Humic Acid-Induced Enhancement of Root Architecture in *Glycine max* (L.) Merr.

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Received: 14 Dec 2023
Revised: 30 Dec 2023
Accepted: 16 Jan 2024
Published: 02 Feb 2024

E-ISSN: 2989-5340
DOI: https://doi.org/10.54660/jafi.2024.4.1.30-34

ABSTRACT
Background: Soybean (*Glycine max* (L.) Merr.) is a highly important legume crop known for its protein, oil, and industrial utility. The structure of its root system plays a crucial role in acquiring water and nutrients, thereby affecting crop productivity. Humic acid, one of the components of humic substances found in soil, is increasingly considered a natural biostimulant that can alter root architecture and the functioning of the rhizosphere.
Objectives: The aim of this paper is to study the impact of soil-applied humic acids applied separately and in combination with recommended fertilization on the parameters of the soybean root architecture, physiological performance, nutrient uptake, and yield.
Methods: The present study was conducted in a field experiment using a randomized complete block design with 7 treatments (control, recommended dose of fertilizers, 4 doses of humic acid at different levels, and one combined treatment) replicated four times each. Root architecture was measured using WinRHIZO image analysis, physiological parameters were measured using SPAD chlorophyll meter and gas-exchange systems, nutrient concentrations were measured with the help of Kjeldahl digestion and ICP-OES, while yield components were calculated based on the physiological maturity of soybean.
Result: Humic acid application led to a notable rise in root length, root area as well as lateral roots number in relation to the control group. Furthermore, when combined with fertilizers, humic acid achieved the greatest improvement of root structure. Moreover, this treatment was not only followed by improvements in other roots-related, but also physiological processes.
Conclusion: The use of humic acid in soils has positive effects on root structure development of soybean plants and therefore enhances their physiological efficiency as well as yield.

Keywords: Soybean (*Glycine max* (L.) Merr.), humic acid, root architecture, soil biostimulant, nutrient uptake, photosynthetic performance, crop yield

1. Introduction
The soybean, or *Glycine max* (L.) Merr., is classified as one of the world's most valuable leguminous plants, as it serves as an excellent supplier of edible oil and plant-based protein for humans and animals. Moreover, soybean has important uses in biodiesel production, food additives, and industrial oleochemicals and is key to agricultural economies in major production areas, contributing significantly to global food and protein security. As the demand for agricultural products rises, while dependence on synthetic inputs is reduced, the interest in biological measures of increasing crop productivity has grown.
Root architecture, defined as the spatial pattern of roots and their structure, has a significant role in determining how well a particular crop is capable of obtaining water and minerals from a given soil environment. Some characteristics of root architecture, including overall root length, surface area, density of lateral roots, and number of root tips allow understanding the quantity of soil that has been worked and the efficiency of their uptake. As a result, agronomic approaches that can improve root architecture can be valuable for enhancing utilization efficiency and stability of yields.
The importance of soil organic matter, particularly humic substances, for maintaining soil fertility is emphasized. Humic acid, which is the alkali-soluble, acid-insoluble part of humic substances is a mixed structure with heteroatoms and hydrophobes, with carboxyl, phenolic, and quinonic functional properties [14, 15]. These functional types give humic acid unique features, such as ion exchange capacity, metal ion binding ability, and surface activity, and allow it to influence nutrient absorption.
Besides its indirect effects through the soil, humic acid is also directly bioactive in the plant parts. There are some hypotheses that the mechanism of humic acid plant impact is associated with the appearance of auxin-like responses in plants, activation of H+-ATPase systerm of plasma membrane, and modulation of nitric oxide action [7, 19]. This gives humic acids the role of a biostimulant and gives them a possibility to facilitate root growth in the early stages of its development.
The soil microbial ecosystem reacts quickly to the application of humic acid because several studies have indicated that the populations of rhizobacteria involved in plant growth and their enzymatic performance associated with carbon and nutrient cycling have increased after the addition of humic acid [9, 17].

Since legumes like soybean depend upon symbiotic rhizobial associations for a significant proportion of their nitrogen needs, any effects of humic acid that enhance rhizosphere microbial functioning become significant not only for nodulation but nitrogen fixation as a whole.

Considering the growing interest in sustainable intensification and less dependency on mineral fertilizers, there is an apparent need to understand, in controlled field conditions, the impacts that the application of humic acid can have on the architecture of soybean roots and possible physiological and yield implications of this process. Hence, the aim of the current research was to implement the study through which the impact of humic acid applied to soil or in combination with recommended fertilization would be analyzed in terms of its influence on the morphology of soybean roots, nutrient acquisition, physiological performance, and yield, which would contribute to sustainable production of soybean and management of soil health.

