.19d	2.23bc
	Average	**51.96a**	**22.47a**	**1163.95a**	**4.08a**	**2.83a**
2022	Control	65.2a	31.8a	1985.20a	4.42a	3.52a
	V1 33%	56.4ab	27.6ab	1341.10ab	4.38a	3.43a
	V1 67%	61.1ab	26.9ab	1642.50ab	4.33a	3.21ab
	V1 100%	62.0ab	30.7a	1652.90ab	4.18ab	1.92d
	R1 33%	54.3ab	17.6bc	1187.40bcd	3.79bc	2.11cd
	R1 67%	21.4cd	5.4cd	456.30de	3.36cd	2.01cd
	R1 100%	11.8d	0.0d	0.0e	3.02d	2.61abc
	Average	**47.46b**	**20.05b**	**1180.20a**	**3.84b**	**2.42b**

Different letters within each column indicate statistically significant differences among treatments (Tukey's HSD test, $p \leq 0.05$). TFW – total fresh plant weight; RTLW – residual trifoliate leaf weight; RTLA – residual trifoliate leaf area; LAI at R3 – leaf area index measured at the pod formation stage (R3); LAI at R5 – leaf area index measured at the beginning of seed filling stage (R5)

Table 2. Vegetative traits show compensatory growth at V1 and branching increase under R1 stress.

Year	Treatment	PW	PH	SD	FPH	NLB
2021	Control	21.80ab	82.80a	0.62a	16.77abcd	1.13bcd
	V1 33%	20.27ab	82.53a	0.59ab	18.93ab	0.73d
	V1 67%	18.08b	81.03a	0.53abc	18.30ab	1.07bcd
	V1 100%	24.81a	83.00a	0.61a	14.93cd	1.07bcd
	R1 33%	21.37ab	83.23a	0.58ab	17.97abc	1.27b
	R1 67%	22.39ab	83.87a	0.54abc	19.40a	1.23b
	R1 100%	22.56ab	83.77a	0.58ab	19.70a	2.13a
	Average	**21.61a**	**82.89a**	**0.58a**	**18.00a**	**1.23a**
2022	Control	19.63ab	70.38b	0.55abc	15.70bcd	0.83bcd
	V1 33%	18.25ab	70.15b	0.50abcd	18.13abc	0.77cd
	V1 67%	16.27b	68.88b	0.40de	17.13abcd	1.13bcd
	V1 100%	22.52ab	66.40b	0.46cde	14.27d	1.17bcd
	R1 33%	19.24ab	66.59b	0.41de	17.03abcd	1.17bcd
	R1 67%	20.15ab	67.09b	0.38e	18.37ab	1.20bc
	R1 100%	20.31ab	67.01b	0.38e	18.27ab	1.87a
	Average	**19.48a**	**68.07b**	**0.44b**	**16.99a**	**1.16a**

Different letters within each column indicate statistically significant differences among treatments (Tukey's HSD test, $p \leq 0.05$). PW – plant weight; PH – plant height; SD – stem diameter; FPH – first pod height; NLB – number of lateral branches

Plant weight (PW): In 2021, the control treatment recorded a PW of 21.80 g. The highest value was observed in V1 100% (24.81 g), while the lowest was in V1 67% (18.08 g). Treatments applied during the R1 showed stable values ranging from 21.37 g (R1 33%) to 22.56 g (R1 100%), indicating no deviation from the control. In 2022, the control recorded 19.63 g. V1 100% again showed the highest value (22.52 g), while V1 67% recorded the lowest (16.27 g). R1 treatments remained within a narrow range (19.24–20.31 g), comparable to the control.

Plant height (PH): In 2021, PH was uniform across treatments, ranging from 81.03 cm (V1 67%) to 83.87 cm (R1 67%), with the control at 82.80 cm. No significant differences were observed. In 2022, the control measured 70.38 cm. V1 treatments ranged from 66.40 cm (V1 100%) to 70.15 cm (V1 33%), while R1 treatments ranged from 66.59 cm (R1 33%) to 67.09 cm (R1 67%). All treatments showed slightly lower values than the control, without statistical significance.

Stem diameter (SD): In 2021, the control recorded a SD of 0.62 cm. V1 67% had the lowest value (0.53 cm), while V1 33% and V1 100% were close to the control (0.59 cm and 0.61 cm). R1 treatments ranged from 0.54 cm (R1 67%) to 0.58 cm (R1 33% and R1 100%), showing no major deviation. In 2022, the control measured 0.55 cm. V1 67% recorded the lowest value (0.40 cm), while V1 33% and V1 100% measured 0.50 cm and 0.46 cm, respectively. R1 treatments showed reductions: R1 33% (0.41 cm), R1 67% and R1 100% (0.38 cm).

