

Article

Foliar Application of Protein Hydrolysates to Soybean [*Glycine max* (L.) Merr.] Parental Plants and Effects on Progeny Seed Germination, Vigor, and Stress Tolerance

Xiaohang Wang ¹, Madelyn Cunningham ¹, Rae Adeimy ¹, Cassidy L. Sharpe ¹, Zachary B. Stanley ¹, Carole Saravitz ², Carol A. Wilkinson ³, Guillaume Pilot ⁴ and Lori Unruh Snyder ^{1,*}

¹ Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC 27695, USA; xwang246@ncsu.edu (X.W.); mmcunningham33@gmail.com (M.C.); raadeimy@ncsu.edu (R.A.); clsharpe@ncsu.edu (C.L.S.); zbstanle@ncsu.edu (Z.B.S.)

² Department of Phytotron, North Carolina State University, Raleigh, NC 27695, USA; carole@ncsu.edu

³ Southern Piedmont Agricultural Research and Extension Center, Virginia Tech, Blackstone, VA 23824, USA; wilki@vt.edu

⁴ School of Plant and Environmental Sciences, Virginia Tech, Blacksburg, VA 24061, USA; gpilot@vt.edu

* Correspondence: ljunruh@ncsu.edu

Abstract

Soybean [*Glycine max* (L.) Merr.] productivity is often limited by abiotic stresses such as drought, heat, and suboptimal temperatures during germination and early establishment. Protein hydrolysates (PHs) are biostimulants that can improve crop stress resilience, but whether foliar PH application to parental plants influences untreated F1 progeny seed performance remains poorly understood. This study evaluated whether parental foliar application of three PHs differing in amino acid composition (EZGro 16-0-0, EZGro 18-0-0, and iQForte), an equal-N foliar urea comparator, or a base-fertilizer/no-foliar control altered progeny germination, seedling vigor, and stress responses. Progeny seeds were tested using AOSA rolled-towel germination assays and controlled-environment phytotron assays under drought and temperature stress. Responses were composition-dependent and stress-specific. EZGro 16-0-0 produced PH-specific positive responses for 2023 AOSA germination, vigor index, and root length, and for 2024 root:shoot ratio. EZGro 18-0-0 produced PH-specific responses for germination in both years and for selected dry-matter traits. iQForte produced a PH-specific response for germination under well-watered phytotron conditions. Several additional responses were greater than urea only. These findings indicate that parental foliar PH application can alter selected progeny seedling traits in product-specific and environment-dependent manners, but the underlying biochemical or epigenetic mechanisms require further identification.

Keywords: abiotic stress; biostimulants; crop productivity; plant growth; protein hydrolysates; seed vigor; sustainable agriculture



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1. Introduction

Soybean [*Glycine max* (L.) Merr.] is one of the most important legume crops because of its high protein and oil content and its use in food, livestock feed, industrial products, and bio-based materials [1–4]. Productivity depends not only on final yield potential but also on rapid and uniform stand establishment. Poor germination, delayed emergence, and weak early seedling growth can reduce plant populations, increase stand variability, and limit the crop's ability to compete for water, nutrients, and light. Improving seed quality

and early vigor is an important target for increasing soybean resilience under variable field conditions.

Seed germination is a complex physiological process that begins with water uptake and proceeds through metabolic reactivation, reserve mobilization, radicle protrusion, and seedling establishment [5,6]. These processes are influenced by intrinsic seed characteristics and the post-sowing environment. Soybeans generally germinate most effectively near 25–30 °C [7,8], but they may encounter lower temperatures during early planting or higher temperatures during delayed planting or heat events. Low temperatures can slow imbibition, enzyme activation, and seedling elongation, whereas high temperatures can increase respiratory demand and oxidative stress [9–11].

Water availability is another major factor regulating soybean germination and early seedling growth. Adequate moisture is required for imbibition, enzyme activation, and embryo growth, but both water deficit and excessive water can impair germination and seedling establishment [12–15]. Water deficit can delay or inhibit radicle emergence, restrict hypocotyl and root elongation, and reduce seedling biomass accumulation. During early establishment, seedlings often respond to water limitation by altering biomass partitioning, including increased allocation to roots relative to shoots, which may improve water acquisition under moderate stress. Because soybean planting environments increasingly involve irregular rainfall and temperature extremes, strategies that improve germination and early seedling vigor under these conditions are increasingly relevant for sustainable production.

Seed performance under stress is not determined solely by post-sowing conditions. The environment and management experienced by the parent plant (the terms parent and parental effect are used here to refer to effects associated with the plant on which the seeds developed) can influence seed size, reserve composition, physiological quality, and subsequent seedling performance. In soybeans, seed protein, oil, fatty acid composition, amino acid composition, and mineral content can vary with growing-season conditions, especially temperature and water availability during seed filling [16–18]. These seed composition traits may affect energy availability, membrane stability, nitrogen supply, and early seedling growth. Thus, foliar management of parent plants may influence both harvested-seed composition and progeny performance.

Plant biostimulants are increasingly used as crop management tools to improve nutrient-use efficiency, stress tolerance, and crop quality under adverse environmental conditions [19–22]. Protein hydrolysates (PHs) are an important class of biostimulants composed of free amino acids, oligopeptides, polypeptides, and related organic compounds produced through hydrolysis of plant or animal protein sources [23,24]. Recent reviews have summarized their effects on nutrient acquisition, carbon and nitrogen metabolism, root architecture, photosynthesis, and crop temperature stress [25–28]. Protein hydrolysates influence plant physiological processes beyond their direct nutrient contribution, including root growth, nitrogen metabolism, antioxidant defense, osmotic adjustment, and stress-responsive signaling pathways [29–31]. For instance, PHs improve drought recovery in tomato (*Solanum lycopersicum* L.) through changes in biomass dynamics, dipeptides, fatty acids, and phenolic metabolism; reprogram lettuce (*Lactuca sativa* L.) transcriptomic and metabolomic responses under low N availability; and regulate growth-related metabolic and molecular pathways in Arabidopsis [*Arabidopsis thaliana* (L.) Heynh] [32–34]. They also alter stress-related gene expression and agronomic efficiency in rice (*Oryza sativa* L.) [35], overall plant development and nutrition with increased pod number or grain weight, and changes in plant nitrogen metabolism [36,37]. Those studies and the numerous reviews dedicated to this topic predominantly represent within-generation responses, in which PHs were applied to foliar tissue, roots, or the rhizosphere of the same plants, subsequently assessed for growth, physiology, yield, or stress tolerance. For example, seed or foliar

application of a PH to soybeans altered N-related metabolites and gene expression and increased several productivity traits of the treated plants [37]. Other studies have applied PHs directly to seeds before germination and demonstrated effects on seedling establishment and abiotic-stress tolerance [24]. Although these studies establish direct biostimulant activity, treating a crop or seed directly is biologically distinct from treating a parental plant and subsequently evaluating seeds that receive no further PH application. Recent PH reviews similarly concentrate on treated-plant metabolism, yield, and stress physiology rather than on germination of the next generation.

Yet parental growing conditions can influence progeny seed physiology through changes in assimilate availability, seed mass, reserve composition, hormonal balance, seed-coat properties, dormancy, and potentially stable regulatory or epigenetic processes [38,39]. Evidence for parental environmental effects is particularly relevant in soybeans and other legumes. Parental drought during soybean reproductive development reduced progeny germination and seedling vigor, whereas parental temperature altered subsequent soybean seed germination responses [40,41]. More recently, parental temperature and elevated CO₂ were shown to modify soybean seed composition, progeny emergence, and seedling vigor [42]. Combined parental drought and herbivory and sequential herbivore exposure have also produced treatment-specific effects on soybean progeny physiology and fitness [43,44]. In chickpea (*Cicer arietinum* L.), seeds developed under high-temperature summer conditions recently exhibited enhanced progeny drought tolerance, demonstrating that the seed-development environment can modify stress responses during the following growth cycle [45].

