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Assessment of seed size and planting depth on seedling growth and agronomic performance of berseem (*Trifolium alexandrinum*)

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ABSTRACT

The present study was carried out during winter (*rabi*) season of 2023 –2024 at ICAR-Indian Grassland and Fodder Research Institute, Jhansi, Uttar Pradesh to explore the effects of seed size and sowing depth on the emergence and morphological development of berseem (*Trifolium alexandrinum* L.). Seeds were categorised into large and small seed lots to assess the impact of seed size on germination and vigour using Petri plate tests. The experiment was laid out in a split-plot completely randomised design in a 2 × 4 factorial arrangements, with JBSC-1 and Wardan as the main factor and four sowing depths (0.0, 2.0, 4.0, and 6.0 cm) as the sub-factor, replicated thrice. Large seed size showed more seedling vigour as compared to small seed irrespective of berseem. Seeds sown at the surface (0.0 cm) exhibited the highest germination percentage, followed by those sown at 2.0 cm and 4.0 cm depths. However, seeds sown at a 6.0 cm depth failed to germinate in both varieties. Sowing at a depth of 2.0 cm significantly enhanced seedling establishment, plant height, and root-to-shoot ratio. Additionally, plants sown at 2.0 cm depth produced the tallest plants and superior fresh and dry leaf weights. Conversely, deeper sowing depths (4.0 cm and 6.0 cm) delayed germination and reduced plant vigour, while surface sowing and 2.0 cm depth resulted in early germination and better overall plant development. Root length and SPAD values were not significantly influenced by sowing depth. This study demonstrates that both seed size and sowing depth play a crucial role in the early establishment and growth performance of berseem. Large seeds consistently exhibited greater seedling vigour than small seeds, regardless of variety. Among the sowing depths evaluated, 2.0 cm emerged as the optimal depth, promoting superior germination, early establishment, plant height, and biomass accumulation. These insights can guide improved agronomic practices for enhancing stand establishment and fodder yield in berseem cultivation.

Keywords: Biomass yield, Root length, Seedling vigour, Sowing depth, Stand establishment

Berseem (*Trifolium alexandrinum* L.) is a vital *rabi* season leguminous fodder crop, extensively cultivated in India (2 mha) followed by Egypt (1.1 mha), Pakistan (0.71 mha) and other countries of Middle East, Mediterranean, Asia Minor regions, and the Indian sub-continent (Mohammad *et al.* 2014, NITI Aayog 2018). Area under berseem cultivation is increasing in many states of India, viz. Maharashtra, Rajasthan, Madhya Pradesh and Gujarat (Singh *et al.* 2020). Its prominence is attributed to its multi-cut nature (4–6 cuts), long duration of green fodder availability (November to April), high green fodder yield (85 t/ha), superior forage quality (20% crude protein), high digestibility (up to 65%), and exceptional palatability (Singh *et al.* 2019). Berseem is classified into three distinct ecotypes, namely Miscavi, Saidi, and Fahli (Singh *et al.* 2020). Most Indian berseem varieties are derived from the Mescavi ecotype, with the exception of JBSC-1, which originates from the Fahli ecotype (Chand *et al.* 2024).

The influence of seed size on seedling growth has been extensively studied across various plant species. Generally, larger seeds tend to produce more vigourous seedlings due to greater food reserves, although the relationship can vary depending on species and environmental conditions. For instance, in soybeans, large seeds exhibited higher germination rates and more robust seedling growth compared to smaller seeds (Wang *et al.* 2025). The relationship between seed size and seedling growth can vary among species, highlighting the importance of context-specific studies. The successful cultivation of berseem is critically influenced by various agronomic practices, among which sowing depth plays a pivotal role. In India, climatic and soil conditions vary markedly hence, understanding the optimal sowing depth is

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essential for enhancing both emergence rates and subsequent morpho-physiological development. Shallow and deep sowing strategies can significantly impact seed germination, root establishment, and ultimately the yield potential of the crop. Furthermore, the relationship between sowing depth and the plants adaptation to environmental stresses underscores the need for targeted research. Investigating these dynamics not only contributes to effective agricultural practices but also aids in maximising the utility of berseem in sustainable farming systems. Research has demonstrated the importance of sowing depth in various crops, such as pea, cowpea, mung bean, soybean, and maize (El-Abady 2015, Thiyam *et al.* 2017).