First pod height (FPH): In 2021, the control recorded a FPH of 16.77 cm. V1 100% had the lowest value (14.93 cm), while V1 33% and V1 67% showed slightly higher values (18.93 cm and 18.30 cm). R1 treatments exceeded

the control, with R1 100% reaching 19.70 cm. In 2022, the control measured 15.70 cm. V1 100% again showed the lowest value (14.27 cm), while V1 33% and V1 67% recorded 18.13 cm and 17.13 cm, respectively. R1 treatments showed increased values: R1 33% (17.03 cm), R1 67% (18.37 cm), and R1 100% (18.27 cm).

Number of lateral branches (NLB): In 2021, the control recorded 1.13 NLB. V1 33% showed a reduction (0.73), while V1 67% and V1 100% were similar to the control (1.07). R1 treatments showed increased branching, with R1 100% reaching 2.13, and R1 33% and R1 67% recording 1.27 and 1.23, respectively. In 2022, the control recorded 0.83 branches. V1 33% had the lowest value (0.77), while V1 67% and V1 100% showed increases (1.13 and 1.17). R1 treatments showed enhanced branching: R1 33% (1.17), R1 67% (1.20), and R1 100% (1.87).

Generative response of soybean to simulated defoliation: The generative response of soybean to simulated defoliation was evaluated using four yield-related indicators: number of pods per plant (NP), pod weight per plant (WP), number of seeds per plant (NS), and seed yield (SY). Data are presented for two consecutive growing seasons, with comparisons across treatments applied at vegetative (V1) and reproductive (R1) developmental stages. Table 3 summarizes the effects of each treatment on reproductive traits relevant to yield formation and allocation efficiency following leaf area reduction.

Number of nodes (NN): In 2021, the control treatment recorded 14.33 NN. V1 treatments showed minimal variation: 14.40 (V1 33%), 14.00 (V1 67%), and 14.00 (V1 100%). R1 treatments remained close to the control: 14.40 (R1 33%), 14.13 (R1 100%), and 14.20 (R1 67%). All treatments maintained nodal stability relative to the control. In 2022, the control treatment recorded 12.33 NN. V1 treatments showed minimal variation: 12.47 (V1 33%), 12.33 (V1 67%), and 12.13 (V1 100%). R1 treatments remained close to the control: 12.40 (R1 33%), 12.20 (R1 67%), and 12.13 (R1 100%). All treatments preserved nodal integrity, with no significant deviations from the control.

Number of nodes with pods (NNP): In 2021, the control treatment recorded 10.50 NNP. V1 treatments showed slight reductions: 10.30 (V1 33%), 10.33 (V1 67%), and 10.23 (V1 100%). R1 treatments ranged from 10.53 (R1 33%) to 9.93 (R1 100%), with R1 67% recording 10.10. Most treatments remained within a range close to the control, indicating preserved pod-setting capacity despite defoliation. In 2022, the control treatment recorded 8.60 NNP. V1 treatments ranged from 8.80 (V1 33%) to 9.23 (V1 100%), with V1 67% showing 8.97. R1 treatments varied slightly: 9.60 (R1 100%), 9.10 (R1 33%), and 8.83 (R1 67%). All values remained within a range close to the control, indicating preserved pod-setting capacity under simulated defoliation.

Number of pods (NP): In 2021, the control treatment recorded 34.00 NP. V1 treatments showed moderate reductions: 31.37 (V1 33%), 30.23 (V1 67%), and 29.07 (V1 100%). R1 treatments exhibited more pronounced declines: 30.60 (R1 33%), 26.20 (R1 100%), and 24.83 (R1 67%). In 2022, the control recorded 27.13 NP. V1 treatments ranged from 21.63 (V1 33%) to 22.67 (V1 100%), while R1 treatments showed lower values: 23.00 (R1 33%), 18.83 (R1 100%), and 18.63 (R1 67%).

Pod weight (WP): In 2021, WP in the control was 17.59 g. V1 treatments ranged from 15.50 g (V1 67%) to 16.46 g (V1 33%), with V1 100% at 16.27 g. R1 treatments showed more substantial reductions: 15.88 g (R1 33%), 13.15 g (R1 67%), and 12.67 g (R1 100%). In 2022, the control recorded 14.04 g. V1 treatments ranged from 13.47 g (V1 67% and V1 100%) to 13.93 g (V1 33%). R1 treatments showed further declines: 13.13 g (R1 33%), 12.17 g (R1 67%), and 11.20 g (R1 100%).

Number of seeds (NS): In 2021, the control recorded 72.43 NS. V1 treatments ranged from 64.43 (V1 100%) to 71.67 (V1 33%), while R1 treatments showed lower values: 63.77 (R1 33%), 55.23 (R1 100%), and 53.57 (R1 67%). In 2022, the control recorded 63.10 seeds. V1 treatments ranged from 53.43 (V1 100%) to 59.37 (V1 33%), while R1 treatments showed further reductions: 52.43 (R1 33%), 49.57 (R1 67%), and 48.57 (R1 100%).