Despite these advances, the two research areas, PH biostimulants and parental regulation of progeny seed performance, have rarely been connected. In a search of recent literature, we did not identify a peer-reviewed study that compared foliar application of multiple PH formulations to soybean parental plants and evaluated the resulting untreated progeny seeds under standardized germination, drought, and temperature conditions. Consequently, it remains unclear whether parental PH application produces reproducible progeny responses, whether such responses differ among PH formulations, whether they are distinct from an equivalent nutritional N response, and whether they are expressed under drought or temperature stress. These questions define the principal research gap addressed by the present study. Recent seed physiology reviews also emphasize that parental effects must be distinguished from demonstrated transgenerational epigenetic inheritance, particularly when only a single progeny generation is evaluated [38,39].

Accordingly, the objective of the study was to evaluate whether foliar application of three amino acid-based PHs to soybean plants affected the germination, vigor, and early stress responses of their untreated F1 progeny seeds. An equal-N foliar urea comparator was included to determine whether progeny responses could be explained by N supply alone or were associated with the distinct composition of the PH formulations. We tested the following hypotheses: (H1) progeny from parental plants treated with each PH formulation would differ from the no-foliar control in germination percentage, seedling vigor, root and shoot growth, and biomass accumulation; (H2) at least one PH treatment would produce greater progeny performance than the equal-N urea treatment, indicating effects not explained solely by the quantity of N applied; (H3) responses would differ among EZGro 16-0-0, EZGro 18-0-0, and iQForte because of differences in amino acid and peptide composition; and (H4) parental PH effects would interact with the progeny growth environment, with treatment differences becoming more pronounced under water deficit or temperature stress depending on product and trait.

2. Materials and Methods

2.1. Plant Material and Seed-Parent Field Treatments

The soybean cultivar used in both years was Asgrow® AG54XF0 (BayerCropScience, MO, USA), a mid-maturity group 5, medium-tall, indeterminate soybean with good canopy coverage. The study was conducted each year at the Southern Piedmont Agricultural Research and Extension Center in Blackstone, VA, USA. Weather data, including rainfall and temperature, were obtained from the Dyacon Weather Station in Blackstone, VA, USA, at 37.096007, −77.963806; the 2023 field was located at 37.099005, −77.963149, and the 2024 field at 37.094094, −77.961549. The cultivar carries XtendFlex® and SR® herbicide-tolerance traits and is described as having good tolerance to sudden death syndrome, very good field tolerance to Phytophthora root rot, and very good tolerance to southern stem canker. Seed was commercially treated with fluxapyroxad, imidacloprid, metalaxyl, and pyraclostrobin, but was neither pelleted nor inoculated. The previous crop was tall fescue (*Lolium arundinaceum* L.), and soybean was established under a tilled production system. Soil test results differed slightly between experimental fields. In 2023, the study was conducted in a sandy loam soil (Spotsylvania-Cecil-Bourne) with pH 5.9, 11 ppm P, 72 ppm K, and 1.4% organic matter. In 2024, the experiment was conducted in sandy loam soil (Helena) with pH 6.1, 10 ppm P, 53 ppm K, and 1.4% organic matter. Soil was tested through the Virginia Tech Soil Testing Laboratory in Blacksburg, VA, USA.

In 2023, soybeans were planted on 24 May 2023 and harvested with a combine on 16 November 2023, and in 2024, they were planted on 30 May 2024 and harvested with a combine on 6 November 2024. Both years had the same granular 5-10-10 fertilizer applied on 22 May 2023 and 7 May 2024 at 672.5 kg ha^{-1} , supplying approximately $33.6 \text{ kg N ha}^{-1}$, $67.3 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$, and $67.3 \text{ kg K}_2\text{O ha}^{-1}$. Plots were established with 76.2 cm row spacing, 6.1 m row length, and a target seeding density of $395,000 \text{ seeds ha}^{-1}$. Parental foliar biostimulant treatments were applied on 7 July 2023, 26 July 2023, 27 June 2024, and 29 July 2024. Weeds were controlled using Broadaxe (sulfentrazone + S-metolcholor; Syngenta) at 2.34 L ha^{-1} on 26 May 2023 (30 May 2024) and glyphosate at 2.34 L ha^{-1} on 12 July 2023 (10 July 2024) for ragweed control. No foliar insecticides or foliar fungicides were applied during the 2023 or 2024 growing seasons.

Parental soybean plants received one of five treatment regimes: (1) base-fertilizer/no-foliar control, (2) foliar urea N comparator, (3) EZGro 16-0-0, (4) EZGro 18-0-0, or (5) iQForte (Table 1). All plots received the same base granular fertilizer. The no-foliar control received no supplemental foliar spray, whereas urea and PH treatments were applied with a manual sprayer twice during vegetative growth: at 21 and 63 days after planting in 2023, and at 28 and 60 days after planting in 2024. Foliar treatments were applied at an equivalent N rate of $0.34 \text{ kg N ha}^{-1}$ per application, for a total supplemental foliar N input of $0.68 \text{ kg N ha}^{-1}$ across the two applications. The free amino acid composition of each product was determined by chromatographic separation. Individual free amino acids were quantified relative to certified standards and expressed as percentages by weight of total detected free amino acids. Standard pest- and crop-management practices were applied uniformly across years and treatments. Harvested seed lots from each field plot were sent to the North Carolina State Seed Laboratory, Raleigh, NC, USA, for progeny seed-quality and seedling vigor evaluation.

Table 1. Free amino acid compositions (expressed as a *w/w* percentage of all free amino acids) of the protein hydrolysate biostimulant used in the study in 2023 and 2024.

Amino Acid	EZGro 16-0-0 (<i>w/w</i> %)	iQForte (<i>w/w</i> %)	EZGro 18-0-0 (<i>w/w</i> %)
Glycine	26.1	16.4	100
Proline	15.4	4.3	—
Glutamic acid	12.3	21.3	—
Alanine	10.9	3.2	—
Arginine	7.5	1.2	—
Aspartic acid	6.5	2.5	—
Lysine	4.0	11.2	—
Leucine	3.6	2.4	—
Valine	2.9	5.7	—
Phenylalanine	2.4	2.6	—
Ornithine	1.5	0.3	—
Isoleucine	1.8	4.2	—
Serine	1.5	2.5	—
Threonine	1.1	19.5	—
Methionine	0.9	0.4	—
Histidine	0.8	0.5	—
Hydroxylysine	0.6	—	—
Tyrosine	0.2	0.8	—
Cysteine	0.1	1.2	—

2.2. Seed Composition Analysis

Seed composition was analyzed using a DA 7250 near-infrared spectrometer (Perten Instruments, Springfield, IL, USA). Approximately 40 g of seed from each plot was scanned three times, and the mean scan value was used. Measurements included oil, protein, starch, ash, selected fatty acids and amino acids, and energy-related traits on a dry-matter basis. Fatty acids included palmitic, stearic, oleic, linoleic, and linolenic acids; amino acids included aspartic and glutamic acids. Nutrient mass per seed and related derived traits were calculated from NIR output values and average weight of one seed. Treatment-level values were retained and analyzed each year, rather than pooling treatments for inferential testing.

2.3. Experiment I: Paper Roll Method

To measure seed germination, a laboratory experiment was conducted in accordance with the “Rules for Testing Seeds” of the Association of Official Seed Analysts (AOSA) [46]. Clean, healthy, and undamaged seeds from each treatment were surface disinfested for 2 min in 2% OxiDate® 2.0, rinsed with deionized water, and placed on moistened germination towels. Standard warm germination tests were conducted in January 2024 and January 2025 using seeds harvested in 2023 and 2024, respectively.

Four replicates of 50 seeds were placed on moistened germination towels for each treatment. Towels were covered, loosely rolled, secured, placed upright in ventilated plastic bags, and incubated in a Hoffman Manufacturing (Corvallis, OR, USA) germinator under an alternating 30/20 °C regime, with 30 °C for 8 h and 20 °C for 16 h for 7 d. At 7 d, normal germination percentage, root length, hypocotyl length, total seedling length, root:shoot ratio, and seedling dry matter were recorded. Normal and abnormal seedlings were classified according to AOSA soybean standards, and when the radicle was ≥ 2 mm

in length, germination percentage and seedling vigor index (SVI) were calculated using Equations (1) and (2) [47]:

$$\text{Germination percentage} = (\text{number of germinated seeds} / \text{total seeds used}) \times 100. \quad (1)$$

$$\text{Seedling vigor index (SVI)} = \text{germination percentage} \times \text{hypocotyl length}. \quad (2)$$

2.4. Experiment II: Phytotron Drought-Stress Experiment

The drought-stress experiment was conducted in May 2024 at the NC State University Phytotron using seeds harvested from the 2023 parental field trial. All experiments were conducted using the growth chamber facility at the North Carolina State University Phytotron in Raleigh, NC, USA, under a consistent light intensity of $550\text{--}600 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ using up to T5 54 W fluorescent and up to 12×40 W incandescent lamps. Seeds were planted 2–3 cm deep in trays containing steam-sterilized, washed gravel mixed with a peat-lite propagation medium. Chambers were maintained under a 30/20 °C (day/night) temperature regime with an 8 h photoperiod.

