

## Low-temperature seed storage optimizes seedling traits, vegetative growth and flowering in *Antirrhinum majus*

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### Abstract

Snapdragon, belonging to the family Scrophulariaceae, is a hardy plant, but the unfavourable seed storage conditions can fluctuate the timing of flowering, which is a key factor in floriculture. Seed physiology, morphology and environmental conditions significantly influence the germination of ornamental plants, while improper handling and processing reduce seed emergence. This study investigates the effects of different seed storage temperatures (0, 4, 6, and 8 °C) and durations (30, 45, and 60 days) on seed germination, seedling traits, and flowering time of snapdragon (*Antirrhinum majus*). The results concluded that seeds stored at 4°C for 60 days showed early seed germination (2.67 days) and maximum seed germination percentage (93.33 %). The treatment also led to early flowering, 30 days earlier than the control. Therefore, storing antirrhinum seeds at 4°C for 60 days enhances seed germination, resulting in improved seedling traits and early flowering, and provides valuable insights for optimising cultivation practices in floriculture.

**Key words:** Storage temperature, storage duration, snapdragon, vernalization, seed germination

### Introduction

Snapdragon (*Antirrhinum majus*), a popular ornamental plant valued for its vibrant flowers and prolonged blooming period, belongs to the family Scrophulariaceae (Abdullah *et al.*, 2020). Native to the Mediterranean region, it is widely grown for its vibrant, showy flowers that resemble a dragon's mouth, giving rise to its common name. Snapdragons are available in a variety of colours, making them a versatile choice for gardens, borders and floral arrangements (Ahmad and Dole, 2014). They thrive in well-drained soils under full sunlight and are particularly valued for their extended blooming period. In addition to their ornamental appeal, snapdragons also tolerate cool climates, making them suitable for seasonal cultivation in diverse regions. Snapdragon seeds have inherent germination issues, including uneven germination and low emergence rates (Bhargava *et al.*, 2015). These problems may arise due to seed coat hardness, physiological dormancy, or suboptimal storage conditions (Çelikel *et al.*, 2010). Many annual flowers like antirrhinum have small seed size, reducing the germination percentage, especially when sown deep. This leads to uneven germination and flowering, reducing the next years seed vigour. The seed survival is better when storage temperature and moisture content is low.

Low temperature (vernalization) is subjecting seeds or propagules to a period of low temperatures, which has proven to be an effective method for improving growth and flowering in many temperate species (Ami *et al.*, 2013). Cold temperatures downregulate the production of abscisic acid (ABA) and FLC (FLOWERING LOCUS C) gene, inhibiting germination and delaying flowering. At the same time, it simultaneously upregulates the production of gibberellic acid (GA), which promotes seed germination and regulates flowering (Penfield, 2024). This process helps break seed dormancy, enhance germination rates and promote uniform flowering by mimicking the natural cold exposure required by

certain plants. Seed storage duration also plays a critical role in determining the vigour and viability of snapdragon seeds. Over time, improper storage conditions can lead to reduced germination rates, weakened seedling growth and the overall decline in plant performance. Factors such as temperature, humidity and storage duration must be carefully managed to preserve seed quality (Aghilian *et al.*, 2014). The proper seed storage conditions of annual flower crops are usually not considered and lack significant scientific knowledge. While storage of snapdragon is easy under temperate conditions due to low temperature, specific temperature and duration effects on snapdragon seed viability, seedling vigour and flowering manipulation remain underexplored in the semi-arid and subtropical regions.

Understanding and optimizing vernalization and storage practices offer promising solutions for overcoming these challenges, ensuring improved growth and superior seed quality in snapdragon cultivation. Growers can achieve more consistent plant establishment and superior floral displays by improving germination rates and seedling vigor. This research aimed to optimize seed storage conditions for improved germination, quality seedling and early flowering in snapdragon, helping in precision floriculture.

### Material and methods

The experiment was carried out at the experimental farm in the subtropical region of India, at 30°54'N latitude and 75°48'E longitude, with an elevation of 247 meters above mean sea level. The region experiences a typically semi-arid climate characterized by hot and dry conditions from April to June, hot and humid conditions from July to September, and occasional ground frost during the winter months of December and January. The average annual rainfall in this area is around 700 mm. Snapdragon (*Antirrhinum majus*) seeds were collected from the experimental