Seed yield (SY): In 2021, the control recorded a SY of 3648 kg ha^{-1}. V1 treatments showed slight increases: 3858 kg ha^{-1} (V1 33%), 3760 kg ha^{-1} (V1 67%), and 3736 kg ha^{-1} (V1 100%). R1 treatments exhibited divergent responses: R1 67% recorded the highest SY (4528 kg ha^{-1}), while R1 100% showed a reduction (3225 kg ha^{-1}). In 2022, the control recorded 3317 kg ha^{-1}. V1 treatments ranged from 3567 kg ha^{-1} (V1 100%) to 3617 kg ha^{-1} (V1 33%), with V1 67% at 3575 kg ha^{-1}. R1 67% again showed the highest SY (4483 kg ha^{-1}), while R1 100% recorded the lowest (3075 kg ha^{-1}).

Multivariate analysis of soybean traits under simulated defoliation: The biplot illustrates the spatial distribution of defoliation treatments applied at V1 and R1 stages during the 2021 and 2022 growing seasons, in relation to principal components capturing variability in vegetative and generative traits (Fig. 2). Axes 1 and 2 jointly explain the majority of total variance in the dataset. Treatment positioning reveals a distinct separation by year and defoliation intensity. Treatments from 2021 cluster near vectors representing SY, NS, PW, and FPH, whereas treatments from 2022—particularly R1 100% and R1 67%—are located opposite to these vectors. Trait vectors SY, NS, PW, and FPH are aligned in the same direction, indicating a strong positive association among these parameters. Vectors for SD, PH, and NLB form smaller angles relative to SY, suggesting moderate correlation, while NNP and NP define a distinct axis, implying a separate contribution to overall variability. Treatments with higher defoliation intensity at the reproductive stage in 2022, especially R1 100%, are positioned furthest from the SY and NS vectors, consistent with reduced yield performance observed in quantitative data. In contrast, the R1 67% treatment from 2021 is located closer to the generative trait vectors, suggesting a more stable physiological response under those conditions.

Table 3. Reproductive traits show developmental-stage-dependent responses to defoliation with yield compensation under moderate conditions.

Year	Treatment	NN	NNP	NP	WP	NS	SY
2021	Control	14.33a	10.50a	34.00a	17.59a	72.43a	3648abcd
	V1 33%	14.40a	10.30abc	31.37ab	16.46ab	71.67ab	3858abcd
	V1 67%	14.00ab	10.33ab	30.23ab	15.50abcd	64.93abc	3760abcd
	V1 100%	14.20a	10.23abc	29.07abc	16.27ab	64.43abcd	3736abc
	R1 33%	14.40a	10.53a	30.60ab	15.88abc	63.77abcd	4104abc
	R1 67%	14.20a	10.10abcd	24.83bcde	13.15def	53.57abcd	4528a
	R1 100%	14.13a	9.93abcde	26.20bcd	12.67ef	55.23cdef	3225cd
	Average	**14.24a**	**10.28a**	**29.47a**	**15.33a**	**64.00a**	**3837a**
2022	Control	12.33c	8.60e	27.13abcd	14.04bcde	63.10abcde	3317cd
	V1 33%	12.47bc	8.80de	21.63de	13.93bcde	59.37abcde	3617bcd
	V1 67%	12.33c	8.97bcde	22.60cde	13.47cdef	58.63bcdef	3575cd
	V1 100%	12.13c	9.23abcde	22.67cde	13.47cdef	53.43cdef	3567cd
	R1 33%	12.40bc	9.10abcde	23.00cde	13.13def	52.43def	3916abcd
	R1 67%	12.20c	8.83cde	18.63e	12.17ef	49.57ef	4483ab
	R1 100%	12.13c	9.60abcde	18.83e	11.20f	48.57f	3075d
	Average	**12.29b**	**9.02b**	**22.07b**	**13.06b**	**55.01b**	**3650a**

Different letters within each column indicate statistically significant differences among treatments (Tukey's HSD test, $p \leq 0.05$). NN – number of nodes; NNP– number of nodes with pods; NP – number of pods; WP – pod weight; NS – number of seeds; SY – seed yield

Table 4. Seed composition, protein yield, and oil yield of soybean under simulated defoliation.

Treatment	Year							
	2021				2022			
	P (%)	PY (kg ha^{-1})	O (%)	OY (kg ha^{-1})	P (%)	PY (kg ha^{-1})	O (%)	OY (kg ha^{-1})
Control	37.10a	1353abcd	18.91a	690abcd	36.95a	1226bc	18.90a	627bc
V1 33%	36.97a	1427abc	18.95a	729abc	37.00a	1338abc	18.91a	684abc
V1 67%	37.07a	1394abc	18.98a	710abc	36.90a	1319abc	18.89a	675abc
V1 100%	37.10a	1386abc	18.89a	706abc	36.97a	1319abc	18.89a	674abc
R1 33%	36.87a	1513ab	18.77a	770ab	37.25a	1459ab	18.90a	740ab
R1 67%	36.99a	1675a	18.91a	856a	36.99a	1659a	18.89a	847a
R1 100%	37.10a	1196cd	18.89a	609cd	36.81a	1132c	18.90a	581c
Average	**37.03a**	**1421a**	**18.90a**	**724a**	**36.98a**	**1352b**	**18.90a**	**690b**

