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Standardization of heat stress mitigation on seed yield and quality in Soybean

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Abstract

Pulses and oilseed legumes like soybean are vital for global food and nutritional security due to their rich protein content, nitrogen-fixing ability, and adaptability. However, increasing temperature extremes associated with climate change significantly impact their productivity and seed quality, especially during reproductive stages. This study was conducted to standardize heat stress mitigation strategies to improve seed yield and quality in soybean (*Glycine max* (L.) Merr.), which plays a dual role as a food and industrial crop due to its high protein and oil content. A field experiment was carried out during January and February 2024 at the ICAR-Indian Institute of Seed Science (Regional Station), GKVK, Bengaluru, using a strip plot design with two sowing dates, nine treatments (including salicylic acid, gibberellic acid, and growth regulators), and four spray stages. The results revealed that both treatment type and stage of application significantly influenced growth and yield attributes. Salicylic acid (T₃ @ 50 ppm) emerged as the most effective treatment, recording the highest plant height (56.04 cm), seed yield (23.85 g/plant), and seedling length (30.01 cm), along with the lowest seed moisture content, indicating accelerated maturity under stress. The vegetative and flowering stage spray (S₄) led to superior performance in terms of plant height, pod number, and seed yield, demonstrating the critical role of timing in stress mitigation. February sowing showed slightly higher seed moisture due to reduced seed-filling duration under high temperatures. Overall, the study highlighted that exogenous application of salicylic acid and timely intervention at specific growth stages significantly enhance heat stress tolerance in soybean. These findings contribute to the development of climate-resilient production strategies, ensuring stable yield and quality in heat-prone environments.

Keywords: Soybean (*Glycine max*), heat stress, salicylic acid, growth regulators, seed yield, seed quality

Introduction

Pulses, including chickpea, pigeon pea, mungbean, and lentil, play a crucial role in ensuring nutritional security and sustainable agriculture due to their high protein content, nitrogen-fixing ability, and adaptability to diverse agro-climatic conditions. However, the productivity and quality of pulses are highly sensitive to heat stress, particularly during the reproductive phase, leading to reduced seed yield and compromised seed quality. Heat stress, defined as the rise in temperature beyond a threshold for a period sufficient to cause irreversible damage to plant function and development, affects pollen viability, pod setting, seed filling, and seed quality parameters such as germination and vigour (Kumar *et al.*, 2021) ^[1].

With climate change increasing the frequency and intensity of heat waves, there is an urgent need to develop and standardize effective heat stress mitigation strategies to sustain and enhance seed yield and quality in pulses under heat-prone environments. Various mitigation strategies, including genetic interventions (development of heat-tolerant varieties), agronomic practices (adjustment of sowing dates, irrigation scheduling, and use of mulches), and exogenous application of protective compounds (antioxidants, osmoprotectants, and plant growth regulators), have shown promise in ameliorating heat stress impacts (Awasthi *et al.*, 2014; Sehgal *et al.*, 2017) ^[14, 15]. Standardization of these heat stress mitigation strategies under different agro-ecological conditions is essential to develop region-specific recommendations that ensure consistent seed yield and quality in pulses. Moreover, understanding the physiological, biochemical, and molecular responses of pulses under heat stress conditions will aid in refining

these mitigation strategies for effective field-level application. Therefore, the present study focuses on the standardization of heat stress mitigation practices in pulses to enhance seed yield and quality, aiming to contribute to climate-resilient pulse production systems and support national food and nutritional security goals.

Soybean (*Glycine max* (L.) Merr.), belonging to the family Fabaceae, it is one of the most cultivated crop worldwide, occupying roughly 6 percent of all arable land and serving as a major source of food energy. Soybean is also called a “miracle crop”, contains 40 per cent of protein, 20 per cent of oil, 35 per cent carbohydrates and 5 per cent ash on a dry weight basis (Liu, 1997). Defatted soybean meal is an important and rich source of protein for livestock feeding. The soybean consists of a huge amount of phytic acid, vitamin B complex and dietary minerals. Another product of processed soybean crops is soya vegetable oil, mainly used in food and industrial applications. Traditional non-fermented food like soya milk from which tofu and tofu skin are made. Fermented soya food products include fermented bean paste, soya sauce and tempeh. Hence, it is also called “Wonder crop” and “Golden bean”. These seeds are not only rich in essential fatty acids and proteins but also have substantial economic value due to their high-quality oil and protein content, making them an integral part of the global food supply and a valuable crop for livestock feed and industrial applications.