2. Literature Review

Humic substances have been extensively investigated as agricultural amendments capable of improving soil physicochemical properties and stimulating plant growth [1, 3, 4]. Meta-analytical evidence indicates that humic acid application consistently increases root and shoot biomass across diverse crop species, with root responses generally more pronounced than shoot responses [4, 10].

Mechanistic studies have demonstrated that humic acid fractions of low molecular weight can penetrate root tissues and elicit auxin-like physiological responses, including enhanced lateral root emergence and increased root hair density [7, 8]. Nitric oxide has been identified as a key intermediate signaling molecule linking humic acid perception to activation of plasma-membrane proton pumps and subsequent root cell elongation [7].

In legumes, several investigations have reported that humic substances enhance nodulation and biological nitrogen fixation, potentially through improved rhizobial colonization and increased carbohydrate allocation to root nodules [11, 18]. Concurrently, humic acid amendments have been shown to modify rhizosphere microbial community composition, favoring plant-growth-promoting taxa and enzymatic activity associated with nutrient cycling [9, 17, 18].

Reviews addressing humic substances in the broader context of soil quality highlight their contribution to aggregate stability, water-holding capacity, and long-term carbon sequestration, positioning humic acid application as compatible with climate-smart and sustainable nutrient management objectives [3, 16]. Despite this substantial body of evidence, comparatively few field-scale studies have quantitatively characterized dose-dependent root architectural responses specifically in soybean, and information linking such architectural change to nutrient uptake efficiency and yield formation under representative agronomic conditions remains limited [23, 24]. This gap forms the rationale for the present investigation.

3. Materials and Methods

This study was conducted to evaluate root architectural, physiological, and yield responses of soybean to soil-applied humic acid under field conditions.

3.1. Experimental Site

The trial was conducted over one growing season at a research farm located at approximately 30.7°N, 76.8°E, in a semi-arid subtropical zone characterized by a mean growing-season temperature of 26-32°C and total seasonal rainfall of approximately 420 mm, supplemented by irrigation. The soil was classified as a sandy clay loam (Typic Haplustept) with initial characteristics of pH 7.6, organic carbon 0.52%, available nitrogen 218 kg ha^{-1}, available phosphorus 14.6 kg ha^{-1}, available potassium 168 kg ha^{-1}, and cation exchange capacity of 12.4 cmol(+) kg^{-1}.

Table 1: Experimental treatments, humic acid application rates, and management schedule

Treatment	Description	HA rate (kg ha^{-1})	Fertilizer (N:P:K, kg ha^{-1})	Application timing
T1	Control	0	0:0:0	—
T2	Recommended fertilizer dose (RFD)	0	25:75:40	Basal, at sowing
T3	Humic acid, low rate	5	0:0:0	Sowing + R1
T4	Humic acid, medium-low rate	10	0:0:0	Sowing + R1
T5	Humic acid, medium-high rate	15	0:0:0	Sowing + R1
T6	Humic acid, high rate	20	0:0:0	Sowing + R1
T7	Humic acid + RFD (combined)	15	25:75:40	Sowing + R1

3.2. Experimental Design

The experiment followed a randomized complete block design (RCBD) with seven treatments and four replications, giving 28 plots of 4 m × 3 m (12 m²) separated by 0.75 m buffer strips. The crop was grown over a 105-day season from sowing to physiological maturity.

3.3. Humic Acid Characteristics

A commercially extracted potassium humate derived from leonardite was used, containing 68% total humic acid, 32% organic carbon, pH 9.2 (1% aqueous solution), and complete water solubility, applied as a granular soil formulation.

3.4. Treatments and Crop Management

Treatments comprised: T1, control (no humic acid, no fertilizer); T2, recommended fertilizer dose (RFD: 25:75:40 kg $N:P_2O_5:K_2O$ ha^{-1}); T3-T6, humic acid applied to soil at 5, 10, 15, and 20 kg ha^{-1}, respectively, without fertilizer; and T7, humic acid at 15 kg ha^{-1} combined with RFD. Humic acid was broadcast and incorporated at sowing, with a second split applied at the flowering stage (R1). The soybean cultivar JS-95-60 was used, seeds were inoculated with *Bradyrhizobium japonicum* prior to sowing, and rows were spaced 45 cm apart with 5 cm intra-row spacing. Irrigation was applied at critical growth stages, weeds were controlled through pre-emergence herbicide application supplemented by one hand weeding, and standard integrated pest management practices were followed.