Three water regimes were imposed in separate phytotron chambers: well-watered control (watered daily, meaning the soil was saturated per pot); moderate drought (watered three times per week); and severe drought (watered once per week) to simulate water constraints similar to growing conditions in the southeastern USA [48]. Three replications per treatment of the seeds from the parental plant were included within each water regime. Day of emergence, germination percentage, and SVI were recorded. Plant height (cm) was measured repeatedly on the same experimental units and analyzed with measurement date as the repeated factor. Seedlings were harvested 15 d after planting, when root and hypocotyl length (cm), root:shoot ratio, root, shoot, and hypocotyl dry mass (g), and leaf area ($\text{cm}^2\cdot\text{seedling}^{-1}$) were measured for the first true leaf. Data were analyzed using a repeated-measures design, with measurement date as the repeated factor and experimental unit, nested within parental treatment and water regime, as the subject. Leaf area was measured with an LI-3100C Area Meter (LI-COR Environmental, Lincoln, NE, USA), and dry mass was measured using a forced-air oven at 60 °C at the NC State Seed Laboratory, Raleigh, NC, USA.

2.5. Experiment III: Phytotron Temperature-Stress Experiment

The temperature-stress experiment was conducted using seeds harvested from the 2024 parental field trial. All experiments were conducted using the growth chamber facility at the North Carolina State University Phytotron in Raleigh, NC, USA, under a consistent light intensity of $550\text{--}600 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ using up to T5 54W fluorescent and up to 12×40 W incandescent lamps. Seeds without visible damage or disease were planted 2–3 cm deep in cups containing a sterilized 2:1 mixture of gravel and potting medium. Nine cups per parental treatment were arranged in trays. A concurrent AOSA rolled-towel germination test (see description 2.3 above) was also conducted under the same chamber temperature regimes to compare germination performance to the soil-based media, conducted in the same phytotron chamber.

Three temperature regimes were imposed in separate chambers to simulate early-, optimal-, and late-season planting conditions: low temperature (LT), 20/12 °C (day/night); optimal temperature (OT), 30/20 °C; and high temperature (HT), 38/25 °C, with an 8 h photoperiod. Day of emergence, germination percentage, and SVI were recorded. Seedlings were harvested 15 d after planting, and leaf area ($\text{cm}^2\cdot\text{seedling}^{-1}$) was measured using the first true leaf. In addition, the root and hypocotyl lengths (cm), root:shoot ratio, root dry mass (g), and other biomass traits were measured. Leaf area was determined using the

LI-3100C Area Meter as previously described, and dry mass was determined after drying at 60 °C. Plant height was measured on multiple dates and analyzed as a repeated measure.

2.6. Statistical Analysis

Data were analyzed using JMP Pro (vC19) (SAS Institute Inc., Cary, NC, USA). Linear mixed models were fitted separately for seed composition, germination, drought, and temperature experiments. Treatment, year, water regime, temperature regime, and their interactions were treated as fixed effects as appropriate; field block or experimental replication was treated as a random effect. Seed composition traits were analyzed within each year. For germination traits, year $\times$ treatment interactions were evaluated, and treatment means were compared within each year when appropriate. Drought and temperature assay means were compared within each imposed regime. Repeated plant-height observations were analyzed with date as the repeated factor and experimental unit nested within treatment and regime as the subject. There were four field replications, and each field replication was accompanied by four laboratory replications. Mean separation was conducted using Tukey's HSD test at $p \leq 0.05$. Standard deviation was calculated for each measured variable.

3. Results

3.1. Rainfall and Thermal Accumulation During the 2023 and 2024 Soybean Growing Seasons

Monthly rainfall and cumulative growing degree days (GDD) were evaluated for the soybean growing seasons from May to November in 2023 and 2024 at the Virginia field site to characterize the parental environments in which F1 seeds were produced. The two field seasons differed primarily in water availability. Total seasonal rainfall was substantially higher in 2023 (840.7 mm) than in 2024 (495.8 mm), indicating that the 2024 parental growing season was markedly drier (Figure 1A). In contrast, cumulative thermal accumulation was similar between years, with 1953 GDDs in 2023 and 1924 GDDs in 2024 (Figure 1B). Because the number of GDDs differed only slightly between seasons, whereas rainfall differed substantially, water availability, rather than heat accumulation, appeared to be the main environmental factor distinguishing the two parental growing seasons. Therefore, progeny seed germination and seedling vigor responses should be interpreted as treatment responses under contrasting parental moisture conditions, with 2023 representing a wetter parental environment and 2024 a drier one. This environmental contrast provides important context for interpreting year-dependent responses to parental foliar biostimulant applications.

3.2. Seed Composition Analysis for 2023 and 2024

3.2.1. Seed Weight, Protein and Selected Amino Acid Contents

Parental foliar treatment did not significantly affect the 1000-seed weight, protein concentration, protein per seed, aspartic acid, or glutamic acid within either year (Table 2). Treatment-level means are reported to document the observed ranges. Across-treatment means were numerically lower in 2024 than in 2023 for 1000-seed weight, protein concentration, protein per seed, aspartic acid, and glutamic acid. Because years were analyzed separately, year-to-year patterns are descriptive and should not be interpreted as formal year effects. Additional tables were provided for each variable tested, with p -values from the ANOVA analysis (Supplemental Tables S1 and S2).

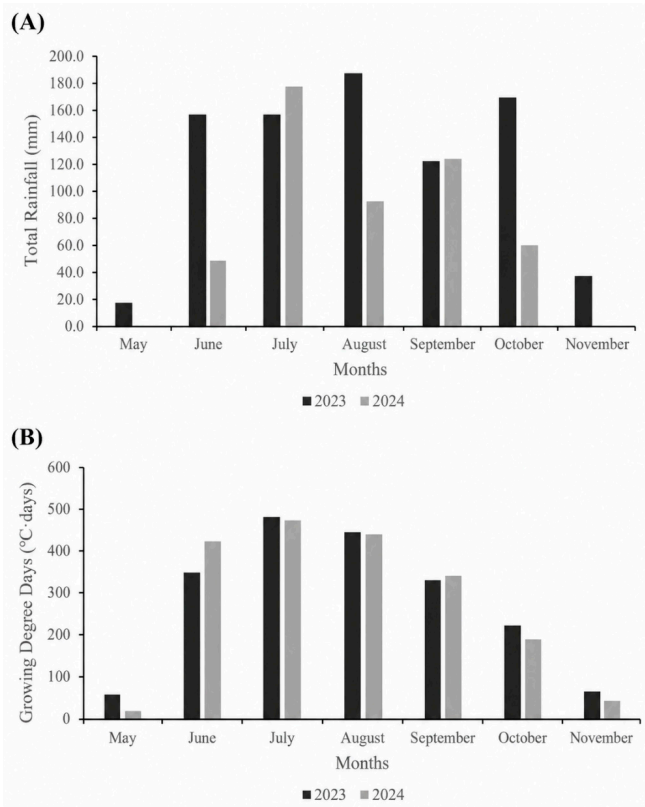


Figure 1. (A) Total rainfall (mm) by field season months, and (B) cumulative growing degree days (GDD) for the soybean growing seasons at Blackstone, VA, 2023–2024.

Table 2. Treatment-level means for 1000-seed weight, protein percentage, protein per seed, and aspartic and glutamic acid amino acids in F1 soybean progeny seeds. Each cell reports the 2023/2024 mean. Years were analyzed separately; no significant paternal-treatment effects were detected within either year (ANOVA, $p > 0.05$).

