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Assessing the effect of bio-formulations and nano urea on phenology and growth of mungbean under the Western agroclimatic region of Haryana

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Abstract
Mungbean (*Vigna radiata* L. Wilczek), also known as green gram and golden gram, is considered the hardiest among all the pulses and is cultivated throughout the country. It is an important catch crop in the rice-wheat cropping system. A field trial was conducted during *Kharif* 2022 and 2023 at Pulses Research Farm, Department of Genetics & Plant Breeding, CCSHAU, Hisar to evaluate the effect of seed treatment with bio-formulations and nano urea on phenology and growth of mungbean. The field experiment was planned in factorial randomized block design with the following treatments: Factor A: Seed treatment comprising control, nano urea, *Rhizobium* + PSB and *Beejamrit*; Factor B: Nitrogen management including control, 100% RDN through conventional fertilizer, soil application of nano urea @ 500 ml ha^{-1}, nano urea at 35 DAS @ 250 ml ha^{-1}, 50% RDN + nano urea at 35 DAS @ 250 ml ha^{-1}, urea @ 2% at 35 DAS. It was evident that *Rhizobium* + PSB reported a maximum CGR of 11.17 g m-2 day-1 at 20-40 DAS among seed treatments. 50% RDN + nano urea @ 250 ml/ha at 35 DAS reported CGR of 10.55 and 10.52 g m-2 day-1 in the years 2022 and 2023, respectively at the flowering stage of mungbean which was statistically at par with 100% RDN (11.04 and 11.10 g m-2 day-1 in the years 2022 and 2023, respectively) and can be adopted as one of the best alternatives of nitrogen management. It will reduce the demand for conventional fertilizers without compromising production potential and also help in enhancing nutrient use efficiency.

Keywords: *Beejamrit*, nano urea, seed treatment, *Rhizobium*, CGR

Introduction
Pulses play a significant role in achieving agricultural sustainability by enhancing soil carbon sequestration, improving soil quality and crop diversity, and producing protein-rich food in this era of vigorously growing population (Abberton, 2010) [1]. India is one of the largest producers and consumers of pulses in the world (Rawal and Navarro, 2019) [24]. Mungbean (*Vigna radiata* L. Wilczek), also known as green gram, is the third most important pulse crop after chickpea and pigeonpea growing in the arid and semi-arid regions of the country after chickpea and pigeonpea, accounting about 5.13 million ha area and 3.08 million tonnes production with productivity of 601 kg ha^{-1} among pulses in India during 2020-21 (Anonymous, 2021). Being a short-duration pulse crop, it is cultivated throughout the country during summer (March–June) as well as the *Kharif* season (June–September) (Rawal and Navarro, 2019) [24]. It is considered the hardiest among all the pulse crops because of its low water requirement and capacity to withstand harsh climate. Nutritionally, seeds are excellent source of protein (~23%), particularly rich in lysine, minerals, carbohydrates and vitamins (Dahiya *et al*., 2015) [6], meeting the dietary requirements of vegetarian people of the country. It has the capacity to fix the atmospheric nitrogen in symbiosis with *Rhizobium* and helps in rejuvenating soil health and fertility (Markos and Yoseph, 2024) [17] by enriching the soil with nitrogen. It is also cultivated as an important catch crop in the rice-wheat cropping system in Northern India for enhancing soil fertility.
Despite being the leading pulse growing nation of the world, growing pulses on marginal lands with fewer inputs under rainfed conditions with inappropriate nutrient management practices has been one of the major constraints of meagre productivity in India (Yadav *et al.*, 2016) [36].