3.5. Root Architecture Measurements

Root samples were collected destructively at the R3 (pod-initiation) stage from a 20 cm × 20 cm × 20 cm soil monolith per plot. Roots were washed free of soil, scanned using an Epson flatbed scanner, and analyzed with WinRHIZO Pro software to determine total root length, surface area, volume, average diameter, lateral root number, and root tip density. Root samples were subsequently oven-dried at 70°C to constant weight for root dry biomass determination.

3.6. Plant Growth, Physiological, and Nutrient Measurements

Plant height and stem diameter were measured on ten tagged plants per plot, leaf area index was determined using a canopy analyzer, and dry matter accumulation was recorded at physiological maturity. Nodule number and nodule dry weight were assessed at R2 stage. Chlorophyll content was estimated using a SPAD-502-meter, net photosynthetic rate and stomatal conductance were measured with a portable infrared gas analyzer, and relative water content was calculated from fresh, turgid, and dry leaf weights. Nitrogen concentration in plant tissue was determined by the Kjeldahl method, while phosphorus, potassium, calcium, magnesium, iron, and zinc concentrations were determined using inductively coupled plasma optical emission spectroscopy (ICP-OES), with selected micronutrients cross-verified by atomic absorption spectroscopy (AAS).

3.7. Yield Parameters and Statistical Analysis

At harvest, pods per plant, seeds per pod, hundred-seed weight, grain yield, biological yield, and harvest index were recorded from net plot areas. Data were subjected to analysis of variance (ANOVA) appropriate to the RCBD structure, and treatment means were separated using Tukey's Honestly Significant Difference (HSD) test at $P < 0.05$, using R statistical software (version 4.3).

4. Results

4.1. Root Architecture

Humic acid application significantly increased root architectural parameters relative to the control (Table 2). Total root length increased progressively with humic acid rate up to 15 kg ha^{-1}, beyond which further gains were marginal. The combined treatment (T7) produced the greatest total root length, surface area, and lateral root number among all treatments, followed by T6 (20 kg ha^{-1} humic acid alone). Average root diameter showed comparatively small and statistically non-significant differences among treatments, indicating that humic acid primarily promoted root elongation and branching rather than radial thickening.

Table 2: Effects of humic acid on soybean root architectural characteristics (R3 stage, mean ± SE, n = 4)

Treatment	Total root length (cm)	Root surface area (cm²)	Root volume (cm³)	Avg. diameter (mm)	Lateral roots (no. plant^{-1})	Root dry weight (g plant^{-1})
T1	412 ± 14a	68.5 ± 2.1a	2.1 ± 0.1a	0.53 ± 0.02a	38 ± 3a	1.24 ± 0.08a
T2	486 ± 16b	81.2 ± 2.4b	2.6 ± 0.1b	0.55 ± 0.02a	47 ± 3b	1.58 ± 0.09b
T3	468 ± 15b	77.4 ± 2.2b	2.4 ± 0.1b	0.54 ± 0.02a	45 ± 3b	1.49 ± 0.08b
T4	512 ± 17c	86.9 ± 2.6c	2.7 ± 0.1bc	0.55 ± 0.02a	52 ± 4c	1.67 ± 0.09c
T5	561 ± 18d	95.3 ± 2.8d	3.0 ± 0.2cd	0.56 ± 0.02a	58 ± 4d	1.82 ± 0.10d
T6	574 ± 19d	97.8 ± 2.9d	3.1 ± 0.2d	0.56 ± 0.02a	60 ± 4d	1.87 ± 0.10d
T7	628 ± 20e	106.4 ± 3.1e	3.4 ± 0.2e	0.57 ± 0.02a	67 ± 5e	2.05 ± 0.11e

Means followed by different letters within a column differ significantly (Tukey's HSD, $P < 0.05$).

4.2. Plant Growth and Nodulation

Plant height, leaf area index, and total dry matter accumulation were significantly greater in humic acid-treated plots compared with the control (Table 3). Nodule number and nodule dry weight increased with increasing humic acid rate, with T7 exhibiting the highest nodulation, consistent with enhanced biological nitrogen fixation potential.