Treatment	1000-Seed Weight (g)	Protein (%)	Protein per Seed (mg·seed ^{−1})	Aspartic Acid (%)	Glutamic Acid (%)
EZGro 16-0-0	192.10/163.35	40.30/36.67	77.40/59.94	4.60/4.19	7.10/6.44
EZGro 18-0-0	190.68/164.00	39.92/35.85	76.09/58.79	4.60/4.10	7.11/6.24
iQForte	185.18/163.55	40.55/36.70	75.18/60.05	4.65/4.17	7.19/6.41
Urea	197.23/158.25	40.33/36.24	79.58/57.35	4.63/4.12	7.13/6.39
No-foliar control	188.33/164.80	40.03/36.39	75.40/59.98	4.62/4.20	7.14/6.51
Across-treatment mean	191.30/162.79	40.23/36.37	76.73/59.22	4.62/4.16	7.13/6.40

3.2.2. Seed Oil and Fatty Acid Contents

Across all treatments, oil content trended to be higher in seeds from the drier 2024 season, increasing by approximately 3.85% (Table 3). Despite the slight increase in oil concentration in 2024 compared with 2023, the substantially smaller seed size in 2024 resulted in lower absolute oil content per seed. Concurrently, the proportions of palmitic, linoleic, and linolenic acids in the oil fraction trended upward in 2024. Palmitic acid rose from an average of 11.7% in 2023 to 12.1% in 2024, linoleic acid increased from 55.4% to 57.1%, and linolenic acid showed the largest relative increase, from 7.5% to 8.6%.

Table 3. Treatment-level means for oil concentration, oil per seed, and fatty acid composition in F1 soybean progeny seeds. Each cell reports the 2023/2024 mean. Years were analyzed separately; no significant parental-treatment effects were detected within either year (ANOVA, $p > 0.05$).

Treatment	Oil (%)	Oil per Seed (mg·seed ^{−1})	Linoleic Acid (%)	Linolenic Acid (%)	Oleic Acid (%)	Palmitic Acid (%)	Stearic Acid (%)
EZGro 16-0-0	19.80/20.93	38.10/34.20	55.64/57.16	7.60/8.60	23.77/21.25	11.44/11.91	3.96/3.76
EZGro 18-0-0	19.90/20.87	37.90/34.20	55.20/58.43	7.63/8.88	22.55/20.36	11.98/12.39	4.04/3.91
iQForte	19.90/20.70	36.80/33.80	55.25/55.14	7.47/8.54	24.57/21.29	11.75/12.00	3.90/3.76
Urea	20.10/20.53	39.60/32.50	55.42/57.40	7.26/8.59	23.41/21.18	11.82/12.24	3.96/3.62
Control	20.20/20.73	38.10/34.20	55.32/57.21	7.38/8.38	25.11/23.81	11.61/12.06	3.88/3.65
Across-treatment mean	19.98/20.75	38.10/33.78	55.37/57.07	7.47/8.60	23.88/21.58	11.72/12.12	3.95/3.74

3.3. AOSA Warm Germination Test

Progeny seed performance was evaluated under standard AOSA warm germination conditions for germination percentage, seedling vigor index, root length, shoot length, root:shoot ratio, root dry matter, shoot dry matter, and total dry matter (Figure 2). For PH treatments only, a PH-specific positive response (denoted by † in the Figures) was defined as a response in which the PH treatment was significantly greater than both the foliar urea N comparator and the base-fertilizer/no-foliar control within the same year, assay, stress condition, and response trait. An asterisk (*) indicates that a PH treatment was significantly greater than the foliar urea N comparator only. In the wetter 2023 parental environment, EZGro 16-0-0 produced PH-specific positive responses for germination (92.00%), vigor index (4.19), and root length (10.24 cm). EZGro 18-0-0 produced PH-specific positive responses for germination (84.00%), root dry matter (1.70 g), and total dry matter (9.28 g). iQForte produced a PH-specific response for total dry matter (9.18 g), while its germination response was greater than that of urea only.

In the drier 2024 parental environment, EZGro 18-0-0 led to the highest germination percentage (96.50%) and exceeded both the urea (93.75%) and the no-foliar control (94.75%) treatments (see bars labeled by † in Figure 2). EZGro 16-0-0 and iQForte responses exceeded urea for germination but did not exceed the no-foliar control and are therefore marked with * in Figure 2. EZGro 16-0-0 also produced a PH-specific positive response for root:shoot ratio (3.36), exceeding that of both urea (2.38) and the no-foliar control (2.70). No PH-specific responses were detected for root dry matter in 2024 or for shoot dry matter.

3.4. Phytotron Drought-Stress Experiment in Soil Medium (2023 Seeds)

Progeny seeds from the wetter 2023 parental season were evaluated under severe drought (watered 1 day/week), moderate drought (watered 3 days/week), and well-watered conditions (watered 7 days/week) (Figure 3). Day of emergence showed no PH-specific response. Under severe drought, EZGro 16-0-0, iQForte, and EZGro 18-0-0 exceeded urea for germination (98.30%, 95.60%, and 92.39%, respectively) (see bars labeled with * in Figure 3). EZGro 16-0-0 and EZGro 18-0-0 treatments exceeded urea for seedling vigor index (5.28 and 4.95, respectively). No PH treatment exceeded both reference treatments for day of emergence, root length, hypocotyl length, root:shoot ratio, or root dry weight under severe drought.