The challenges in the initial establishment phase of berseem exacerbate productivity issues. Proper management during this phase, such as timely irrigation within 3–6 days of sowing and ensuring optimal conditions for germination, is critical. Among agronomic practices, sowing depth is a crucial factor influencing seed germination, seedling establishment, initial crop growth, and eventual yield. Deviations from the optimal sowing depth can result in subpar plant performance, yield reductions, and even crop failure.

Given the limited research on the impact of sowing depth on the germination and growth of berseem, there is a critical need to address this knowledge gap. Understanding the relationship between sowing depth and key growth parameters, such as germination rate, seedling vigour, and chlorophyll content, can provide essential insights for optimising agronomic practices. Such knowledge will also provide strategies to mitigate seed exposure to suboptimal conditions during planting, ensuring improved growth and development. Therefore, the present study aims to evaluate the effects of seed size and different sowing depths on crop establishment, seedling growth, and biomass yield in berseem.

MATERIALS AND METHODS

Seeds: Fresh seeds of two berseem varieties i.e. Wardan and JBSC-1 were used for the study. Wardan variety of berseem is high yielding, multi-cut, small seeded with test weight of 2.5 g while JBSC-1 is a single cut, high yielding and bold seeded variety having test weight- 3.0 g). Bold seeds of Wardan (test weight-3.3 g) and JBSC-1 (3.7 g), small seeds of Wardan (2.3 g) and JBSC-1 (2.9 g) were manually separated.

Germination studies under laboratory condition: Seed germination studies were conducted by following procedure given by ISTA (2015). Fifty seeds of each Berseem variety were placed on two layers of Whatman No. 1 filter papers in sterile plastic Petri dishes (90 mm diameter, 15 mm height). Filter papers were moistened with 5 mL of distilled water to provide sufficient moisture for germination, with additional water added as needed to maintain consistent moisture. Petri dishes were covered with lids for the first three days to retain moisture and promote germination, and then left uncovered to allow aeration and prevent fungal growth. The experiment was conducted at room temperature (25 ± 2°C) under a 12 h light/dark cycle (light intensity: ~200 µmol /m²/s, provided by cool white fluorescent lamps). Germination percentage was recorded on the sixth day after sowing, whereas seedling parameters; root length (mm), shoot length (mm), root weight (mg), and shoot weight (mg) were measured on the ninth day after sowing.

Seed germination dynamics under glasshouse condition: The experiment was conducted in a pot under glasshouse at ICAR-Indian Grassland and Fodder Research Institute, Jhansi (25.5114°N, 78.5337°E; 275 m amsl), Uttar Pradesh, during winter (*rabi*) season of 2023–2024. The experiment was laid out in a completely randomised design (CRD) arranged in a 2 × 4 factorial scheme in three replications. The main factor comprised two varieties of berseem, Wardan and JBSC1. The sub-factor included four sowing depths, viz. 0.0 cm (surface sowing), 2.0 cm, 4.0 cm, and 6.0 cm. Each treatment combination (2 varieties × 4 depths × 3 replications) resulted in 24 experimental units (pots). Plastic pots (20 cm diameter, 25 cm height) were filled with a soil mixture comprising three parts soil (collected from the 0–20 cm layer) and one part vermicompost, mixed thoroughly. The physico-chemical properties of soil sample was analysed in Soil Laboratory of Crop Production Division, ICAR-Indian Grassland and Fodder Research Institute, Jhansi, Uttar Pradesh (Table 1). Fifty seeds of each berseem variety were sown at the specified depths (0.0 cm, 2.0 cm, 4.0 cm, 6.0 cm) in each pot. Surface-sown seeds (0.0 cm) were lightly pressed into the soil to ensure contact, while other depths were achieved by placing seeds in pre-measured holes and covering with the soil mixture. Initially, all pots were saturated with water and allowed to drain for 4–6 days to stabilise soil moisture at approximately 80% of available soil moisture (ASM). Immediately after sowing, pots were irrigated to field capacity (approximately 200 mL water per