Considering the scenario, suitable nutrient management practices including seed treatment and fertilizer management, should be framed for improving crop productivity and soil health. Biofertilizers are low-cost renewable sources of nutrients (Singh *et al*., 2016) [31] that play vital role in encouraging plant growth and sustaining soil fertility (Kumar *et al*., 2018) [35]. When applied to soil, seed and plant surfaces, these microbial formulations colonize mainly in the rhizosphere and help in increasing nutrient supply and availability (Bhattacharjee and Dey, 2014) [5]. *Rhizobium* is a symbiotic nitrogen fixer, which serves as a source of ammonia (Havugimana *et al*., 2016) [11], while phosphorus-solubilizing microorganisms (PSB) including *Bacillus*, *Pseudomonas*, *Aspergillus* and *Penicillium* (Sharma *et al*., 2013) [28], solubilize the insoluble phosphatic compounds by secreting several organic acids (Yadav, 2017) [37]. Enhanced availability of nutrients like nitrogen and phosphorus to plants reflects improved root nodulation and better yield (Kumawat *et al*., 2009) [15]. *Beejamrit* is one of the ancient organic formulations employed in organic agriculture. This low-cost formulation is the source of microbial niche, capable of synthesizing plant growth regulators (PGRs) such as IAA, and minimizing phytopathogenic microbes in rhizosphere (Mukherjee *et al*., 2022) [18]. Its direct role as bio-stimulant and antimicrobial properties deliberates its inclusion in conventional agricultural practices (Sarma and Dutta, 2024) [26].

To improve nitrogen-use efficiency, several strategies have been suggested in the past few decades. Among these, nano fertilizers emerged as an alternative to conventional fertilizers with the potential to revolutionize the agricultural system, designed to deliver nutrients in correspondence with crop demand. Nanotechnology-based fertilizers effectively penetrate the plant leaves, activate nutrient pathways and help in accomplishing higher nutrient use efficiency (NUE) than traditional fertilizers with reduced environmental footprint (Kumar *et al*., 2021) [14] and also helps in reducing nitrogen leaching along with emissions (Kannoj *et al*., 2022) [12]. These are effective and efficient tool for plant nutrition which ultimately increased the crop production with enhanced quality (Kumar *et al*., 2021) [14] and improved nutrient availability to plants (Sharma *et al*., 2022) [29]. For the semi-arid conditions of Haryana, an attempt has been made for recommendation of latest production technology including nano urea to improve profitability in mungbean production.

Materials and Methods

The field experiment was conducted during *Kharif* season in the year 2022 and 2023 at Pulses Research Farm, Department of Genetics & Plant Breeding, CCSHAU, Hisar, Haryana, geographically positioned as 29° 08' 42" N latitude and 75° 41' 01" E longitude at an elevation of 230 meters above mean sea level. Under Western agroclimatic region of Haryana, crop was grown in moderately alkaline sandy soil having low, medium and high available nitrogen, phosphorus and potassium, respectively. Different nutrient management practices were evaluated including seed treatments with bio-formulations and nitrogen management including nano urea foliar application. Design of the experiment was factorial randomized block design (FRBD) which replicated three times with treatments as follows: Factor A: Seed treatment comprising i) Control (S1), ii) Nano Urea (S2), iii) *Rhizobium* + PSB (S3) iv) *Beejamrit* (S4); Factor B: Nitrogen management comprising i) Control (T1) ii) 100% RDN through chemical fertilizer (T2) iii) Soil application of nano urea @ 500 ml ha^{-1} (basal application) (T3) iv) Foliar spray of nano urea at 35 DAS @ 250 ml ha^{-1} (T4) v) 50% RDN (basal application) + Foliar spray of nano urea at 35 DAS @ 250 ml ha^{-1} (T_5) and vi) Foliar spray of Urea @ 2% at 35 DAS (T6). Mungbean cultivar MH 1468 was sown at row-to-row distance of 30 cm at 4-5 cm depth by *pora* method at the rate of 15-18 kg seed ha^{-1}. Seed was treated with bio-formulations *viz*. *Rhizobium*, PSB and *Beejamrit* and nano urea, a day before sowing. Nitrogen fertilizers were applied as per proposed treatments along with the recommended dose of P and K fertilizers before sowing of the crop and meticulously incorporated into soil. Field was irrigated by providing light irrigation during the crop growing season to dodge the consequences of dry spell. One spray of Rogor @ 625 ml/ha was done at 25 DAS for dealing the insect-pests along with one manual thinning cum-weeding to maintain optimum plant stand. Observations for crop phenological stages and growth rate were done periodically. Crop growth rate defined as the increase of plant dry matter per unit of time, was calculated as per the standard formula proposed by Radford (1967).

$$CGR\ (g/m^2/day) = \frac{W_2 - W_1}{(t_2 - t_1)}$$

Where, W2 = Dry weight of plant at time interval T_2
W1 = Dry weight of plant at time interval T_1

Crop was harvested manually, leaving 3-4 border lines from each side and tied properly in bundles for sun-drying in the plot. Recorded data was statistically analyzed by Fisher's analysis of variance (ANOVA) technique (Fisher, 1950). Significance of different treatments was evaluated by "F" test and critical difference was computed at 5% level of significance among the treatments where significant differences were observed as per the procedure outlined (Panse and Sukhatme, 1967) [20].