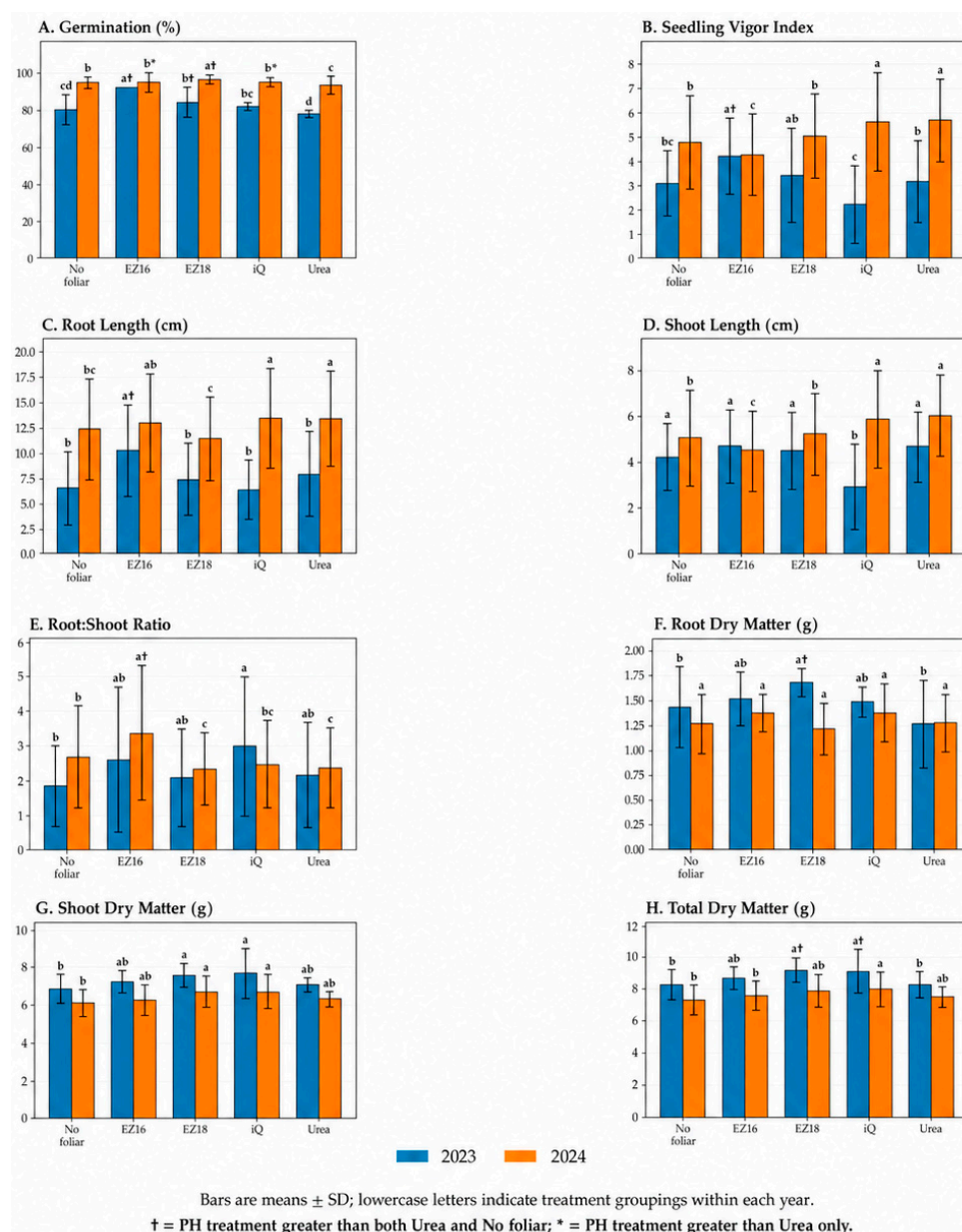


Figure 2. Effects of parental foliar treatments on (A) germination, (B) seedling vigor index, (C) root length, (D) shoot length, (E) root:shoot ratio, (F) root dry matter, (G) shoot dry matter, and (H) total dry matter of F1 soybean progeny seeds evaluated under AOSA warm germination conditions in 2024 and 2025 for seeds in 2023 and 2024. Bars represent treatment means ± standard deviation from four replications of 50 seeds per treatment. Different lowercase letters indicate significant differences among parental foliar treatments within each year and response variable according to the mean-separation test at $p \leq 0.05$. The symbol † indicates a response significantly greater than both the urea N comparator and the base-fertilizer/no-foliar control treatments. An asterisk (*) indicates, for PH treatments only, a response significantly greater than the urea treatment. EZ16 = EZGro 16-0-0; EZ18 = EZGro 18-0-0; iQ = iQForte.

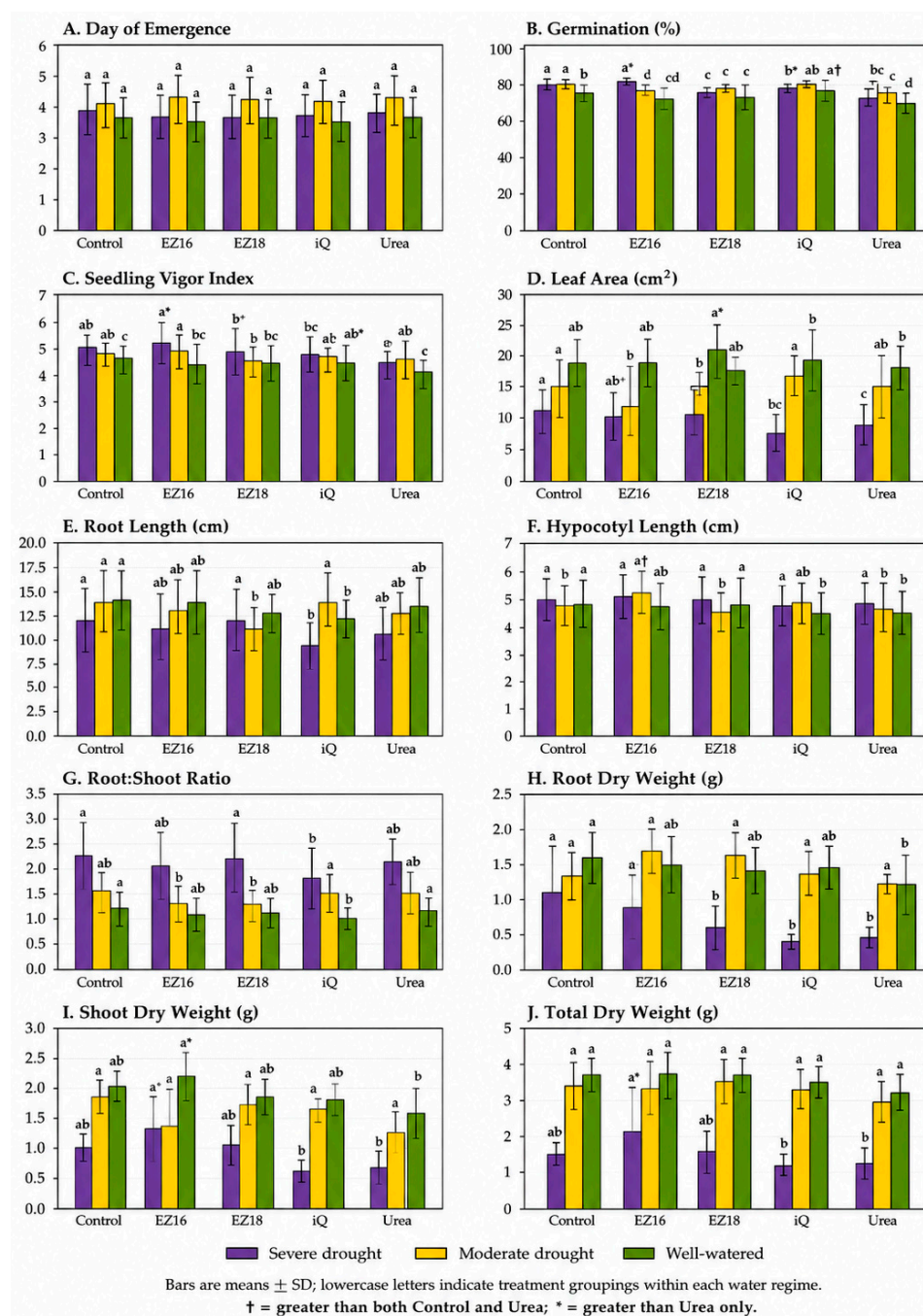


Figure 3. Effects of parental foliar treatments on F1 soybean progeny seeds collected in the 2023 growing season and grown in the phytotron under severe and moderate drought and well-watered conditions. Bars represent treatment means $\pm$ standard deviation for (A) day of emergence, (B) germination %, (C) seedling vigor index, (D) leaf area (cm²) (E) root length (cm), (F) hypocotyl length (cm), (G) root:shoot ratio, (H) root dry weight (g), (I) shoot dry weight (g), and (J) total dry weight (g). Lowercase letters indicate significant differences among treatments within each water regime based on the mean-separation test at $p \leq 0.05$. The symbol † indicates, for PH treatments only, a response significantly greater than that of both the foliar urea N comparator and the base-fertilizer/no-foliar control. An asterisk (*) indicates, for PH treatments only, a response significantly greater than that of the urea treatment only.

Under moderate drought, a broader range of PH-specific effects emerged. EZGro 16-0-0 was the only treatment to achieve a PH-specific response (†), producing a significantly greater hypocotyl length (5.49 cm) than both urea (4.89 cm) and the control (5.00 cm). There was no significant difference for germination, seedling vigor index, leaf area, root length,

root:shoot ratio, root dry weight, shoot dry weight, and total dry weight between any of the treatments.

Under well-watered conditions, iQForte was the only treatment to achieve a PH-specific response, producing the highest germination percentage (96.49%) and exceeding both urea (87.56%) and the no-foliar control (94.49%). EZGro 18-0-0 showed several * responses different from urea only, outperforming it for germination percentage (89.71%), leaf area (24.31 cm² vs. 20.31 cm²), hypocotyl length (5.05 cm vs. 4.72 cm), and seedling vigor index (4.56 vs. 4.15), although these traits did not exceed those of the no-foliar control. EZGro 16-0-0 also showed a better response than urea for shoot dry matter (2.45 g vs. urea at 2.02 g). Overall, drought-stress responses were water-regime-specific rather than uniform across traits.

3.5. Phytotron Temperature-Stress Experiment in Soil Medium (2024 Seeds)

Progeny seeds from the 2024 parental season were evaluated under three temperature regimes: low (20/12 °C), optimal (30/20 °C), and high (38/25 °C) using a soil-based cup system. As in the previous two assays, PH-specific responses were marked as “*” when a PH treatment was significantly greater than the urea treatment only, and as “†” when significantly greater than both urea and the no-foliar control. In this temperature-stress experiment, no PH treatment met the PH-specific criterion for any trait under any temperature regime; however, several PH products exhibited significant advantages over the urea treatment alone under suboptimal temperatures (Figures 4 and 5).

