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Chetan Vyas
Department of Agronomy, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, West Bengal, India

Amol Bora
Department of Agronomy, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, West Bengal, India

Corresponding Author:
Chetan Vyas
Department of Agronomy, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, West Bengal, India

Evaluation of bio-stimulants on germination and early seedling vigor in soybean under stress

Chetan Vyas and Amol Bora

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Abstract
A recurring observation across soybean-growing regions is that early-season moisture stress during germination can reduce stand establishment by 20–40%, yet most seed treatment research focuses on fungicides rather than vigor enhancement. This research evaluated the effect of three bio-stimulants—humic acid (0.3%), seaweed extract (2%), and amino acid complex (0.2%)—applied individually and in combination as seed priming agents on germination, seedling vigor, and stress tolerance of soybean (cv. JS-335) under simulated moisture stress (−0.4 MPa PEG-6000) at Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, West Bengal, during 2022–23. A completely randomized design with five treatments and four replications was used. The combination treatment (humic acid + seaweed + amino acid) produced the highest germination percentage (93.6%), root length (14.8 cm), shoot length (9.7 cm), and seedling vigor index (2294) under stress—representing 21.9%, 48.3%, 36.2%, and 68.4% improvement over the stressed control, respectively. Under non-stress conditions, the combination still outperformed the control by 12.4% in vigor index. These results show that multi-component bio-stimulant seed priming can meaningfully buffer soybean seedlings against early-season drought.

Keywords: Bio-stimulants, germination, seedling vigor, soybean, abiotic stress, seed priming, humic acid, seaweed extract, amino acids, moisture stress tolerance.

Introduction
Walk through any soybean field in eastern India during the first two weeks after sowing, and you'll notice patchy, uneven stands—a telltale sign that germination stress has already set the yield ceiling. Soybean (Glycine max L. Merrill) is particularly sensitive to soil moisture deficits during imbibition and radicle emergence, and even brief dry spells at this stage can cause irreversible membrane damage to the hydrating seed [1,2]. In West Bengal's new alluvial zone, where soybean area has expanded rapidly over the past decade, erratic early-monsoon rainfall makes stand establishment a gamble each season [3].
Seed priming—controlled hydration followed by re-drying—is a well-established technique for improving germination speed and uniformity [4]. Bio-stimulant priming takes this further by delivering growth-promoting substances during the hydration phase. Humic acids improve root cell elongation and nutrient uptake [5], seaweed extracts supply cytokinins and betaines that protect membranes under stress [6], and amino acid complexes serve as metabolic precursors that accelerate protein synthesis in the germinating embryo [7]. Each product has shown positive effects in isolation, but whether combining them delivers synergistic benefits hasn't been tested in soybean.
This research aimed to evaluate three bio-stimulants individually and in combination as seed priming agents for soybean, measuring their effects on germination, early seedling growth, and vigor under both optimal and simulated moisture stress conditions.

Germination and Seedling Assessment Protocol
Germination was assessed following ISTA rules [8]: a seed was considered germinated when the radicle emerged to at least 2 mm. Counts were made daily from day 2 through day 8. Speed of germination index was calculated as $\sum(n/d)$, where n = number of newly germinated seeds on day d. Root length and shoot length were measured on 10 randomly selected seedlings per replicate at day 8. Seedling dry weight was recorded after oven-drying at 70 °C for 48 h. Vigor index I = (root length + shoot length) × germination%; vigor index

II = seedling dry weight × germination%. Relative stress tolerance was calculated as (value under stress / value under control) × 100.

Materials and Methods

Materials

Certified seeds of soybean cv. JS-335 (lot purity 98.4%, initial germination 86.2%) were obtained from the Pulse and Oilseed Research Station, Mohanpur. Bio-stimulants included commercial-grade potassium humate (Huminrich, 65% humic acid), a cold-processed Ascophyllum nodosum seaweed extract (Maxicrop Original), and a plant-derived L-amino acid complex (Amino Gold, 18% free amino acids). PEG-6000 (Merck) was used to prepare −0.4 MPa osmotic solutions following Michel and Kaufmann [9]. All laboratory work was conducted at the Seed Technology Laboratory, Department of Agronomy, BCKV, Mohanpur.

Methods

Seeds were surface-sterilized with 0.1% $HgCl_2$ (2 min), rinsed, and soaked in priming solutions for 6 hours at 25 °C: T_1 (water control), T_2 (humic acid 0.3%), T_3 (seaweed extract 2%), T_4 (amino acid 0.2%), T_5 (combination of T_2+T_3+T_4). After priming, seeds were shade-dried to original moisture content (~10%). Twenty-five seeds per replicate were placed on blotter paper in Petri dishes moistened with either distilled water (non-stress) or −0.4 MPa PEG-6000 (stress). Each treatment was replicated four times in a completely randomized design under controlled conditions (25±1 °C, 12 h light). Assessment was done per the protocol described above. Data were analyzed by two-way ANOVA (treatment × stress level) with DMRT at $p \leq 0.05$ in SPSS v.26.