Different letters within each column indicate statistically significant differences among treatments (Tukey's HSD test, $p \leq 0.05$). P – protein content; PY – protein yield; O – oil content; OY – oil yield

Table 5. Statistically significant trait correlations in soybean (Pearson's r) under simulated defoliation

	SY	NS	WP	NP	PW	FPH	PY	OY
SY	1	0.91	0.88	0.86	0.85	0.76	0.89	0.86
NS	0.91	1	0.84	0.87	0.78	0.72	0.82	0.80
WP	0.88	0.84	1	0.83	0.79	0.75	0.81	0.78
NP	0.86	0.87	0.83	1	0.76	0.70	0.79	0.77
PW	0.85	0.78	0.79	0.76	1	0.79	0.77	0.75
FPH	0.76	0.72	0.75	0.70	0.79	1	0.74	0.72
PY	0.89	0.82	0.81	0.79	0.77	0.74	1	0.81
OY	0.86	0.80	0.78	0.77	0.75	0.72	0.81	1

Presented correlations are statistically significant at the $p<0.05$ level
PW – plant weight; FPH – first pod height; NP – number of pods; WP – pod weight; NS – number of seeds; SY – seed yield; PY – protein yield; OY – oil yield

Seed composition response of soybean to simulated defoliation: The compositional response of soybean seeds to simulated defoliation was evaluated using protein content, protein yield (PY), oil content, and oil yield (OY). Data are presented for two consecutive growing seasons, with comparisons across treatments applied at vegetative (V1) and reproductive (R1) developmental stages. Table 4 summarizes the effects of simulated defoliation on seed composition and derived yield-quality traits.

Protein and oil concentrations remained relatively stable across all treatments in both growing seasons. Protein content ranged from 36.87% to 37.10% in 2021 and from 36.81% to 37.25% in 2022, while oil content varied between 18.77% and 18.98% in 2021 and between 18.89% and 18.91% in 2022. These results indicate that simulated defoliation had little effect on the concentration of major seed storage compounds.

In contrast, protein yield (PY) and oil yield (OY) differed significantly among treatments. In 2021, PY ranged from 1196 kg ha^{-1} in R1 100% to 1675 kg ha^{-1} in R1 67%, while OY ranged from 609 kg ha^{-1} to 856 kg ha^{-1} for the same treatments. Similar trends were observed in

2022, where R1 67% produced the highest PY (1659 kg ha^{-1}) and OY (847 kg ha^{-1}), whereas R1 100% resulted in the lowest values (1132 and 581 kg ha^{-1}, respectively).

Vegetative-stage treatments (V1) generally maintained PY and OY values comparable to or slightly higher than the control. The highest protein and oil yields were consistently recorded under moderate reproductive-stage defoliation (R1 67%), whereas complete reproductive-stage defoliation (R1 100%) significantly reduced both parameters.

Overall, the observed variation in protein yield and oil yield primarily reflected differences in seed yield among treatments, while protein and oil concentrations remained largely unaffected by simulated defoliation.

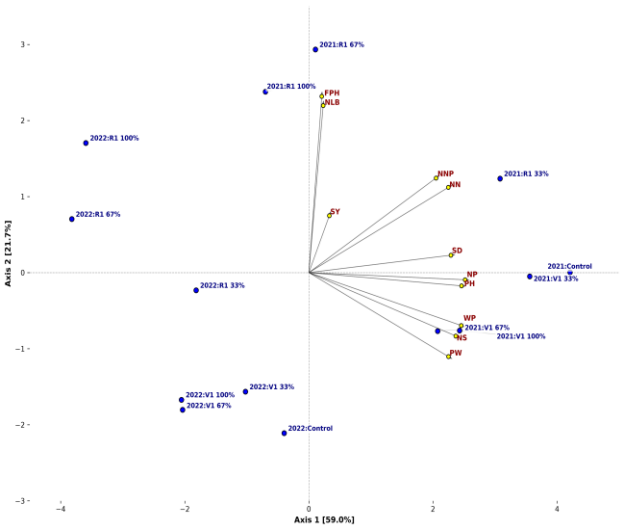

Fig. 2. PCA biplot of soybean trait responses to simulated defoliation at V1 and R1 stages.
PW – plant weight; PH – plant height; SD – stem diameter; FPH – first pod height; NLB – number of lateral branches; NN – number of nodes; NNP– number of nodes with pods; NP – number of pods; WP – pod weight; NS – number of seeds; SY – seed yield.