Table 1 Characteristics of experimental soil filled in pot

Soil parameters	Value	References
Sand (%)	43.68	Gee and Bauder (1986)
Silt (%)	24.72	
Clay (%)	31.60	
Soil Class: Clay Loam (USDA Textural Triangle)		
Soil *p*H (1:2.5 Soil: Water)	7.56	Jackson (1958)
EC (dS/m) (25°C) 1:2.5	0.18	Richards (1954)
Organic carbon (%)	0.68	Walkley and Black (1934)
Available nitrogen (kg/ha)	154.29	Bremner (1960)
Available phosphorus (kg/ha)	24.00	Bray and Kurtz (1945)
Available potash (kg/ha)	268.80	Jackson (1958)
Field capacity (%)	27.00	Milks *et al.* (1989)
Permanent wilting point (%)	13.00	Milks *et al.* (1989)
Available moisture (%)	14.00	Milks *et al.* (1989)

pot). Thereafter, soil moisture was maintained at 70–80% of field capacity by irrigating at 2–3 day intervals, based on periodic soil moisture monitoring.

Data recording: Data on seed quality and agronomic parameters were systematically recorded for both varieties across large and small-seeded seed lots. The data on seed quality and agronomic parameters were recorded.

Seed quality traits: Germination, seedling establishment, seedling vigour were recorded on large and small seed lots of both varieties. Germination percentage was assessed on the 5th day after sowing (DAS). Seeds with a radicle emergence of ≥2 mm were counted as germinated. Germination percentage was calculated as:

$$\text{Germination percentage (G\%)} = \frac{\text{Seeds germinated}}{\text{Total seeds}} \times 100$$

Seedling establishment (SE) was recorded on the 15th DAS. A seedling was considered established when the first trifoliate leaf was fully opened. SE was calculated as:

$$\text{SE (\%)} = \frac{\text{Total seedlings established}}{\text{Total seeds germinated}} \times 100$$

$$\text{Seedling vigour index-I} = \text{Germination percentage} \times \text{Mean seedling length (cm)}$$

$$\text{Seedling vigour index-II} = \text{Germination percentage} \times \text{Seedling dry weight.}$$

Agronomic traits: The agronomic traits including plant height, root length, root and shoot biomass and relative chlorophyll content were measured at 60 DAS on five randomly selected plants per pot. Plant height was measured five times at 10-days intervals and final plant height was recorded at 60 DAS. The root and shoot biomass were recorded on gently uprooted plants. Roots and shoots were attenuated at the root-shoot junction and recorded fresh biomass; oven-dried at 70°C for 48 h to constant weight, and weighed using a precision analytical balance (accuracy: 0.1 mg). The chlorophyll content was measured at 60 DAS using a SPAD-502 chlorophyll meter. Three readings were taken from the youngest fully expanded leaf of five plants per pot, and the mean SPAD value was recorded.

Data analysis: Data on GP, SE, plant height, root and shoot biomass, and SPAD readings were recorded for each replication. Means were calculated, and data were subjected to two-way analysis of variance (ANOVA) to assess the effects of variety, sowing depth, and their interaction. Means were separated using Tukey's Honestly Significant Difference (HSD) test at $p<0.05$, performed in R software (version 4.3.0). Normality and homogeneity of variance were verified using Shapiro-Wilk and Levene's tests, respectively. Results were presented as means ± standard error (SEMI), with significant interactions visualised using interaction plots.