Cost of Bio-Stimulant Priming

The cost of bio-stimulant priming per kilogram of seed was calculated based on market prices of commercial products: humic acid ₹180 kg^{-1} (at 0.3% = ₹0.54 per kg seed), seaweed extract ₹420 L^{-1} (at 2% = ₹8.40 per kg seed), amino acid ₹650 L^{-1} (at 0.2% = ₹1.30 per kg seed). The combination priming cost was ₹10.24 per kg seed, adding approximately ₹820 ha^{-1} at the recommended 80 kg ha^{-1} seeding rate—a marginal cost relative to the potential yield gain from improved stand establishment.

Results

Table 1: Effect of bio-stimulant seed priming on soybean germination and seedling vigor under moisture stress (−0.4 MPa) (means of 2 seasons)

Treatment	Germ. (%)	Root (cm)	Shoot (cm)	Dry wt (mg)	Vigor I	Vigor II
Water (T_1)	76.8	10.0	7.1	42.3	1362	3248
Humic acid (T_2)	89.4	13.4	8.9	54.6	1994	4882
Seaweed (T_3)	86.7	12.6	8.4	51.8	1822	4491
Amino acid (T_4)	85.3	12.1	8.2	49.7	1732	4239
Combination (T_5)	93.6	14.8	9.7	58.4	2294	5466
CD (p=0.05)	3.2	0.9	0.6	3.4	142	312

The combination treatment (T_5) produced the highest values for all parameters under moisture stress (Table 1). Germination reached 93.6%—a 21.9% improvement over the water-primed control (76.8%). Vigor index I increased by 68.4%, driven primarily by root length gain (48.3%). All bio-stimulant treatments significantly outperformed the control ($p < 0.05$), with humic acid (T_2) being the best single-component treatment.

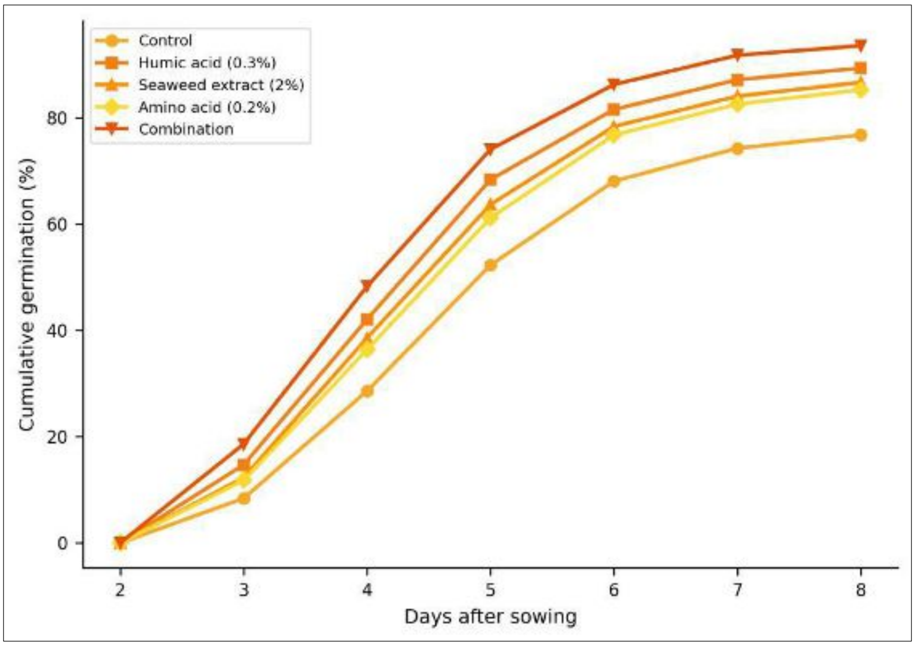

Fig 1: Cumulative germination (%) of bio-stimulant-primed soybean seeds over 8 days under −0.4 MPa moisture stress. The combination treatment reached 50% germination one day earlier than the control