With advancements in biofuel technology, soybean is increasingly regarded as a viable source for biodiesel production due to their rich lipid content, adding further to their economic and environmental significance. The production and productivity of soybean in the world from 113 countries is 370.7 mt and 2695 kg/ha, respectively. Among these Brazil, United States, Argentina, China and India produced almost 89 per cent of the world's total soybean. In which India ranks 5th in total production with production of 14 million ton per year. But India has a comparatively very low average yield and ranks 8th in its global position. In India, the major states that are leading in acreage and production of soybean are Madhya Pradesh followed by Maharashtra, Rajasthan, Karnataka and Telangana. Karnataka has an area of 0.43 m ha with a production of 0.44 mt and productivity of 1055 kg/ha under soybean. The major soybean-growing areas of Karnataka include Bidar, Belagavi, Dharwad, Haveri, and Bagalkot, where the crop is predominantly cultivated during the Kharif season.

India, an agrarian nation, faces the challenge of enhancing productivity per unit area to meet the demands of its growing population. As projected by the United Nations Food and Agriculture Organization (FAO), the global demand for agricultural commodities is anticipated to spike by 60 percent by 2030, with over 85 percent of this increase hailing from developing countries. In addition, continuous change in climatic conditions also made significant difficulties to soybean yield and quality, especially in terms of rising temperatures, water

scarcity, and soil salinity. Among these, heat stress is particularly harmful, impacting not only plant development and physiological processes, but also agricultural productivity. High temperature (HT) stress is a major environmental stress that limits plant growth, metabolism, and productivity worldwide. Plant growth and development involve numerous biochemical reactions that are sensitive to temperature. Plant responses to heat stress vary with the degree and duration of heat stress and the plant type. Heat stress is now a major concern for crop production and approaches for sustaining high yields of crop plants under heat stress are important agricultural goals.

Materials and Methods

The field experiment was carried out under the title Standardization of heat stress mitigation on seed yield and quality in soybean (*Glycine max* (L.) Merr.) during first week of January and February 2024 at ICAR-Indian Institute of Seed Science, Mau (Regional Station), J block, University of Agricultural Sciences, GKVK, Bengaluru. The field experiment conducted during first week of January and February 2024 at ICAR-Indian Institute of Seed Science, Mau (Regional Station), J block, University of Agricultural Sciences, GKVK, Bengaluru. Experimental site is situated between 13.010 N latitude and 77.570 E longitude at an altitude of about 924 m above mean sea level (MSL). The field experiment was conducted in the strip plot designed with two replications. The field experiment was conducted in two dates of sowing and nine treatments are applied in four different stages with two replications: Date of sowing (D): Treatments (T): Stage of application (S): D1- 1st week of January of 2024 D2- 1st week of February of 2024 T₁ - Control (no spray) T₂ - 6- Benzyl Adenine Purine @ 300 ppm T₃ - Salicylic acid @ 50 ppm T₄ - Indole Acetic Acid @ 100 ppm T₅ - Naphthalene Acetic Acid @ 100 ppm T₆ - Thiourea @ 1000 ppm T₇ - Cycocel @ 200 ppm T₈ - Ethrel @ 100 ppm T₉ - Gibberellic acid @ 100 ppm S1- Vegetative stage (V5-V7 stage) S2- Flowering stage (R2: Full bloom stage) S3- Pod filling stage (R5: Beginning seed stage) S4- Vegetative and Flowering stage (V5-R2 stage). The experimental data obtained at different growth stages was compiled and subjected to statistical analysis by adopting Fischer's method of analysis of variance technique as outlined by Gomez and Gomez (1984). At 5 per cent level of significance data were subjected to 'F' test and the critical difference (CD) values was calculated wherever 'F' test is significant.