Significant trait correlations in soybean under simulated defoliation: A comprehensive analysis of interrelationships among vegetative, generative, and nutritional traits in soybean subjected to simulated defoliation revealed pronounced physiological connectivity among key components of adaptation and productivity. Table 5 presents the most relevant trait correlations recorded across treatments and growing seasons, highlighting statistically significant positive associations among selected parameters. SY was highly correlated with NS (r = 0.91), WP (r = 0.88), PW (r = 0.85), as well as PY (r = 0.89) and OY (r = 0.86). These relationships confirm that the generative potential of the plant is closely linked to the efficiency of pod formation and filling, as well as to overall vegetative biomass. NS showed strong correlations with number of NP (r = 0.87) and PW (r = 0.84), indicating a functional dependency between pod structure and reproductive output. PW was consistently associated with FPH (r = 0.79), suggesting that morphological vigor contributes to more favorable distribution of generative organs. Nutritional traits—PY and OY—were mutually correlated (r = 0.81) and also strongly associated with seed yield, confirming that quantitative productivity is accompanied by qualitative enhancement. This linkage is particularly relevant for the selection of genotypes with high nutritional potential. Leaf area parameters, including TFW, RTLW, RTLA, and LAI, did not exhibit statistically significant correlations with generative or nutritional traits. This lack of association may be attributed to the early storage of defoliation and the plant's compensatory capacity to redistribute resources toward reproductive structures.

Discussion

Physiological response of soybean to simulated defoliation: The results indicate that the developmental stage at which simulated defoliation is applied significantly influences soybean physiological responses. Treatments imposed at the vegetative stage (V1) maintained stability across all measured parameters (TFW, RTLW, RTLA, and LAI), even under high defoliation intensities (V1 67% and V1 100%). This suggests effective activation of compensatory mechanisms, including leaf regeneration and redistribution of photosynthetic resources. These findings are consistent with previous reports by (Dollete *et al.,* 2024) and (Wang *et al.,* 2020), who demonstrated that early-stage defoliation in leguminous crops can stimulate vegetative recovery through regenerative and resource-reallocation processes.

In contrast, R1-stage treatments, particularly at higher defoliation intensities, resulted in pronounced reductions in all physiological parameters. The decline in TFW, together with progressive reductions in RTLW and RTLA, reflects limited regenerative capacity during reproductive development, when assimilates are preferentially allocated to reproductive organs. Complete defoliation at R1 (100%) severely compromised photosynthetic capacity, corroborating findings by (Slattery *et al*., 2017) and (Poni *et al.,* 2006), who reported that late-stage defoliation substantially reduces photosynthetic efficiency and yield in crops characterized by strong reproductive sink demand.

LAI remained stable under V1 treatments, confirming the maintenance of functional leaf area despite trifoliate removal. In R1 treatments, LAI declined proportionally with defoliation intensity, with the lowest values recorded in R1 100%. The higher LAI observed at R5 compared with R3 in R1 100% suggests partial leaf regeneration following initial defoliation, indicating a degree of delayed compensatory growth. This response warrants further investigation into senescence dynamics, assimilate redistribution, and potential activation of latent meristematic activity.

Overall, soybean exhibited greater physiological tolerance to simulated defoliation when applied at the vegetative stage (V1), whereas reproductive-stage defoliation (R1) led to substantial reductions in vegetative biomass and photosynthetic surface. These response patterns have direct implications for agronomic management under stress conditions and for the selection of genotypes with improved regenerative capacity, in agreement with (Zhang *et al.,* 2017) and (Valliyodan *et al.,* 2016), who emphasized the importance of exploiting genetic variability for stress adaptation.

These findings refine current understanding by demonstrating a clear developmental threshold in soybean defoliation tolerance, characterized by near-complete physiological compensation at V1 and marked sensitivity at R1, a pattern that has not been consistently resolved in previous studies.

Vegetative response of soybean to simulated defoliation: Across both growing seasons, the V1 100% treatment produced the highest plant weight (PW), exceeding the control. This indicates a clear over-compensatory growth response following complete early defoliation (Carrera *et al.,* 2022). Severe leaf removal at the V1 stage likely triggers reorganization of source–sink relationships, resulting in preferential allocation of assimilates toward structural biomass rather than immediate restoration of leaf area. This shift promotes stem thickening and enhanced lateral branching during the recovery phase.

In addition, complete defoliation at V1 likely reduces apical dominance and stimulates axillary meristem activation through hormonal regulation, particularly changes in the auxin–cytokinin balance. Increased cytokinin activity relative to auxin may enhance bud outgrowth and vegetative proliferation during recovery (Waldie *et al.,* 2014). Similar compensatory responses after early defoliation have been reported in legumes, where initial biomass loss is followed by accelerated structural growth under favorable recovery conditions (Anten and Ackerly, 2001).

In contrast, the V1 67% treatment consistently showed the lowest PW, suggesting that intermediate defoliation intensity imposes sufficient stress to reduce photosynthetic capacity but does not strongly activate compensatory growth. This supports a threshold-dependent response, where only complete early defoliation induces full compensatory mechanisms (Borrás *et al.,* 2004).