4.3. Physiological Responses and Nutrient Uptake

SPAD chlorophyll values, net photosynthetic rate, stomatal conductance, and relative water content were all significantly improved under humic acid treatments relative to the control, with the combined treatment consistently producing the highest values (Table 3). Nitrogen, phosphorus, potassium, iron, and zinc uptake increased significantly with humic acid application, while calcium and magnesium uptake showed smaller, though still statistically significant, increases.

Table 3: Influence of humic acid on plant growth, physiological characteristics, and nodulation

Treatment	Plant height (cm)	LAI	SPAD value	Photosyn. rate (µmol CO_2 m^{-2} s^{-1})	RWC (%)	Nodules (no. plant^{-1})
T1	58.2±1.8a	2.4±0.1a	38.6±1.1a	18.4±0.6a	72.1±1.4a	22±2a
T2	66.7±2.0b	3.1±0.1b	43.2±1.2b	21.6±0.7b	78.4±1.5b	26±2b
T3	64.1±1.9b	2.9±0.1b	42.0±1.2b	20.8±0.6b	77.0±1.5b	27±2b
T4	68.9±2.1bc	3.3±0.1c	45.1±1.3c	22.9±0.7c	79.6±1.6bc	31±3c
T5	73.5±2.2c	3.6±0.2c	47.8±1.3d	24.5±0.8d	81.9±1.6c	35±3d
T6	74.8±2.3c	3.7±0.2c	48.3±1.4d	24.9±0.8d	82.5±1.7c	36±3d
T7	80.4±2.5d	4.1±0.2d	51.6±1.5e	27.3±0.9e	85.7±1.8d	42±3e

LAI, leaf area index; RWC, relative water content. Means followed by different letters within a column differ significantly (Tukey's HSD, $P < 0.05$).

4.4. Yield Performance

Pods per plant, seeds per pod, hundred-seed weight, grain yield, biological yield, and harvest index were significantly higher under humic acid treatments than the control (Table 4). The combined humic acid-fertilizer treatment (T7) produced the highest grain yield, exceeding the control by approximately 34% and the recommended fertilizer-alone treatment by approximately 12%, indicating a synergistic effect between humic acid and mineral fertilization.

Table 4: Effects of humic acid treatments on yield components, grain yield, and harvest index

Treatment	Pods plant⁻¹	Seeds pod⁻¹	100-seed wt. (g)	Grain yield (kg ha⁻¹)	Biological yield (kg ha⁻¹)	Harvest index (%)
T1	38.4 ± 1.5a	2.1 ± 0.1a	12.6 ± 0.3a	2185 ± 62a	5980 ± 145a	36.5 ± 0.9a
T2	45.2 ± 1.7b	2.4 ± 0.1b	13.8 ± 0.3b	2612 ± 68b	6740 ± 158b	38.8 ± 1.0b
T3	43.6 ± 1.6b	2.3 ± 0.1b	13.4 ± 0.3b	2498 ± 65b	6520 ± 152b	38.3 ± 1.0b
T4	47.9 ± 1.8bc	2.5 ± 0.1bc	14.1 ± 0.3c	2734 ± 70c	6960 ± 160c	39.3 ± 1.0bc
T5	52.1 ± 1.9c	2.7 ± 0.1c	14.7 ± 0.3c	2891 ± 72cd	7210 ± 165c	40.1 ± 1.1c
T6	53.0 ± 2.0c	2.7 ± 0.1c	14.8 ± 0.4c	2925 ± 73d	7280 ± 167c	40.2 ± 1.1c
T7	59.8 ± 2.2d	3.0 ± 0.1d	15.9 ± 0.4d	3268 ± 78e	7860 ± 172d	41.6 ± 1.2d

Means followed by different letters within a column differ significantly (Tukey's HSD, $P < 0.05$)

5. Discussion

The observed enhancement of soybean root architecture under humic acid treatment is consistent with the hormone-like bioactivity attributed to humic substances, particularly their capacity to elicit auxin-mimetic signaling and activate plasma-membrane H+-ATPase, thereby promoting root cell elongation and lateral root initiation [7, 19]. The disproportionate increase in root length and lateral root number relative to root diameter observed in this study aligns with previous reports describing humic acid as preferentially stimulating root branching architecture rather than secondary thickening [8, 24].

The concurrent improvement in nutrient uptake efficiency likely reflects both the expanded root surface area available for absorption and the chelating and cation-exchange properties of humic acid, which can increase the bioavailability of micronutrients such as iron and zinc in the rhizosphere [9, 14]. Enhanced rhizosphere microbial activity associated with humic acid amendment may further have contributed to nutrient mineralization and availability, complementing the direct physiological effects on the root system [9, 17, 18]. The increase in nodulation and inferred biological nitrogen fixation potential under humic acid treatment corroborates earlier findings in legume systems and suggests a mechanism through which humic acid indirectly supports plant nitrogen economy without additional synthetic nitrogen input [11, 18].