Results and Discussion

Growth and development of plants are greatly influenced by seed treatment and nitrogen management under definite climatic *viz*., temperature, relative humidity, rainfall and sunshine hours, and edaphic conditions *viz*., soil pH, EC, organic carbon and nutrient availability in soil (Rao *et al*., 2018) [23].

Phenology

Phenology of crop play vital role in functioning of agricultural ecosystem. Timely sowing ensures optimum vegetative and reproductive growth reflecting grain quality and quantity. Table 1 presented the number of days taken to different phenological stages of mungbean influenced by various bio-formulations and nano urea during *Kharif* 2022 and 2023. Among the different seed treatments, *Rhizobium* + PSB (S3) encouraged the seedling emergence at the earliest within 5.4 and 5.2 DAS during 2022 and 2023, respectively as compared to control (S1) (5.9 and 5.7 DAS, respectively). Maximum number of days taken to 50% flowering and 50% pod formation was observed with seed inoculation of *Rhizobium* + PSB (S3), 38.8 and 47.5 DAS during 2022 and 38.9 and 47.7 DAS during 2023, respectively. These results are in agreement with Yadav *et al.* (2018) [35]. It was evident from pooled data that significantly higher number of days taken to physiological maturity was observed under seed treatment of *Rhizobium* + PSB (S3) (64.3) which was statistically at par with *Beejamrit* (S4) and Nano urea (S2), attaining physiological maturity at 64.0 and 63.6 DAS,

respectively. Minimum number of days taken to emergence was observed under 100% RDN through conventional chemical fertilizers (T2) (5.3 and 5.2 DAS during 2022 and 2023, respectively) among different nitrogen management practices. It was obvious from data that days taken by crop to reach 50% flowering, 50% pod formation and physiological maturity markedly increased by application of nitrogenous fertilizers in rising temperature by extending their growing seasons (Bashir *et al*., 2024 [4]). Based on pooled data analysis, it was observed that mungbean sown under 100% RDN (T_2) took significantly higher number of days to 50% flowering (39.0), being statistically at par with crop sown under 50% RDN + Nano Urea at 35 DAS @ 250 ml/ha (T_5) (38.7), Nano Urea at 35 DAS @ 250 ml/ha (T_4) (38.5) and Urea @ 2% at 35 DAS (T_6) (38.4). The crop took a greater number of days to 50% pod formation and physiological maturity under 100% RDN (T_2) (47.7 and 64.4, respectively) in comparison to crop sown under 50% RDN + Nano Urea at 35 DAS @ 250 ml/ha (T_5) (47.4 and 64.0, respectively). Inoculation of nitrogen-fixing bacteria and N application delays the process of senescence and ultimately, extends the time taken to physiological maturity due to their constructive interaction in comparison to control (Din *et al.*, 2021) [8].

Crop growth rate

Crop production was determined mainly by crop growth rate (CGR), being a function of light interception through the leaf area of crop (Whigham, 1983) [34]. Different bio-formulations and nitrogen management produced significant variation in crop growth rate (CGR) of mungbean up to 40 DAS while no significant difference was observed afterwards till harvesting during both years of field trial and in pooled analysis (Table 2 & Fig. 1). Similar trend was observed by Sritharan *et al.* (2015) [32] in blackgram with foliar application of different fertilizers.