Reproductive-stage (R1) treatments showed relatively stable PW across defoliation intensities, indicating reduced vegetative plasticity during reproductive development. At this stage, assimilate partitioning is primarily directed toward reproductive sinks, limiting structural compensation (Raza *et al.,* 2021; Poudel *et al.,* 2025).

Plant height remained largely unaffected across treatments, indicating stable apical growth. However, stem diameter showed slight reductions under V1 67% and R1 100%, likely reflecting reduced carbon availability for structural development.

Interestingly, R1 100% increased first pod height (FPH), suggesting an adaptive architectural adjustment that may improve pod positioning and suitability for mechanical harvest (Board & Harville, 1992).

The most pronounced vegetative plasticity was observed in the number of lateral branches (NLB). In both seasons, R1 100% significantly increased branching, indicating strong compensatory architectural reprogramming under severe reproductive stress. Increased branching may partially offset reproductive loss by increasing potential pod-bearing sites (Board & Kahlon, 2011).

Overall, reproductive-stage defoliation primarily induced architectural reprogramming, while vegetative-stage defoliation triggered over-compensatory biomass production only under complete leaf removal. These responses highlight strong developmental control over soybean plasticity under defoliation stress (Egli & Bruening, 2006).

Generative response of soybean to simulated defoliation: Soybean generative parameters exhibited clear stage-specific sensitivity to simulated defoliation, with responses dependent on both developmental stage and defoliation intensity. Across both growing seasons, NP was highest in the control treatment. V1 treatments induced moderate reductions in NP, particularly at higher defoliation intensities, whereas R1 treatments caused a pronounced decline, especially under R1 67% and R1 100%, indicating a strong inhibitory effect of reproductive-stage defoliation on pod formation. WP followed a similar pattern, with V1 treatments maintaining values close to the control, while R1 treatments showed significant reductions, with the lowest values consistently recorded under R1 100%. These results align with Raza *et al.,* (2022), who reported impaired assimilate allocation to reproductive organs under defoliation during reproductive development.

NS displayed a comparable trend: control plots showed the highest values, V1 treatments caused only slight reductions, whereas R1 treatments—particularly R1 67% and R1 100%—resulted in markedly lower NS, confirming disruption of source–sink synchrony during reproductive development (Parvej *et al.,* 2025).

Seed yield (SY) showed a more complex, non-linear response. In both seasons, the highest SY was observed in R1 67%, indicating a compensatory yield response under moderate reproductive-stage defoliation. However, this increase was not accompanied by increases in yield components such as pod number or seed number, which both decreased under this treatment. This suggests that yield formation may have been driven by improved efficiency of assimilate allocation per reproductive unit and/or changes in unmeasured yield components such as seed size (e.g., 100-seed weight, not assessed in this study). Such a response indicates a threshold-dependent adjustment in source–sink relations, where moderate defoliation enhances allocation efficiency, while severe defoliation exceeds the compensatory capacity of the plant and disrupts reproductive development (Sadras and Denison, 2016). In contrast, R1 100% consistently resulted in the lowest SY, confirming the detrimental effect of severe defoliation.

Overall, moderate reproductive-stage defoliation (R1 67%) may enhance yield through improved allocation efficiency, whereas severe defoliation disrupts reproductive development and significantly reduces all yield components (NP, WP, NS, and SY). These results support the identification of stress-resilient genotypes and the refinement of agronomic strategies under climate-induced stress conditions (Denton *et al.,* 2018).

These findings extend current understanding of soybean reproductive plasticity by demonstrating a threshold-dependent response to defoliation, where moderate reproductive-stage stress can enhance yield via improved assimilate partitioning, while severe stress leads to reproductive failure. Recent studies further indicate that this response is strongly modulated by climatic variability, particularly fluctuations in temperature and precipitation, which amplify stress effects and alter source–sink relationships (Poudel *et al.,* 2025; Raza *et al.,* 2022; Dong *et al.,* 2023). Collectively, these studies support the concept that moderate stress may activate compensatory mechanisms, whereas high-intensity stress results in irreversible yield losses.

In conclusion, soybean exhibits marked plasticity in its generative response to simulated defoliation. While R1 100% severely reduces all yield components, moderate defoliation at R1 (67%) may enhance seed yield through improved assimilate distribution dynamics. These findings have direct implications for cultivar selection and agronomic management in environments prone to climatic stress.

Multivariate analysis of soybean traits under simulated defoliation: Multivariate analysis of soybean traits under simulated defoliation enabled the identification of dominant response patterns across growing seasons and developmental stages. Principal component analysis (PCA) showed that the first two principal components explained the majority of total variance, supporting the use of biplot visualization for interpretation of complex interactions among treatments and morpho-generative traits (Jolliffe & Cadima, 2016; Akshit *et al.,* 2020).

The biplot clearly separated treatments according to season and defoliation intensity. Treatments from the 2021 season clustered in the direction of SY, NS, PW, and FPH vectors, indicating a more favorable overall physiological and generative response. This suggests that agroecological conditions in 2021 supported more effective compensation under defoliation stress, consistent with Parvej *et al*., (2025), who emphasized the strong influence of seasonal variability on soybean stress responses. In contrast, 2022 treatments—particularly R1 100% and R1 67%—were positioned opposite to these vectors, corresponding with reduced yield and reproductive performance observed in univariate analyses.