The synergistic yield response observed when humic acid was combined with recommended fertilization supports the hypothesis that humic acid enhances fertilizer-use efficiency rather than simply substituting for mineral nutrition, a pattern reported in other biostimulant-fertilizer combination studies [3, 20]. From an agronomic and environmental perspective, these findings suggest that humic acid application could allow partial optimization of fertilizer inputs while maintaining or improving yield, contributing to reduced nutrient losses and improved soil organic matter status over time [16].

Economically, the relatively low application rates required to elicit measurable responses suggest a favorable cost-benefit ratio for humic acid adoption in soybean systems, although formal economic analysis incorporating regional input costs and humic acid pricing would be required to confirm feasibility. The magnitude of yield improvement observed at the highest humic acid rates combined with fertilizer suggests that, even after accounting for material and application costs, marginal returns could be positive under typical regional soybean price structures, though this warrant dedicated partial-budget analysis in future work.

Limitations of the present study include its single-season, single-site design, which constrains extrapolation across soil types and climatic conditions, and the absence of long-term monitoring of soil organic carbon and microbial community dynamics beyond the single growing season examined. Additionally, root sampling was restricted to a single growth stage, which, while informative for characterizing architectural response at pod initiation, does not capture the full temporal trajectory of root development across the crop cycle. Future research should prioritize multi-season, multi-location trials across contrasting soil textures and fertility regimes, molecular characterization of humic acid-induced signaling pathways in soybean roots, and precision biostimulant management approaches that optimize humic acid formulation, rate, and timing within site-specific, climate-smart soil fertility programs. Integration of humic acid response data with root phenotyping platforms and remote-sensing-based canopy monitoring could further support the development of decision-support tools for biostimulant scheduling in soybean production systems.

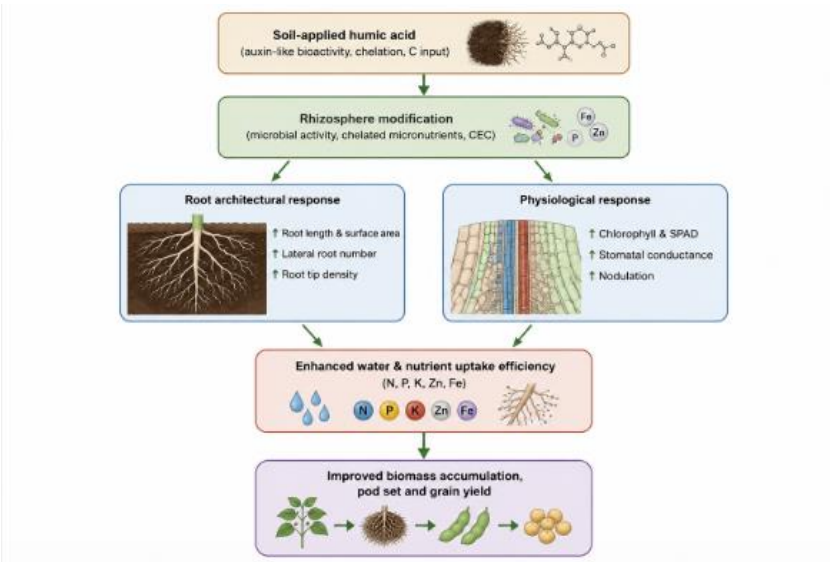

Fig 1: Conceptual pathway illustrating humic acid-induced enhancement of soybean root architecture (increased root length, surface area, and lateral branching), associated rhizosphere and physiological changes, and downstream effects on nutrient/water uptake and grain yield.

6. Conclusion

The study showed that the application of humic acid to soil increased the root system efficiency of soybean plants, as indicated by the increases in root length, root area, and number of branches, with the highest impact achieved when humic acid was used with recommended fertilization. Moreover, these changes in root structure are associated with improvements in chlorophyll content, photosynthesis performance, nodulation, uptake of nutrients, and grain yield. The findings prove that humic acid can be a useful biostimulator that helps improve resource use efficiency in soybean production. As there is an observable interaction with mineral fertilizers and other soil health benefits linked with humic substances, including them into soybean management decisions can be really helpful in sustainable and climate-friendly soybean production practices.

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