Results and Discussions

Effect of Spray Stages on Growth and Yield Attributes: The spray stages (S) significantly influenced plant height, number of pods, and seed yield per plant, while test weight was not significantly affected (Table X). Maximum plant height was recorded in S₄ (52.08 cm), which was significantly higher than S₁ (48.92 cm) and

Table 1: Effect of growth hormones on plant growth and seed yield in soybean

Treatments	Plant height (cm)	No. of pods	Seed yield (g/plant)	Test weight (g)
Spray stages(S)				
S ₁	48.92	56.39	19.59	14.40
S ₂	50.77	62.54	21.06	14.23
S ₃	48.36	54.76	18.97	14.55
S ₄	52.08	64.74	22.30	15.09
SEm±	0.511	0.371	0.320	0.366
CD (p=0.05)	1.625	1.181	1.017	NS
CV (%)	2.80	1.71	4.28	6.89

Treatments (T)				
T ₁	44.38	52.63	15.94	14.06
T ₂	48.61	57.05	19.86	14.39
T ₃	56.04	65.61	23.85	15.17
T ₄	51.63	62.25	21.56	14.78
T ₅	50.37	62.12	20.61	14.75
T ₆	52.87	62.37	22.05	14.54
T ₇	46.52	55.22	19.30	14.29
T ₈	45.64	55.54	18.37	14.10
T ₉	54.23	63.69	22.72	15.01
SEm±	1.055	0.897	0.488	0.279
CD (p=0.05)	2.432	2.069	1.125	0.643
CV (%)	4.47	3.19	5.05	4.06

S₃ (48.36 cm). This indicates that spraying at the appropriate stage can enhance vegetative growth, aligning with findings by Singh *et al.* (2020) [5], who reported increased plant height in pulses with optimal foliar applications. The highest number of pods per plant (64.74) was observed in S₄, significantly superior to S₃ (54.76), indicating that late-stage spraying may enhance reproductive development (Kumar *et al.*, 2021) [1]. Seed yield followed a similar trend, with S₄ (22.30 g/plant) significantly outperforming S₁ and S₃, while S₂ also showed improvement (21.06 g/plant). This demonstrates the critical role of spray timing in optimizing seed yield in pulses (Verma *et al.*, 2018) [6]. Test weight was not significantly influenced by spray stages, although numerically higher values were seen in S₄ (15.09 g).

Effect of Treatments on Growth and Yield Attributes

T₃ (56.04 cm) recorded the highest plant height, which was significantly higher than T₁ (44.38 cm) and T₈ (45.64 cm), indicating treatment effectiveness in promoting vegetative growth. The highest number of pods was found in T₃ (65.61), followed by T₉ (63.69), both significantly higher than T₁ (52.63). This aligns with the report of Sharma *et al.* (2019) [4], emphasizing the role of specific treatments in enhancing pod formation. T₃ also recorded the highest seed yield (23.85 g/plant), significantly higher than other treatments, followed by T₉ (22.72 g/plant) and T₆ (22.05 g/plant). This indicates the treatment's effectiveness in increasing assimilate partitioning towards reproductive structures (Rani *et al.*, 2022) [3]. Test weight was highest in T₃ (15.17 g), followed by T₉ (15.01 g), showing the positive impact of treatments on seed development and quality.

Effect of heat stress mitigation treatments on Seed Moisture Content in Soybean

The data presented in Table 2 indicate that heat stress mitigation treatments and spray stages significantly influenced seed moisture content (%) in soybean sown during the first week of January (D₁) and February (D₂). Under D₁ (January sowing), the seed moisture content ranged from 9.97% (S₄) to 10.88% (S₃) across spray stages, with an overall mean of 10.43%. Under D₂ (February sowing), moisture content ranged from 10.26% (S₄) to 11.16% (S₃), with a higher overall mean of 10.72%. This indicates that February sowing exhibited slightly higher seed moisture content than January sowing, which may be due to shorter seed-filling duration under higher temperatures in late sowing leading to incomplete moisture loss at harvest (Sita *et al.*, 2017) [8].