Among different seed treatments, *Rhizobium* + PSB (S3) reported significantly highest crop growth rate of 5.86 and 6.60 g m-2 day-1 in *Kharif* 2022 and 2023, respectively by 0-20 DAS which was statistically similar to that of *Beejamrit* (S4) (5.73 and 6.22 g m-2 day-1, respectively). Singh and Singh (2021) observed that *Rhizobium* inoculation increased vegetative growth and improved dry matter accumulation

At 20-40 DAS, a maximum CGR of 11.22 and 11.13 g m-2 day-1 in 2022 and 2023, respectively was obtained with seed inoculation of *Rhizobium* + PSB (S3) which was significantly higher than *Beejamrit* (S4) (10.36 and 10.80 g m-2 day-1, respectively), Nano Urea (S2) (8.72 and 9.81 g m-2 day-1, respectively). *Rhizobium* + PSB (S3) recorded numerically higher crop growth rate (7.27 and 7.79 g m- 2 day-1 in 2022 and 2023, respectively) at 40-60 DAS. Likewise, highest CGR was reported in seed treatment of *Rhizobium* + PSB (S3) (2.30 and 2.50 g m-2 day-1 in 2022 and 2023, respectively) at 60 DAS to harvest as compared to *Beejamrit* (S4) (2.24 and 2.15 g m-2 day-1 in 2022 and 2023, respectively) and control (S1) (1.95 and 1.92 g m-2 day-1 in 2022 and 2023, respectively). These results are in conformity with Kundu *et al*. (2013) [16], and Thakur *et al*. (2024) [33]. Application of biofertilizers (*Rhizobium*, PSB) improved the microbial niche of soil. It also enhanced biological nitrogen fixation and phosphorus solubilization along with increased production of plant growth-promoting hormones which eventually increased the plant vigor (Gorade *et al*., 2014) [10]. Seed treatment with *Beejamrit* also improved seed germination and seedling vigor (Dhiman *et al*., 2024) [7]. Sharma *et al*. (2007) [27] reported that these bio-formulations inhibit harmful microorganisms in the rhizosphere and ensure favourable edaphic conditions for the phenomenal development of the plant.

The experimental findings indicated that the application of N fertilizers either conventional or nano urea significantly improved the crop growth rate of mungbean. At 0-20 DAS, crop growth rate was significantly highest under 100% RDN through chemical fertilizers (T_2) (5.96 and 6.70 g m-2 day- 1 in 2022 and 2023, respectively). However, application of 50% RDN + Nano Urea at 35 DAS @ 250 ml/ha (T_5) reported statistically similar CGR (5.86 g m-2 day-1 in 2022 and 6.29 g m-2 day-1 in 2023).

At 20-40 DAS, 100% RDN (T_2) showed crop growth rate of 11.04 and 11.10 g m-2 day-1 which was significantly highest among different nitrogen management practices in 2022 and 2023, respectively, reporting an overall increase of 31.7 and 20.0 percent over control (T_1), respectively. It was statistically at par with 50% RDN + Nano Urea at 35 DAS @ 250 ml/ha (T_5) (10.55 g m-2 day-1) in the year 2022, 50% RDN + Nano Urea at 35 DAS @ 250 ml/ha (T_5) (10.52 g m-2 day-1) and Urea @ 2% at 35 DAS (T_6) (10.47 g m-2 day-1) in 2023. Foliar application of nano urea at the flowering stage of the crop significantly improved the crop growth rate of mungbean. This might be attributed to protein synthesis and increased photosynthetic activity owing to the increased availability of nitrogen (Samanta *et al*., 2022) [25]. These research findings are in agreement with Pathak *et al*. (2024) [22].

Table 1: Effect of different bio-formulations and nano urea on days taken to phenological stages of mungbean