The proximity and similar orientation of SY, NS, PW, and FPH vectors indicate strong positive associations among these traits, confirming their collective role in defining generative performance. This supports the hypothesis that biomass-related and structural traits contribute jointly to reproductive success, in agreement with Sharma *et al*., (2020), who highlighted the importance of vegetative support for yield formation under stress conditions.

Traits such as SD, PH, and NLB showed a moderate association with SY, suggesting an indirect but relevant contribution to yield formation through maintenance of plant structural integrity. In contrast, NP and NS formed a distinct cluster in the ordination space, indicating that pod- and seed-related traits contribute independently to overall variability and reflect sink-related differentiation among treatments (Kim *et al.,* 2022).

Notably, the R1 67% treatment in the 2021 season was positioned closer to the cluster of generative traits, indicating a relatively stable and potentially compensatory physiological response under moderate reproductive-stage defoliation. This pattern is consistent with Dong *et al*., (2023), who reported activation of adaptive allocation mechanisms under moderate stress in soybean.

Overall, PCA effectively discriminated between seasonal effects, developmental stages, and defoliation intensities, while identifying key trait associations underlying soybean adaptive capacity. These results confirm that multivariate approaches provide an integrative framework for understanding defoliation responses that cannot be fully resolved through single-trait analyses. The findings also support the identification of genotypes with enhanced compensatory potential and contribute to the refinement of agronomic strategies under variable climatic conditions.

The observed separation between the 2021 and 2022 growing seasons in the PCA may be partly explained by contrasting weather conditions during critical growth stages. In particular, 2021 was characterized by lower precipitation in June (23.9 mm) compared with 2022 (43.6 mm), which likely resulted in reduced soil moisture availability during early vegetative development. These conditions may have contributed to differences in biomass accumulation, physiological responses, and yield-related traits between the two growing seasons, thereby reinforcing the observed seasonal clustering in multivariate space (Miladinov *et al.,* 2020).

Seed composition response of soybean to simulated defoliation: The analysis of seed composition and derived quality-related yield traits revealed distinct responses to simulated defoliation. Protein and oil concentrations remained relatively stable across all treatments in both growing seasons, indicating that defoliation had limited influence on the accumulation of major storage compounds in soybean seeds (Assefa *et al.,* 2019). In contrast, protein yield (PY) and oil yield (OY) varied significantly among treatments, reflecting differences in seed yield rather than changes in seed composition (Tayade *et al.,* 2023).

Across both growing seasons, treatments applied at the V1 stage maintained protein and oil concentrations comparable to the control, while PY and OY were slightly increased. This suggests that early vegetative-stage defoliation can be effectively compensated, without substantial effects on seed composition or reproductive performance (Fernandes *et al.,* 2022). Similar observations were reported by (Poudel *et al.,* 2025), who highlighted the strong recovery capacity of soybean during early developmental stages following moderate leaf area loss.

The highest PY and OY were consistently observed under the R1 67% treatment. However, this response was not associated with increases in protein or oil concentration, which remained stable across treatments. Therefore, the higher PY and OY under moderate reproductive-stage defoliation were primarily driven by increased seed yield rather than enhanced accumulation of storage compounds per seed. This indicates that moderate stress may improve the efficiency of assimilate partitioning toward existing reproductive sinks, thereby increasing overall production without altering seed composition (Borrás *et al.,* 2004). Similar compensatory responses under moderate defoliation in soybean have been reported by (Aguiar *et al.,* 2023).

In contrast, the R1 100% treatment resulted in the lowest PY and OY in both years. Since protein and oil concentrations remained stable, the reduction in PY and OY can be attributed mainly to the substantial decline in seed yield caused by severe reproductive-stage defoliation (Owen *et al.,* 2013). These results indicate that extensive leaf area loss during flowering disrupts reproductive success and reduces the total production of protein and oil per unit area.

Overall, the results demonstrate that simulated defoliation affected protein and oil yield primarily through changes in seed yield, while protein and oil concentrations remained largely conserved. This stability suggests a strong buffering capacity of soybean seed composition under varying levels of source limitation (Rotundo & Westgate, 2009). Similar stability of seed composition under stress conditions has been reported by (Cui *et al.,* 2023). The findings further indicate that developmental stage and stress intensity jointly determine the extent to which defoliation influences overall protein and oil productivity under field conditions (Duan *et al.,* 2023).

Significant trait correlations in soybean under simulated leaf area reduction: The observed correlations revealed strong physiological integration among vegetative, generative, and nutritional traits in soybean subjected to simulated leaf area reduction. The strong positive associations between SY and NS, WP, and PW, as well as between PY and OY, confirm that reproductive performance is closely linked to pod formation efficiency, seed filling, and overall assimilate allocation to reproductive sinks. This relationship becomes particularly evident under reduced leaf area conditions, where maintenance of yield depends on coordinated functioning of source and sink components despite partial loss of photosynthetic surface.