Among the treatments, Under D₁, T₃ (Salicylic acid @ 50 ppm) recorded the lowest mean moisture (10.02%), followed by T₉ (10.04%) and T₄ (10.13%), while the highest was in T₇ (10.68%) and T₈ (10.70%). Under D₂, T₃ again showed the lowest mean moisture (10.31%), followed by T₉ (10.32%) and T₄ (10.38%), whereas the highest was observed in T₈ (11.04%) and T₇ (10.99%). The lower seed moisture content in Salicylic acid (T₃) and Gibberellic acid (T₉) treatments suggests their effectiveness in improving seed maturity and desiccation under heat stress conditions, consistent with the findings of Khan *et al.* (2021), who reported that salicylic acid enhances antioxidant activity, reducing oxidative stress and accelerating physiological maturity in soybean.

Spray stages influenced seed moisture content, with S₄ (late stage) showing the lowest seed moisture content (9.97% in D₁ and 10.26% in D₂), indicating enhanced seed drying and

Table 2: Effect of heat stress mitigation treatments sprayed at different growth stages on seed moisture (%) content in soybean sown during first week of January and February

Treatments	D1: 1 st week January sowing					D2: 1 st week February sowing				
	S1	S2	S3	S4	Mean	S1	S2	S3	S4	Mean
T1: Control	11.27	10.44	11.27	10.66	10.91	11.63	10.73	11.60	10.93	11.22
T2: 6-Benzyl Adenine Purine @ 300 ppm	10.29	10.52	10.93	9.84	10.39	10.56	10.84	11.19	10.11	10.67
T3: Salicylic acid @50 ppm	10.21	9.56	10.58	9.74	10.02	10.53	9.82	10.82	10.06	10.31
T4: Indole Acetic Acid @100 ppm	10.38	10.06	10.96	9.11	10.13	10.65	10.33	11.21	9.36	10.38
T5: Naphthalene Acetic Acid @ 100 ppm	10.20	9.96	11.20	10.01	10.34	10.49	10.31	11.45	10.36	10.65
T6: Thiourea @1000 ppm	11.18	10.43	11.13	9.86	10.65	11.44	10.75	11.42	10.14	10.94
T7: Cycocel @ 100 ppm	10.56	10.78	11.14	10.24	10.68	10.83	11.15	11.47	10.50	10.99
T8: Ethrel @100 ppm	10.45	11.01	10.14	11.22	10.70	10.75	11.39	10.43	11.60	11.04
T9: Gibberellic acid @ 100 ppm	10.41	10.14	10.57	9.03	10.04	10.66	10.46	10.87	9.32	10.32
Mean	10.55	10.32	10.88	9.97	10.43	10.83	10.64	11.16	10.26	10.72
	S. Em.±		CD (P=0.05)		CV%	S. Em.±		CD (P=0.05)		CV%
T	0.130		0.380		3.59	0.128		0.367		3.38
S	0.090		0.250			0.085		0.245		
T×S	0.260		0.760			0.256		0.735		

Spraying stages (S), S₁: Vegetative stage (V5 -V7 stage), S₂: Flowering stage (R2: Full bloom stage), S₃: Pod filling stage (R5: Beginning seed stage), S₄: Vegetative stage and Flowering stage (V5 & R2), NS: Non-significant

Table 3: Effect of heat stress mitigation treatments sprayed at different growth stages on mean seedling length (cm) in soybean

Treatments	D1: 1 st week January sowing					D2: 1 st week February sowing				
	S1	S2	S3	S4	Mean	S1	S2	S3	S4	Mean
T1: Control	25.95	25.69	25.23	27.54	26.10	25.31	26.71	24.89	25.87	25.69
T2: 6-Benzyl Adenine Purine @ 300 ppm	28.78	28.31	28.27	27.96	28.33	27.77	28.39	26.95	28.52	27.91
T3: Salicylic acid @50 ppm	29.69	29.65	30.65	30.06	30.01	29.59	29.46	29.28	29.77	29.52
T4: Indole Acetic Acid @100 ppm	28.05	28.02	28.02	28.45	28.13	27.8	27.34	27.70	28.30	27.78
T5: Naphthalene Acetic Acid @ 100 ppm	28.93	29.52	29.65	30.39	29.62	29.36	29.49	27.55	30.25	29.16
T6: Thiourea @1000 ppm	28.89	29.28	29.35	31.43	29.63	29.06	29.96	29.59	29.73	29.46
T7: Cycocel @ 100 ppm	27.50	27.25	25.74	28.18	27.16	26.59	26.87	26.68	26.92	26.76
T8: Ethrel @100 ppm	26.77	26.96	26.57	26.93	26.81	26.09	26.92	26.02	26.59	26.40
T9: Gibberellic acid @ 100 ppm	30.02	30.29	29.86	31.57	30.54	30.00	28.69	30.38	30.88	29.99
Mean	28.28	28.33	28.15	29.17	28.48	27.92	28.20	27.65	28.54	28.07
	S. Em.±		CD (P=0.05)		CV%	S. Em.±		CD (P=0.05)		CV%
T	0.327		0.937		3.24	0.337		0.967		3.40
S	0.218		0.624			0.225		0.645		
T×S	0.653		NS			0.674		NS		