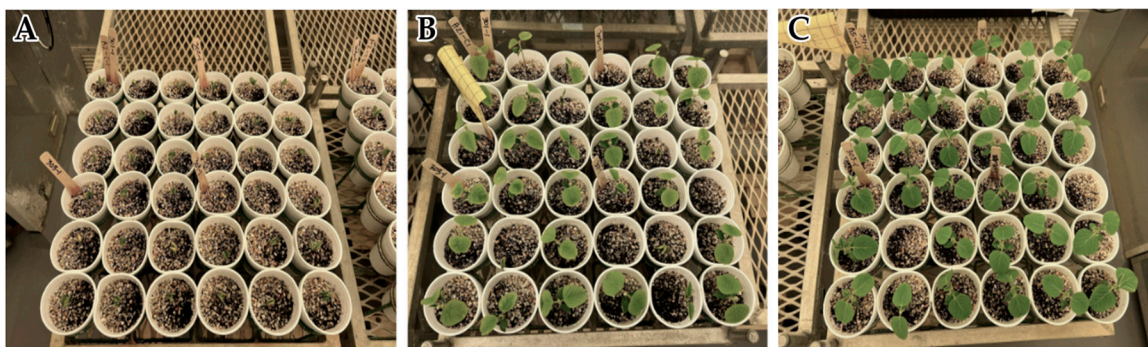
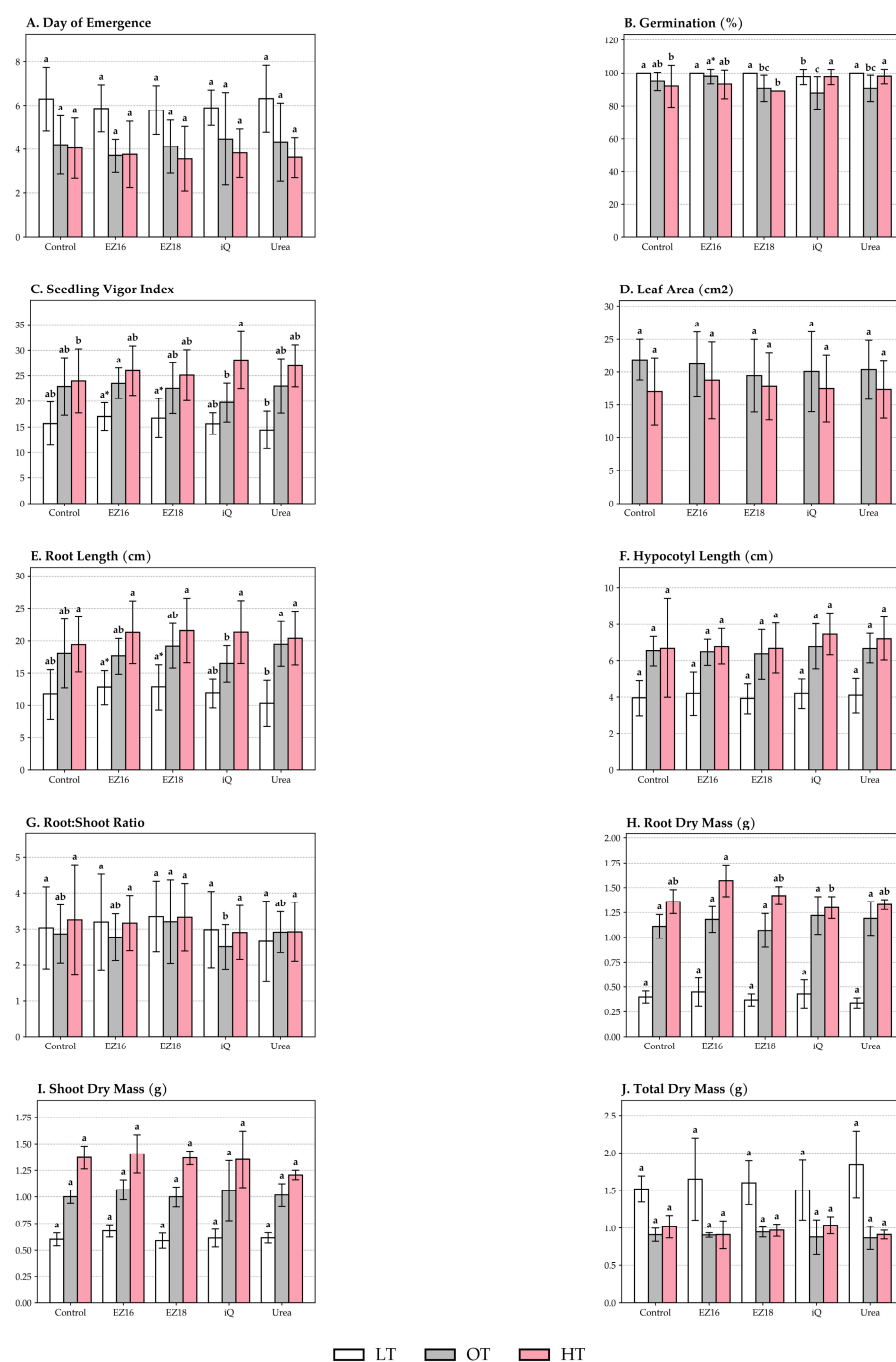


Figure 4. Soybean temperature-stress study at the NCSU Phytotron after 10 days of planting. Temperature settings were (A) low temperature (20/12 °C), (B) optimal temperature (30/20 °C), and (C) high temperature (38/25 °C).

Under low-temperature stress (20/12 °C), EZGro 16-0-0 and EZGro 18-0-0 treatments showed significantly different responses relative to urea. EZGro 16-0-0 produced a seedling vigor index of 17.03, which was significantly greater than that of urea (14.41) (bars marked with * in Figure 5); however, it did not differ from that of the no-foliar control (15.67) (Figure 5). EZGro 18-0-0 resulted in a higher seedling vigor index (16.71) than that of urea (14.41). Both EZGro 16-0-0 and EZGro 18-0-0 were greater than urea for root length (12.74 and 12.77 cm, respectively, vs. 10.31 cm for urea). Leaf area could not be measured under low-temperature conditions because seedling development was limited by the cooler temperature regime, and seedlings lacked a first true leaf.

Under optimal temperature (30/20 °C), few differences among treatments were observed. EZGro 16-0-0 led to a germination percentage of 97.71%, which was significantly higher than that of urea (90.48%), but not different from that of the no-foliar control (94.79%) (bars marked with * in Figure 5). For all other traits measured under optimal temperature, including day of emergence, SVI, leaf area, root length, hypocotyl length, root-to-shoot ratio, root dry mass, shoot dry mass, and total dry mass, no significant differences were detected among any of the parental treatments, and no PH outperformed urea. At high

temperatures (38/25 °C), no significant differences were found in physiological traits, with PH treatments performing no better than urea or both urea and the no-foliar control.



Bars are means ± SD; lowercase letters indicate treatment groupings within each temperature condition.
* = significantly greater than the Urea treatment.

Figure 5. Phytotron temperature-stress responses in F1 soybean progeny seeds collected in the 2024 growing season. Bars represent treatment means ± standard deviation for EZGro 16-0-0, EZGro 18-0-0, iQForte, urea, and the base-fertilizer/no-foliar control under low-temperature (LT; 20/12 °C), optimal-temperature (OT; 30/20 °C), and high-temperature (HT; 38/25 °C) regimes. Traits were (A) day of emergence, (B) germination %, (C) seedling vigor index, (D) leaf area (cm²), (E) root length (cm), (F) hypocotyl length (cm), (G) root:shoot ratio, (H) root dry mass (g), (I) shoot dry mass (g), and (J) total dry mass (g). Lowercase letters indicate significant differences among treatments within each temperature regime based on the mean-separation test at $p \leq 0.05$. An asterisk (*) indicates, for PH treatments only, a response significantly greater than that of the foliar urea N comparator.

In summary, the temperature-stress results showed that parental PH application produced selective progeny responses under specific temperature regimes, but these responses were expressed as advantages over the foliar urea N comparator rather than as responses exceeding both urea and the no-foliar control. The absence of PH-specific responses in this assay indicates that temperature-related PH effects were product- and trait-dependent and were most evident for low-temperature root elongation and seedling vigor index.