RESULTS AND DISCUSSION

Effect of seed size on seedling growth: The effect of seed size on germination and seedling growth parameters in berseem was evaluated using two distinct seed size lots for each of the Wardan and JBSC-1 varieties. While seed size did not significantly influence germination percentage in either variety, notable differences were observed in seedling traits. Specifically, larger seeds produced seedlings with greater root length, shoot length, fresh weight, and dry weight compared to those from smaller seeds (Fig. 1). These findings suggest that, although germination rates remain unaffected, larger seed size confers advantages in early seedling development in berseem, consistent with earlier findings in sorghum (Jung *et al.* 2012), bird's-foot trefoil (McKersie *et al.* 1981), and maize (Kandasamy *et al.* 2020). Seedling vigour (SVI-I and SVI-II) was also recorded to be higher in the large seed lot compared to the small seed lot, regardless of the berseem variety (Table 2). This indicates that larger seeds are beneficial for the robust establishment of the berseem crop. In agreement with the present findings, Dalianis (1980) reported that larger berseem seeds exhibited faster germination, increased seedling elongation, and superior emergence, primarily due to their greater reserve food content.

Larger seeds produced more robust seedlings with greater biomass and elongation, irrespective of variety. This trend has been widely documented in legumes such as chickpea, where larger seed size was associated greater

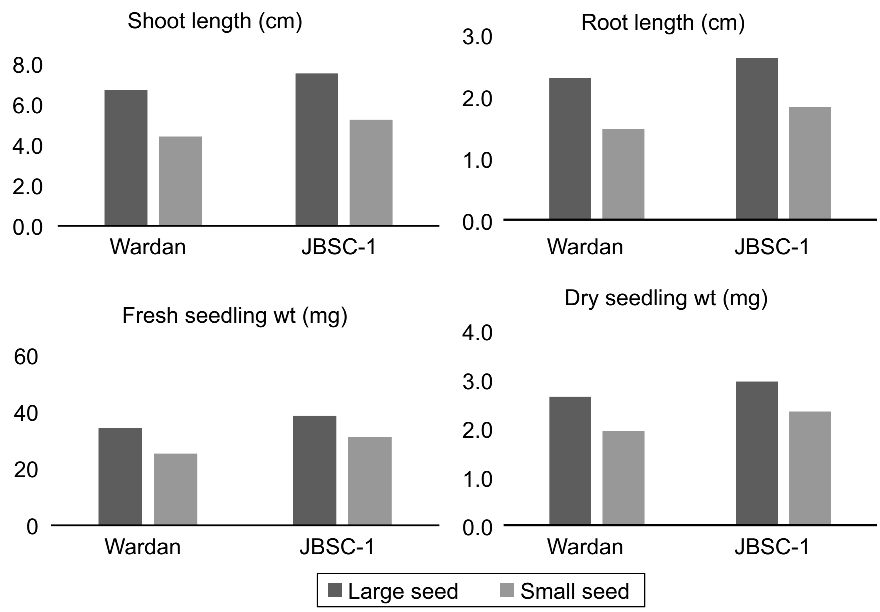

Fig. 1 Effect of seed size on seedling growth parameters in berseem in two distinct seed size lots for each of the Wardan and JBSC-1 varieties.

seedling emergence, and improved growth throughout the crop cycle, ultimately leading to higher yields (Villalobos-Olivera *et al.* 2025). Similar results were observed in soybean, where larger seeds exhibited more vigourous seedlings, and enhanced stress resistance compared to smaller seeds (Wang *et al.* 2025). In cowpea larger seeds contributed to increased seedling biomass and seedling vigour index (Valmik *et al.* 2024). These findings suggest that seed size plays a critical role in early seedling establishment across legumes, possibly due to greater nutrient reserves in larger seeds that support early growth. Therefore, selecting larger seeds can be a practical and low-cost approach in improving crop establishment and performance in berseem and other legume crops.

Effect of sowing depth on crop establishment and growth: Data recorded from pot experiment exhibited significant differences between the two varieties for most of the traits viz. germination, seedling, and agronomic traits, except root length (Table 3). Sowing depth significantly affected most traits, including germination percentage and seedling establishment, but had no significant impact on root length or SPAD values. Interaction effects between varieties and sowing depth were significant for several traits, except for plant height recorded on the first, second, and third dates, root length, shoot length, and SPAD values.