Spraying stages (S), **S1:** Vegetative stage (V5 -V7 stage), **S2:** Flowering stage (R2: Full bloom stage), **S3:** Pod filling stage (R5: Beginning seed stage), **S4:** Vegetative stage and Flowering stage (V5 & R2), **NS:** Non-significant

Faster maturity when treatments were applied at later stages. In contrast, S₃ (mid stage) recorded the highest moisture, suggesting a delay in seed drying, potentially due to physiological responses to mid-stage spraying.

Effect of heat stress mitigation treatments sprayed at different growth stages on mean seedling length (cm) in soybean

T₃ (Salicylic Acid @50 ppm) showed highest seedling length (30.01 cm in D1, 29.52 cm in D2), indicating its significant role in enhancing seedling growth. T₅ (NAA @100 ppm) and T₇ (Cycocel @100 ppm) (Table 3) also showed higher seedling lengths (D1: 29.62, 29.96 cm; D2: 29.16, 29.99 cm). T₁ (Control) recorded the lowest seedling length (26.10 cm in D1, 25.69 cm in D2). Soybean is sensitive to high temperatures during reproductive stages, which affects early vigor, germination, and seedling length. Seedling length is a crucial early indicator of plant vigor and resilience (Djanaguiraman *et al.*, 2013) [10]. SA helps in mitigating oxidative damage under heat stress by enhancing antioxidant enzyme activity (Kumar *et al.*, 2020) [11]. The maximum seedling length with T3 shows that SA likely helped in enhancing cellular elongation and division under heat stress. Both T₅ and T₇ improved seedling length. NAA is known to promote cell elongation and auxin signaling, crucial under stress conditions. Cycocel (chlormequat chloride) improves stress tolerance by regulating growth and enhancing water retention (Yin *et al.*, 2018) [13]. Spraying at R2 (S2) and R5 (S3) stages resulted in better seedling development, likely due to improved translocation of assimilates and cell wall plasticity during flowering and pod development stages. Though T6 data is incomplete, previous studies (Pandey *et al.*, 2017) [12] indicate thiourea boosts germination and seedling vigor by acting as a sulfur donor and antioxidant.

Conclusion

The present study demonstrates that heat stress significantly impairs the growth, yield, and seed quality of soybean, a crop of global nutritional and economic significance. With rising temperatures due to climate change, the development and standardization of effective mitigation strategies are essential to ensure sustainable soybean production. Among the various treatments tested, Salicylic acid @ 50 ppm (T₃) emerged as the most effective in enhancing plant height, pod number, seed yield, seedling length, and reducing seed moisture content,

thereby improving both yield and seed quality under heat stress conditions. Similarly, Gibberellic acid (T₉) and NAA (T₅) also showed beneficial effects on seedling growth and maturity. The timing of spray application played a crucial role, with the combined vegetative and flowering stage (S₄) resulting in the best performance across most parameters, highlighting the importance of stage-specific interventions for optimal results. February sowing was associated with slightly higher seed moisture, suggesting the need for strategic adjustments in sowing time to reduce heat-induced stress during critical reproductive stages. Overall, the integration of exogenous application of plant growth regulators, particularly salicylic acid, and timely intervention at appropriate growth stages can serve as a practical and scalable strategy to mitigate heat stress in soybean. These findings offer valuable insights for developing climate-resilient pulse and oilseed production systems and can support policy formulation and on-field recommendations for sustainable agriculture in heat-prone regions.

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