4. Discussion

4.1. Overview of Principal Findings

This study was designed to address a previously unresolved question: Does foliar application of protein hydrolysate (PH) biostimulants to soybean parental plants influence the germination, vigor, and stress responses of their untreated F1 progeny seeds? While PHs are well documented for direct effects on treated plants, including enhanced nutrient acquisition, root architecture modification, antioxidant defense, and stress-responsive signaling [25,27–29], their potential to generate parent–seed carryover effects remains largely unexplored. Our results provide comparative evidence that parental PH application can alter selected progeny seedling traits in a product-specific and stress-dependent manner that differs from responses to the equal-N foliar urea comparator.

The principal findings are threefold. First, the three PH formulations, namely, EZGro 16-0-0 (rich in glycine, proline, and glutamic acid; Table 1), EZGro 18-0-0 (100% glycine), and iQForte (high in glutamic acid and threonine), exhibited markedly different and non-interchangeable effects on progeny seed germination, vigor, and stress tolerance, confirming that amino acid composition is a key determinant of transgenerational biostimulant activity. Second, PH efficacy was strongly conditioned by the parental growing environment (wet 2023 vs. dry 2024; Figure 1) and by the stress regime imposed during progeny evaluation (drought, low temperature, or high temperature; Figures 3 and 5). Third, the equal-N urea treatment provided a critical comparator, where at times urea occasionally improved progeny performance relative to the no-foliar control (e.g., vigor index in 2024; Figure 2). However, the key focus was the distinction between the observed benefits and the biostimulant properties of PHs, rather than their nitrogen content alone.

4.2. Amino Acid Compositions of PHs Reflect Product-Specific Responses

The composition-dependent responses observed in this study align with the growing literature on PH biostimulants [23,24,27,49]. Our results demonstrate that the amino acid profile of the parental PH treatment can program distinct physiological outcomes in progeny seedlings, a phenomenon not previously documented in soybean.

EZGro 16-0-0 (Table 1) produced the broadest response profile and was particularly effective under low-temperature stress and in promoting root growth. In the 2024 phytotron temperature experiment (Figure 5), responses differed from urea only for seedling vigor index and root length under low temperature and for germination percentage under optimal temperature. In the AOSA test of the seeds from the wetter 2023 season, it delivered PH-specific positive responses (as defined in 3.6) for germination, vigor index, and root length (Figure 2). Proline and glycine are known osmoprotectants and reactive oxygen species scavengers that stabilize membranes under cold stress [50,51]. Glutamic acid serves as a precursor for glutathione, a key antioxidant [51].

iQForte (Table 1) showed targeted responses in germination, seedling vigor, and total dry matter. In the 2023 drought-stress study, iQForte produced a PH-specific positive response for germination under well-watered conditions, and responses were different from urea only under severe drought and for the well-watered seedling vigor index (Figure 3). These responses may be consistent with roles of glutamic acid, threonine, lysine, and

branched-chain amino acids in carbon–nitrogen balance and stress-related metabolism, but this interpretation remains hypothetical because seed metabolite pools and gene expression were not measured.

EZGro 18-0-0 (Table 1) provided comparatively stable broad-spectrum responses. In the AOSA test, it produced PH-specific positive responses for germination in both years and for root dry matter and total dry matter in 2023 (Figure 2). Under low temperature, it produced responses different from urea only for seedling vigor index and root length (Figure 5).

4.3. Parental Growing Season Modulates the Response

A striking finding was the strong year $\times$ treatment interaction: PH efficacy depended markedly on the parental growing season. The two seasons differed primarily in rainfall (840.7 mm in 2023 vs. 495.8 mm in 2024; Figure 1), while thermal accumulation was similar. Thus, water availability during seed development was the main environmental factor distinguishing the two seed lots.

Seeds from the drier 2024 season were smaller (Table 2), had lower protein concentration (Table 2), and showed altered fatty acid profiles (Table 3). These compositional shifts are consistent with known trade-offs under parental drought [16–18,41]. The increase in linolenic acid may enhance membrane fluidity, potentially aiding germination under suboptimal temperatures despite reduced seed size.

Importantly, PH efficacy reversed between years: iQForte performed well in 2023 (Figure 2), whereas EZGro 16-0-0 and EZGro 18-0-0 gained prominence in 2024 (Figures 2 and 5). This suggests that paternal environment “conditions” the response to PHs—perhaps by altering the physiological or epigenetic state of the developing seed. This interpretation is consistent with transgenerational priming, where parental environmental cues can modify progeny stress tolerance through epigenetic mechanisms, including DNA methylation, histone modifications, and small non-coding RNAs, enabling plants to respond to environmental cues and generate stress memory that may be transmitted to subsequent generations [38,39,52–57]. In the context of PH, parental treatments might alter seed hormone balance (e.g., ABA:GA ratios), seed composition, or methyl donor availability (e.g., via methionine content), potentially affecting progeny vigor [58,59]. However, this study did not directly measure DNA methylation, histone modifications, small RNAs, or hormone levels; therefore, epigenetic regulation and other proposed mechanisms remain hypotheses requiring targeted molecular validation in future work.

4.4. Potential Mechanisms and Their Limitations

While our phenotypic data provide strong evidence for paternal carryover effects, underlying mechanisms remain hypothetical. We propose several plausible pathways: (1) epigenetic regulation—paternal stress signals may induce DNA methylation, histone modifications, or small RNA changes in developing seeds [52,55–57]; (2) seed hormone balance altered abscisic acid:gibberellin acid ratios affecting dormancy and stress responses [58,59]; (3) seed composition shifts in fatty acid profiles (Table 3) influencing energy availability and membrane stability; and (4) priming of stress pathways, i.e., foliar amino acids preparing paternal plants for stress, transmitted to progeny via metabolic cues [27,48,49]. We emphasize that these remain hypotheses requiring molecular validation.

Several limitations must be acknowledged. First, the study used a single cultivar at one location; multi-location, multi-year field validation is needed. Second, we did not measure epigenetic marks, hormones, or gene expression; future multi-omics studies are essential. Third, the limited number of treatments and replications reduced the power to detect subtle interactions. Fourth, the 2024 seed lot had lower overall quality, reducing opportunities to

detect responses significantly different from both the control and urea treatments. Fifth, the term “parental effect” describes seed-parent influence without implying a specific mechanism, as distinguishing parental effects from true transgenerational inheritance would require multi-generation evaluation [38,39].

4.5. Comparison with Urea: Distinguishing Biostimulant from Nitrogen Effects

We think that the inclusion of the equal-N foliar urea treatment was a methodological strength because it allowed PH-associated responses to be distinguished from those associated with low-dose foliar N application. Urea sometimes produced responses comparable to PH treatments, indicating that low-dose foliar N may influence parent-plant or progeny seed responses. PH-specific responses occurred only when a PH treatment exceeded both urea and the no-foliar control, indicating that some PH responses could not be attributed solely to the quantity of N supplied, although the specific biochemical mechanisms remain unresolved.

The low application rate (total foliar N < 1 kg ha⁻¹) relative to base fertilization (~33 kg N ha⁻¹, not counting N acquired by biological N fixation through symbiosis) further argues against a nutritional explanation. PHs are more plausibly acting as signaling molecules modifying paternal hormonal pathways or stress-response networks, consistent with the literature emphasizing their hormone-like activity at very low concentrations [27,29,48].

Although urea is an organic molecule, it is conventionally used as a synthetic nitrogen fertilizer. Urea can be absorbed by plants and hydrolyzed by urease, after which the resulting ammoniacal N is incorporated into amino acids primarily through the glutamine synthetase/glutamate synthase pathway [60]. Including urea at an equivalent nitrogen rate, therefore, provided a useful comparison for separating general foliar N effects from product-specific PH responses. Because PH products differ substantially in amino acid composition, their effects on progeny seed germination and seedling vigor may also be product-specific and stress-dependent.