Similar findings were reported by (Bu *et al.,* 2023), who demonstrated that vegetative biomass contributes significantly to reproductive performance in soybean under nitrogen-limited conditions. The positive correlation between PW and FPH further supports the role of morphological vigor in determining reproductive architecture, with higher pod placement offering agronomic advantages such as improved suitability for mechanical harvesting and reduced pre-harvest losses (Lu *et al.,* 2021). The observed associations among NP, NS, and WP indicate a functional dependency between pod structure and overall yield formation, consistent with previous reports in legumes where NP is a strong predictor of productivity (Jarecki & Migut, 2022).

The significant relationships between PY, OY, and SY suggest that higher productivity is generally associated with improved seed quality, indicating the possibility of simultaneous selection for yield and nutritional traits. This is supported by transcriptomic evidence from (Xu *et al.,* 2022), who identified coordinated regulatory networks underlying concurrent increases in yield and protein accumulation.

In contrast, leaf area-related parameters (TFW, RTLW, RTLA, and LAI) showed no significant correlations with generative or nutritional traits. This lack of association may reflect the compensatory capacity of soybean following defoliation, where reductions in photosynthetic area are partially offset by internal resource redistribution and improved source–sink efficiency at the whole-plant level (Wang *et al.,* 2022).

Overall, these results indicate that under simulated leaf area reduction, soybean maintains stable and coordinated relationships among yield and quality traits, while leaf area parameters become partially decoupled from generative performance, reflecting a high degree of physiological buffering and trait integration stability.

Integrated physiological and agronomic response to simulated defoliation: The results demonstrate a clear developmental-stage-dependent response of soybean to simulated defoliation, with V1 treatments showing greater physiological stability, while R1 treatments exhibited stronger effects on reproductive performance. During the vegetative stage, plants maintained relatively stable growth and reproductive potential, indicating a high degree of compensatory plasticity in early development.

In contrast, reproductive-stage defoliation exerted a stronger influence on yield formation. Severe defoliation at R1 (100%) consistently reduced all yield components, confirming the high sensitivity of soybean during reproductive development. However, moderate defoliation at R1 (67%) resulted in the highest seed yield across both growing seasons. This response occurred despite reductions in pod number and seed number, indicating that yield formation was not primarily driven by sink size, but rather by increased efficiency of assimilate partitioning and compensatory adjustments in the development of remaining reproductive structures, including enhanced seed weight (Tayade *et al.,* 2023).

This pattern suggests a threshold-dependent compensatory response under moderate reproductive-stage defoliation, where reduced sink number is offset by improved performance of existing sinks. Similar responses have been reported in soybean under stress conditions, where moderate stress can enhance source–sink efficiency, whereas severe stress leads to reproductive failure (Egli & Bruening, 2006; Denton *et al.,* 2018).

From a physiological perspective, these results indicate that soybean yield stability under moderate stress is likely associated with adaptive regulation of assimilate distribution and maintenance of functional reproductive sinks, whereas excessive defoliation exceeds the compensatory capacity of the plant (Srinivasan & Kumar, 2020; Xu *et al.,* 2023).

From a breeding and agronomic perspective, these findings suggest that genotypes capable of maintaining stable yield under vegetative-stage stress (V1), as well as those expressing controlled compensatory responses under moderate reproductive-stage defoliation (R1 67%), may represent valuable genetic material for improving defoliation tolerance. Key selection traits include stable seed yield, maintenance of reproductive structure integrity, and adaptive plant architectural responses under stress.

A limitation of this study is the evaluation of a single cultivar (‘Fortuna’), which restricts the generalizability of the findings. Future research involving multiple genotypes with contrasting genetic backgrounds is necessary to validate these responses and strengthen their applicability in breeding programs.

Conslusion

This study demonstrates that soybean response to simulated defoliation is strongly dependent on both developmental stage and stress intensity. Defoliation at the vegetative stage (V1) resulted in greater physiological stability and partial maintenance of growth and yield formation, indicating higher tolerance during early development. In contrast, reproductive-stage defoliation (R1) led to more pronounced reductions in seed yield and yield components, particularly under high-intensity defoliation (100%).

A non-linear, threshold-dependent response was observed under moderate reproductive-stage defoliation (R1 67%), which was associated with the highest seed yield and consequently the highest protein and oil yields, despite reductions in yield components. Importantly, protein and oil concentrations remained stable across treatments, indicating that variation in protein and oil yield was driven primarily by differences in seed yield rather than changes in seed composition.

Overall, the results confirm a clear developmental threshold in defoliation tolerance, highlighting the greater sensitivity of soybean during reproductive development. These findings emphasize the importance of maintaining canopy integrity during reproductive stages and provide a basis for identifying genotypes with improved tolerance to leaf area loss under field conditions.

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