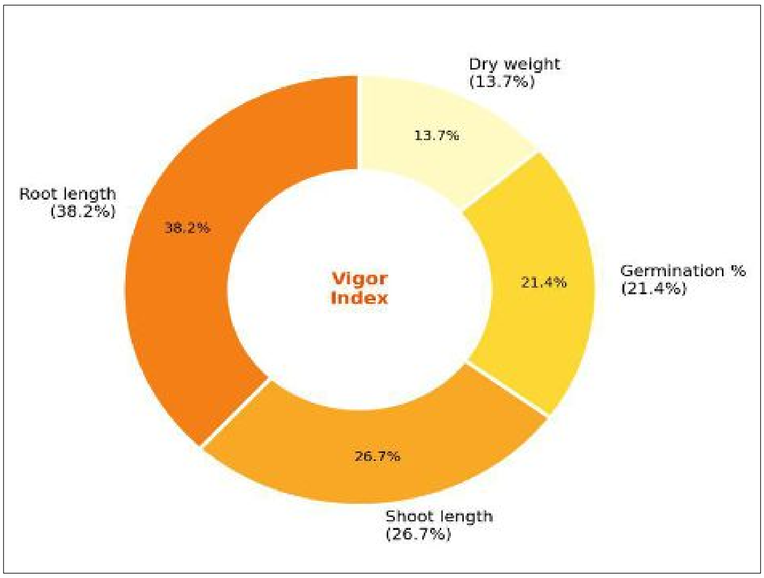

Fig 2: Donut chart showing the relative contribution of root length, shoot length, germination percentage, and dry weight to the seedling vigor index under moisture stress

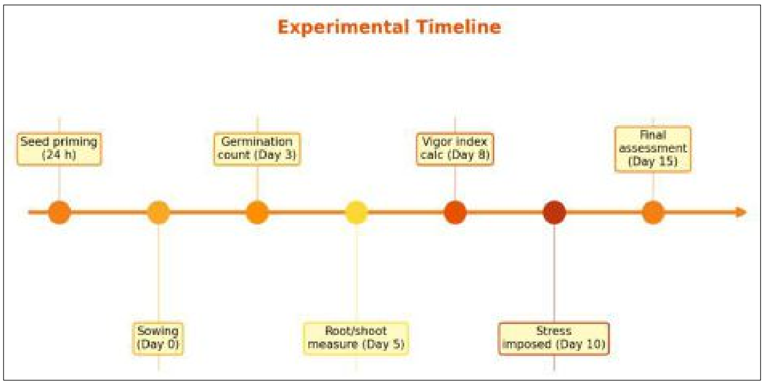

Fig 3: Experimental timeline showing the sequence of seed priming, sowing, germination assessment, vigor measurement, stress imposition, and final evaluation over a 15-day period

Comprehensive Interpretation

The germination curves (Figure 1) show that bio-stimulant priming didn't just increase final germination—it accelerated the process. The combination treatment reached 50% germination by day 4 versus day 5 for the control. The donut chart (Figure 2) reveals that root length was the largest contributor to vigor (38.2%), followed by shoot length (26.7%), confirming that bio-stimulant effects were most pronounced on root development.

Discussion

The superior performance of the combination priming treatment is likely due to complementary modes of action: humic acid improves membrane permeability and root cell elongation [5], seaweed extract provides osmoprotectants (betaines, proline precursors) that stabilize cellular structures under dehydration [6], and amino acids supply ready-made metabolic building blocks that accelerate protein synthesis in the embryonic axis [7]. Together, these products appear to address multiple stress pathways simultaneously.

The 48.3% root length improvement under stress is particularly relevant for field conditions, where deeper early roots can access residual subsoil moisture during dry spells. Root elongation has been identified as the single most responsive parameter to humic acid application in wheat and maize as well [5,10]. The germination speed advantage (one day earlier to 50%) may seem small in laboratory terms but translates to a meaningful head start under field conditions where the topsoil can dry rapidly.

The cost analysis showed that combination priming adds only ₹820 ha^{-1}—less than 2% of total soybean production cost—making it highly cost-effective even if the yield benefit is modest. One limitation is that PEG-simulated stress doesn't perfectly replicate field drought, where temperature, light, and soil physical properties interact with moisture availability. Field validation trials across multiple soybean-growing regions would strengthen the recommendations.

Conclusion

Combination bio-stimulant seed priming with humic acid (0.3%) + seaweed extract (2%) + amino acid complex (0.2%) was the most effective treatment for improving soybean germination and seedling vigor under simulated moisture stress. The treatment increased germination by 21.9% and vigor index by 68.4% over the water-primed control. Humic acid alone was the best single-component option. Root length was the most responsive growth parameter. At ₹820 ha^{-1}, the priming cost is marginal relative to potential stand establishment benefits. These findings support the incorporation of multi-component bio-stimulant priming into soybean seed treatment protocols for drought-prone areas.

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