The equal-N comparison controls the total amount of N applied but does not make the treatments physiologically identical because urea and PHs differ in molecular composition, uptake kinetics, carbon content, and subsequent metabolism. Moreover, urea should not be described as having no signaling activity, as urea, ammonium, nitrate, and whole-plant N status can modulate transporter expression and N-responsive pathways [60,61]. Similarly, a universal energetic advantage of PH-derived N over urea should not be assumed. Organic N may bypass some steps required for inorganic N assimilation, but amino acid and peptide uptake and metabolism also require energy, while urea bypasses nitrate reduction before its N enters ammonium-assimilation pathways [60–63]. Therefore, differences between the PH and equal-N urea treatments would be consistent with PH activity beyond the quantity of supplied N, but they would not independently prove a specific signaling or energetic mechanism.

Protein hydrolysates, in contrast, supply reduced organic N directly as free amino acids and peptides. In addition to serving as N-containing metabolites, PH constituents may alter nutrient uptake, carbon–nitrogen metabolism, root development, hormone-like responses, osmotic adjustment, and antioxidant defense [22,25,26,61]. Thus, parental responses to urea would be expected to arise mainly through altered parental N status, assimilate production, and seed provisioning, whereas PH-mediated responses could involve these nutritional pathways together with amino acid- or peptide-associated biostimulant activity.

4.6. Limitations and Future Directions

The findings therefore have implications for sustainable agriculture. PHs are derived from renewable sources, are biodegradable, and can be applied at low rates, making them potentially useful tools for targeted seed-quality management [19,23,48]. The results suggest that parental PH application can influence selected progeny seedling traits without direct treatment of the seed or seedling, which may be relevant where early-season drought or temperature stress limits soybean establishment.

Our results also highlight formulation specificity: not all PHs are equivalent. The optimal choice depends on target stress and paternal environment, suggesting that “tailored” biostimulant strategies could be more effective than generic applications.

Future research should prioritize: (i) mechanistic studies elucidating molecular, hormonal, and epigenetic bases of paternal PH carryover; (ii) field validation across multiple locations and years; (iii) exploration of synergistic effects by combining PHs with complementary amino acid profiles; and (iv) investigation of seed microbiome roles in mediating paternal effects.

5. Conclusions

Figure 6 displays the proposed parental carryover mechanism by which foliar protein hydrolysate applications to soybean parental plants influenced untreated F1 progeny seed performance. Overall, the results supported the proposed hypotheses. For H1, progeny from parental plants treated with PH formulations differed from the base-fertilizer/no-foliar control for selected traits, including germination, seedling vigor, root length, root:shoot ratio, hypocotyl length, and dry matter accumulation; however, these responses were not consistent across all measured traits. For H2, the inclusion of the equal-N foliar urea comparator showed that several PH responses could not be explained solely by the small quantity of foliar N applied, because each PH produced at least one response that exceeded urea, and several responses exceeded those of both urea and the no-foliar control. For H3, the response patterns differed among EZGro 16-0-0, EZGro 18-0-0, and iQForte, supporting the hypothesis that PH effects were formulation-dependent and likely related to differences in amino acid and peptide composition. EZGro 16-0-0 showed the broadest response profile, particularly for germination, vigor, root length, root:shoot ratio, and selected drought- and low-temperature-associated traits; EZGro 18-0-0 showed more stable responses across germination, dry matter, leaf area, and low-temperature root traits; and iQForte produced more targeted responses in germination, seedling vigor, and total dry matter. For H4, PH effects clearly depended on the progeny growth environment, but the response was not simply stronger under all stress conditions. Instead, treatment effects were stress-specific, with responses occurring under AOSA warm germination, severe drought, moderate drought, well-watered phytotron conditions, low temperature, and optimal temperature depending on the PH product and trait measured. Collectively, these findings indicate that parental plant foliar PH applications can alter F1 soybean progeny performance, but the effects are product-specific, trait-specific, and environment-dependent rather than universal. Future work should determine whether these parent–seed carryover responses are mediated by changes in seed metabolite profiles, hormone balance, reserve mobilization, gene expression, or epigenetic regulation.

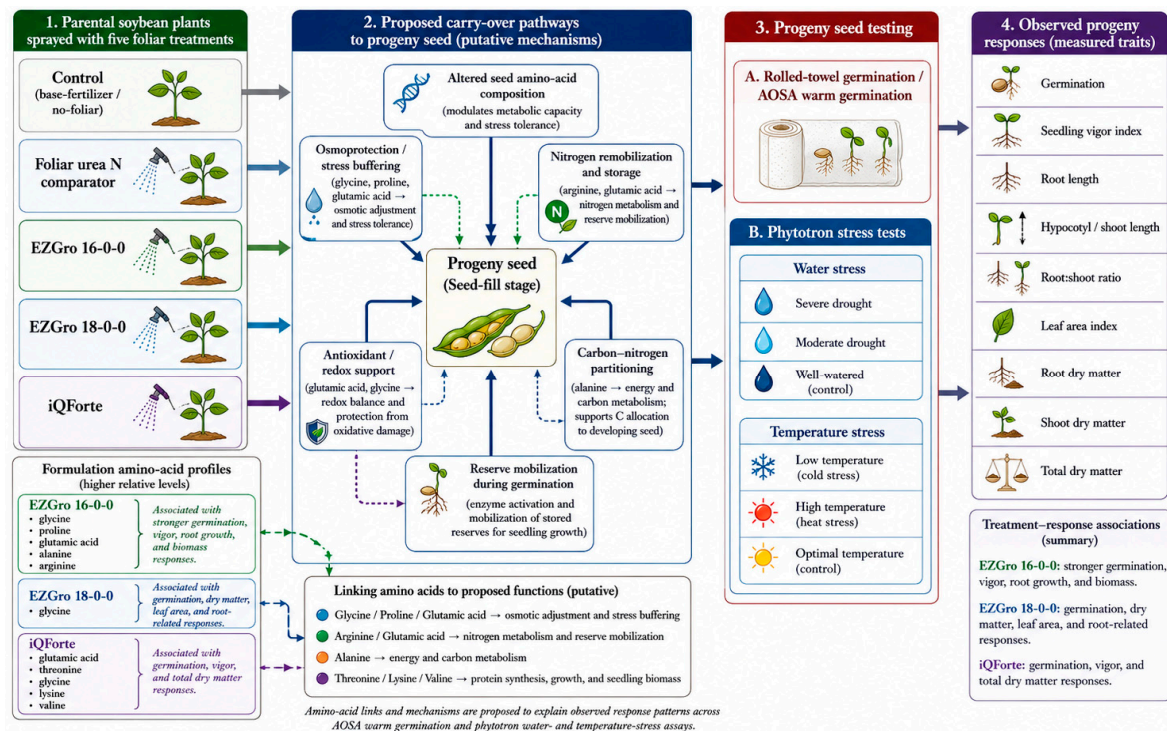


Figure 6. Proposed parent–seed carryover model linking parental plant foliar protein hydrolysate treatments, amino acid-associated pathways, and F1 soybean progeny responses under germination and stress assays.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/agriculture16141515/s1>: Table S1: Seed chemistry concentrations and composition values by treatment and year. Values are mean $\pm$ standard deviation. Saturated Fatty Acid (SFU):Unsaturated Fatty Acid (UFA). The no-foliar control received base fertilizer but no foliar spray. Urea comparator = foliar urea N comparator. p Treat = within-year treatment-effect p -value. p Year and p Year $\times$ Treat are shown to document year differences and year-dependent treatment responses; Table S2: Seed-level calculated values by treatment and year. Values are mean $\pm$ standard deviation. 1000-seed weight in g is numerically equivalent to single-seed mass in mg seed^{−1}. Seed-level masses were calculated as single-seed mass $\times$ concentration/100. Fatty acid-in-seed values were calculated as oil % $\times$ fatty acid %/100; amino acid-in-seed values were calculated as protein % $\times$ amino acid %/100. Kcal values were recalculated uniformly from chemistry percentages and are best interpreted as a comparative energy index unless the final manuscript defines the kcal basis explicitly.

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Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

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Abbreviations

The following abbreviations are used in this manuscript:

AOSA	Association of Official Seed Analysts
GDD	Growing Degree Days
HT	High Temperature
LA	Leaf Area
LT	Low Temperature
NIR	Near-Infrared Spectroscopy
OT	Optimal Temperature
RCBD	Randomized Complete Block Design
SVI	Seedling Vigor Index

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