Treatment details		Emergence			50% Flowering			50% Pod formation			Physiological maturity		
		2022	2023	Pooled	2022	2023	Pooled	2022	2023	Pooled	2022	2023	Pooled
Seed treatment													
S1	Control	5.9	5.7	5.8	37.7	38.2	37.9	46.2	46.9	46.5	63.1	63.3	63.2
S2	Nano Urea	5.7	5.5	5.6	38.1	38.6	38.4	46.6	47.1	46.9	63.6	63.6	63.6
S3	*Rhizobium* + PSB	5.4	5.2	5.3	38.8	38.9	38.8	47.5	47.7	47.6	64.1	64.5	64.3
S4	*Beejamrit*	5.8	5.4	5.6	38.3	38.7	38.5	47.2	47.4	47.3	63.8	64.2	64.0
SE (m)±		0.2	0.1	0.1	0.3	0.3	0.2	0.4	0.2	0.2	0.3	0.4	0.3
C.D. (p=0.05)		NS	NS	0.3	NS	NS	NS	NS	NS	NS	NS	NS	0.8
Nitrogen management													
T_1	Control	6.1	5.6	5.8	37.7	38.1	37.9	46.3	46.7	46.5	62.9	63.3	63.1
T_2	100% RDN	5.3	5.2	5.3	38.8	39.3	39.0	47.6	47.8	47.7	64.3	64.6	64.4
T_3	Nano Urea soil application @ 500 ml/ha	5.8	5.4	5.6	37.8	38.1	37.9	46.6	46.9	46.8	63.4	63.6	63.5
T_4	Nano Urea at 35 DAS @ 250 ml/ha	5.8	5.4	5.6	38.3	38.7	38.5	46.8	47.4	47.1	63.7	63.8	63.8
T_5	50% RDN + Nano Urea at 35 DAS @ 250 ml/ha	5.5	5.5	5.5	38.6	38.8	38.7	47.1	47.7	47.4	63.8	64.2	64.0
T_6	Urea @ 2% at 35 DAS	5.7	5.5	5.6	38.2	38.6	38.4	46.8	47.2	47.0	63.6	63.8	63.7
SE (m)±		0.2	0.2	0.1	0.4	0.4	0.3	0.4	0.3	0.3	0.4	0.4	0.3
C.D. (p=0.05)		NS	NS	NS	NS	NS	0.8	NS	NS	NS	NS	NS	NS

*interaction is non-significant at p=0.05; RDN: Recommended Dose of Nitrogen; DAS: Days After Sowing; NS: Non-Significant

The data related to CGR revealed that different nitrogen management practices could not influence crop growth rate at 40-60 DAS and 60 DAS to harvest during both years and on pooled data basis. Though numerically higher CGR was obtained during *Kharif* 2023 as compared to 2022, depicted in Figure 1. 100% RDN (T2) showed maximum CGR of 7.58 g m-2 day-1 at 40-60 DAS and 2.53 g m-2 day-1 at 60 DAS to harvest as per pooled data over control (T_1), 7.05 and 1.81 g m-2 day-1 at 40-60 DAS and 60 DAS to harvest, respectively. CGR decreased after flowering up to harvest due to leaf senescence and decreasing leaf area index. Similar results were observed by Abisankar *et al.* (2024) [2] in chickpea.