Increasing sowing depth reduced germination percentage in both varieties. However, seedling establishment percentage improved with increasing sowing depth. At all sowing depths, variety JBSC-1 demonstrated higher germination percentages (97%) compared to Wardan (95%). Surface sowing (0.0 cm) and at 2.0 cm depth had significantly higher germination percentages than 4.0 cm and 6.0 cm depths, while the lowest germination were observed at 6.0 cm depth. Plant height measured at successive time intervals under varying sowing depths revealed that the variety JBSC-1 consistently outperformed Wardan. Furthermore, a sowing depth of 2.0 cm resulted in significantly greater plant height across all observations, irrespective of varietal differences (Fig. 2). Among the sowing depths, 2.0 cm resulted in the tallest plants, whereas shallow (0.0 cm) and deeper (4.0 cm) sowing depths led to reduced plant height (Table 4).

Chlorophyll content, as measured by SPAD readings, was not significantly influenced by sowing depth. However, JBSC-1 consistently demonstrated higher chlorophyll content compared to Wardan, irrespective of sowing depth. The variety JBSC-1 is derived from the Fahli ecotype, while Wardan belongs to the Mescavi ecotype. Previous studies have reported that Fahli types possess higher seed yield compared to Mescavi types (Singh *et al.* 2020). Therefore, JBSC-1 may serve as a potential genetic resource for the improvement of Mescavi-type varieties, particularly with respect to seed yield and seed quality traits.

Shoot length was significantly influenced by sowing depth and variety. The maximum shoot length was observed at a sowing depth of 2.0 cm, and the minimum was recorded at 4.0 cm. JBSC-1 produced significantly greater shoot lengths over the Wardan at all depths. Shoot biomass (both fresh and dry weights) also varied significantly with sowing depth and variety, with 2.0 cm sowing depth yielding the highest biomass and 4.0 cm the lowest. JBSC-1 recorded higher shoot biomass than Wardan regardless of sowing

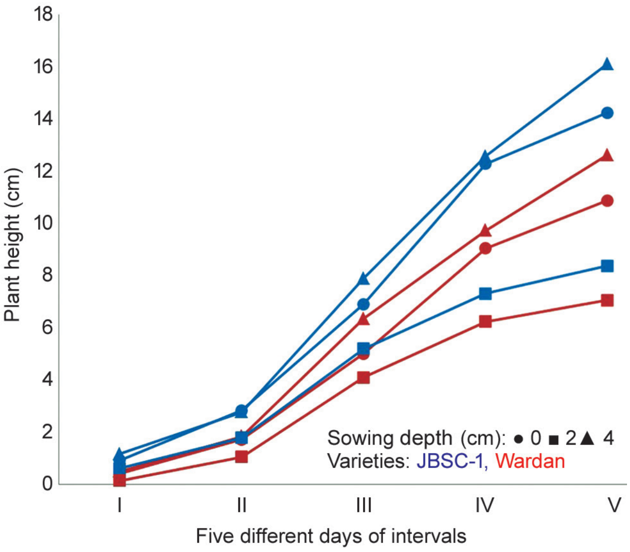

Fig. 2 Temporal variation in plant height over five intervals in two berseem varieties sown at different depth.

Table 2 Effect of seed size on seedling morphometrics of two different varieties

Variety	Germination %		Seedling length		SVI-I		SVI-II	
	Large	Small	Large	Small	Large	Small	Large	Small
Wardan	95.60	95.20	8.85	6.15	846.06	585.48	252.90	183.28
	95.60	94.80	9.18	5.66	877.61	536.57	244.61	176.77
	94.80	95.30	9.00	5.84	853.20	556.55	256.28	189.73
Mean	95.33	95.10	9.01	5.88	858.95	559.51	251.28	183.25
JBSC-1	97.00	96.80	9.86	7.06	956.42	683.41	284.90	223.68
	97.60	97.10	10.11	7.03	986.74	682.61	293.05	222.81
	96.30	96.20	10.47	7.10	1008.26	683.02	279.57	229.82
Mean	96.97	96.70	10.15	7.06	983.89	683.02	285.82	225.45

SVI, Seedling vigour index.