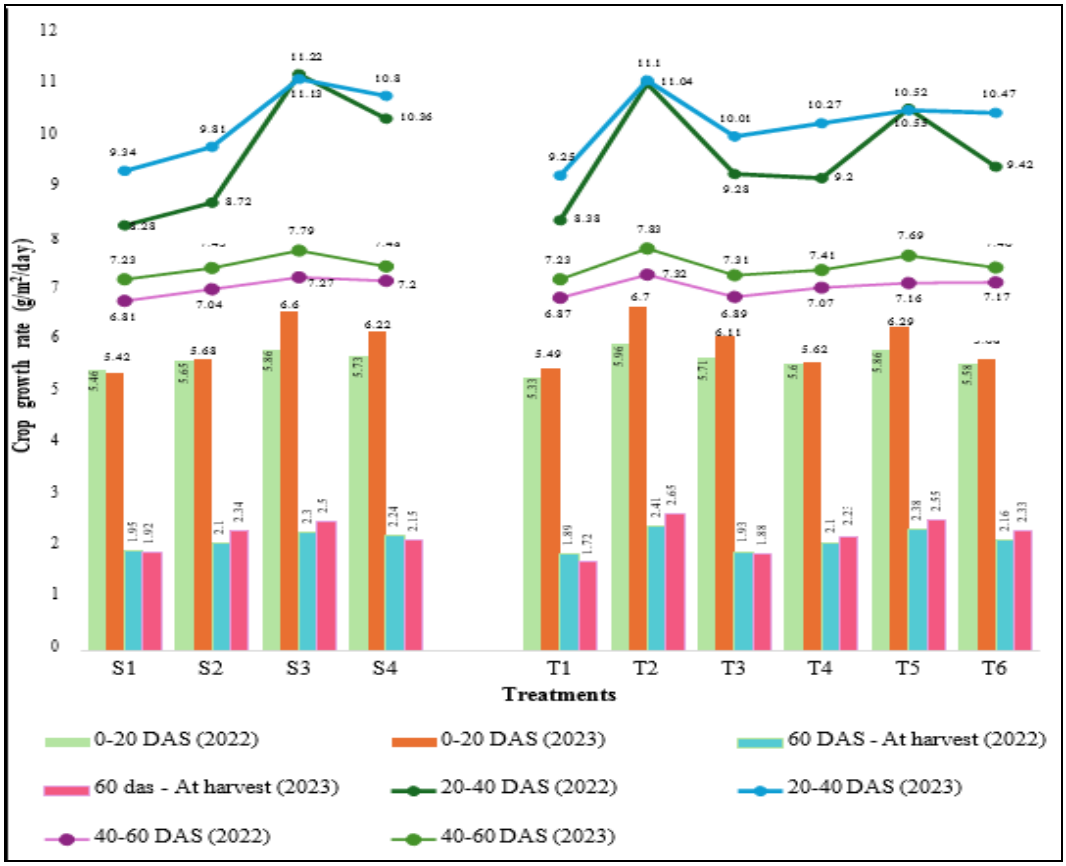


Fig 1: Effect of different bio-formulations and nano urea on the crop growth rate of mungbean

Table 2: Effect of different bio-formulations and nano urea on the crop growth rate of mungbean

Treatment details		Crop growth rate (g m^{-2} day^{-1})											
		0-20 DAS			20-40 DAS			40-60 DAS			60 DAS - At harvest		
		2022	2023	Pooled	2022	2023	Pooled	2022	2023	Pooled	2022	2023	Pooled
Seed treatment													
S_1	Control	5.46	5.42	5.54	8.28	9.34	8.81	6.81	7.23	7.02	1.95	1.92	1.94
S_2	Nano Urea	5.65	5.68	5.67	8.72	9.81	9.26	7.04	7.45	7.24	2.10	2.34	2.22
S_3	*Rhizobium* + PSB	5.86	6.60	6.23	11.22	11.13	11.17	7.27	7.79	7.53	2.30	2.50	2.40
S_4	*Beejamrit*	5.73	6.22	5.98	10.36	10.80	10.58	7.20	7.48	7.34	2.24	2.15	2.19
SE (m)±		0.06	0.08	0.05	0.19	0.19	0.14	0.24	0.23	0.15	0.21	0.32	0.24
C.D. (p=0.05)		0.17	0.24	0.16	0.53	0.54	0.40	NS	NS	NS	NS	NS	NS
Nitrogen management													
T_1	Control	5.33	5.49	5.41	8.38	9.25	8.82	6.87	7.23	7.05	1.89	1.72	1.81
T_2	100 % RDN	5.96	6.70	6.33	11.04	11.10	11.07	7.32	7.83	7.58	2.41	2.65	2.53
T_3	Nano Urea soil application @ 500 ml/ha	5.71	6.11	5.91	9.28	10.01	9.65	6.89	7.31	7.10	1.93	1.88	1.91
T_4	Nano Urea at 35 DAS @ 250 ml/ha	5.60	5.62	5.61	9.20	10.27	9.73	7.07	7.41	7.24	2.10	2.23	2.17
T_5	50 % RDN + Nano Urea at 35 DAS @ 250 ml/ha	5.86	6.29	6.08	10.55	10.52	10.53	7.16	7.69	7.42	2.38	2.55	2.47
T_6	Urea @ 2 % at 35 DAS	5.58	5.68	5.63	9.42	10.47	9.94	7.17	7.46	7.31	2.16	2.33	2.25
SE (m)±		0.07	0.10	0.07	0.23	0.23	0.17	0.29	0.28	0.18	0.26	0.39	0.29
C.D. (p=0.05)		0.22	0.29	0.20	0.65	0.66	0.49	NS	NS	NS	NS	NS	NS

*interaction is non-significant at p=0.05; RDN: Recommended Dose of Nitrogen; DAS: Days After Sowing; NS: Non-Significant

Conclusion

In this study, we concluded that seed treatment with bio-formulations (*Rhizobium*, PSB and *Beejamrit*) and incorporation of nitrogen fertilizers improved the emergence of mungbean and significantly enhanced the crop growth rate of mungbean. 100% RDN reported maximum growth rate of 11.07 g m- 2 day-1 which was statistically at par with 50% RDN + foliar application of nano urea @ 250 ml/ha at 35 DAS (10.53 g m-2 day-1) and can be adopted as one of the best nitrogen management strategies without compromising production potential.

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