Table 3 Analysis of variance for agro-physiological traits in berseem varieties sown at different depth

Source of variation	DF	Mean sum of square													
		Plant height (days) (cm)					Root weight (g)/plant		Shoot weight (g)/plant		Root length (cm)	Shoot length (cm)	SPAD	Dry root/shoot	Fresh root/shoot
		I	II	III	IV	V	Fresh	Dry	Fresh	Dry					
Varieties	1	1.41**	3.85**	10.29**	25.63**	33.42**	2.08**	0.01**	108.53**	1.76**	1.03	106.92**	130.14**	684.75	62.54
Sowing depth	2	0.34**	1.54**	9.19**	34.44**	70.86**	0.93**	0.01**	97.06**	1.48**	2.48	174.93**	18.02	327.71	174.34
Varieties × Sowing depth	2	0.01	0.05	0.22	2.02*	2.21*	2.16**	0.01**	5.93**	0.09**	2.71	5.46	11.48	996.79	267.22
Error	10	0.02	0.19	0.40	0.74	0.42	0.00	0.00	0.06	0.01	1.27	2.56	9.67	684.75	62.54

Table 4 Effect of sowing depth on seedling and agronomic traits in berseem varieties

Variety	Sowing depth (cm)	Germi-nation (%)	Seedling estab-lishment (%)	Plant height (cm) days					Root weight (g)/plant		Shoot weight (g)/plant		Root length (cm)	Shoot length (cm)	SPAD	Dry root/shoot	Fresh root/shoot
				I	II	III	IV	V	Fresh	Dry	Fresh	Dry					
JBSC-1	0.0	97	92	0.93	2.84	6.91	12.28	14.24	1.90	0.46	11.10	1.42	14.83	24.97	34.93	0.33	0.17
	2.0	94	97	1.17	2.78	7.90	12.57	16.11	1.99	0.49	15.17	1.91	14.61	26.94	33.23	0.26	0.13
	4.0	70	98	0.62	1.79	5.21	7.31	8.38	2.30	0.47	5.20	0.67	14.50	16.64	29.03	0.70	0.44
Wardan	0.0	95	86	0.43	1.73	5.02	9.04	10.88	3.40	0.51	5.73	0.73	13.44	17.93	32.40	0.70	0.59
	2.0	94	91	0.50	1.84	6.36	9.73	12.62	3.23	0.49	8.53	1.06	15.68	23.50	27.90	0.464	0.38
	4.0	60	92	0.15	1.06	4.10	6.24	7.06	1.60	0.35	2.47	0.32	13.39	12.50	26.40	1.072	0.65

Fig. 3 Effect of sowing depth of root and shoot growth of berseem plant (A) surface sown, (B) sown at 2.0 cm depth (C) sown at 4.0 cm depth.

depth. Sowing depth did not significantly affect root length (Fig. 3). However, the root-to-shoot ratio was significantly higher at 2.0 cm depth, suggesting enhanced resource allocation to root development at optimal sowing depth. Variety JBSC-1 (Larger seeds) exhibited higher root-to-shoot ratios, which aligns with previous studies (Moles and Westoby 2006, Mendes-Rodrigues *et al.* 2011).

Seed size and sowing depth are critical determinants of germination, seedling establishment, and growth in berseem. Larger seeds, exhibited superior germination, vigour, and biomass, while an optimal sowing depth of 2.0 cm enhanced overall plant performance. In contrast, both shallow and deep sowing depths negatively affected seedling establishment and growth. These results underscore the importance of using bold-seeded varieties and optimal sowing depth to maximise productivity. The findings of this research are anticipated to lay the groundwork for future advancements in berseem cultivation, particularly in the development of specialised sowing machinery and the breeding of bold-seeded varieties. These initiatives aim to enhance productivity and promote sustainability in berseem farming practices.

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