farm of the Department of Floriculture and Landscaping, PAU Ludhiana, during 2023-2024. The seeds were thoroughly cleaned and air dried so no moisture was left. The winter seasonal, annual flower crops require chilling temperatures for seed germination, highlighting the significance of storing their seeds at low temperatures (5°C or below), which tend to slow the aging process. Still, the actual specification for annual flower crops is not present. Hence, the following temperatures and durations were selected for the study. The seeds were placed in plastic bags and kept at different storage temperatures for varying durations 0, 4, 6, 8°C for 30, 45 and 60 days. The seeds in control were stored for 60 days at ambient room temperature after harvesting. The temperature maintained in control (approx. 26.5°C) is the average mean temperature during the two months of storage. All the treatments were taken out from different temperature regimes on 4<sup>th</sup> October 2023 after their desired storage duration was completed, and seeds were sown on 5<sup>th</sup> October 2023 in a raised nursery bed. Then, 10 seedlings at 3-4 true leaf stages of each treatment per replication were transplanted in 8-inch pots on 2<sup>nd</sup> December 2023. The experiment was laid out in a completely randomized design (CRD) with 13 treatments and three replications. The observations for various seed germination and seedling traits, along with flowering time and quality, employed a two-way analysis of variance and data was analyzed using OPSTAT (Sheoran *et al.*, 1988).

## Results and discussion

Effects of different vernalization temperatures (0°C, 4°C, 6°C and 8°C) with varying durations (30, 45 and 60 days) on seed germination and seedling traits along with flowering time and quality of snapdragon are presented in Table 1, 2 and 3.

**Seed germination and seedling traits:** The storage temperature, duration and their interaction were found to be significant ( $P <$

0.05) for days to seed germination, germination percentage (%), and seedling length (cm) (Table 1). The combination of seed storage temperature and duration showed early seed germination at 4°C for 60 days (2.67 days) followed by 4°C for 45 days (3.67 days), whereas delayed germination was observed in control (14.33 days). The germination percentage increased with a decrease in temperature as seen in treatment 0°C for 30 days (100 %), which was statistically at par with 4°C for 60 days (93.33 %), whereas the minimum was observed in control (40 %).

The maximum length of seedlings was observed at 0°C for 30 days (3.90 cm), which was statistically at par with 4°C for 60 days (3.77 cm), while control showed a minimum length (1.33 cm). The observed improvements in germination and seedling growth parameters with optimal storage conditions can be attributed to preserving seed moisture content and reducing metabolic activities during storage, which help maintain seed viability. Moreover, appropriate storage conditions can prevent the deterioration of seed reserves essential for germination and early seedling development. Similar results were reported in pansy (Dhatt 2016; Syssoeva *et al.*, 2010) where low-temperature storage facilitated the enzymatic activity and metabolic readiness for germination. The storage duration results are also conclusive with studies in salvia and petunia (Demir *et al.*, 2020), where prolonged storage of 24 months upregulated the physiological ageing of seeds and reduced seed germination percentage.

**Vegetative growth:** The two-way analysis of variance of storage conditions along with their interaction showed significant ( $P <$  0.05) results for plant height, plant spread and number of branches (Table 2). The effect of storage conditions observed maximum results for plant height in 4°C for 60 days (31.83 cm), which was at par with 6°C for 60 days (30.33 cm) and 8°C for 60 days (30.17 cm), while minimum was observed in control (15.45 cm).

Table 1. Effect of storage temperature (°C) and duration on seed and seedling parameters

Storage temperature	Days to seed germination				Germination percentage (%)				Seedling length (cm)			
	30	45	60	Mean	30	45	60	Mean	30	45	60	Mean
0	5.33	6.33	8.33	6.67	100 (10.00)	46.67 (6.89)	56.67 (7.58)	67.78 (8.17)	3.90	3.77	3.80	3.82
4	5.67	3.67	2.67	4.00	73.33 (8.61)	83.33 (9.18)	93.33 (9.71)	83.33 (9.18)	2.77	3.43	3.77	3.32
6	8.67	10.33	5.33	8.11	53.33 (7.36)	73.33 (8.61)	83.33 (9.18)	70.00 (8.38)	2.63	3.17	3.27	3.02
8	10.33	11.33	8.33	10.00	66.67 (8.22)	56.67 (7.58)	63.33 (8.01)	62.22 (7.94)	3.67	3.87	3.73	3.76
Mean	7.50	7.92	6.17		73.33 (8.61)	65.00 (8.07)	74.16 (8.62)		3.24	3.56	3.64	
Control			14.33				40					1.33
C.D. (0.05)												
Storage temperature			0.57			5.41					0.22	
Storage duration			0.49			4.69					0.19	
Interaction			0.98			9.38					0.38	

Table 2. Effect of storage temperature and duration on growth parameters

Storage temperature (°C)	Plant height (cm)				Plant spread (cm)				Number of branches			
	30	45	60	Mean	30	45	60	Mean	30	45	60	Mean
0	29.33	19.33	16.33	21.67	13.83	14.67	13.50	14.00	4.33	2.67	1.67	2.89
4	26.67	23.67	31.83	27.39	11.50	16.17	16.83	14.83	2.33	3.33	5.33	3.67
6	23.00	23.67	30.33	25.67	16.17	13.67	14.17	14.67	1.70	2.67	4.33	2.90
8	26.33	27.50	30.17	28.00	14.00	15.17	17.67	15.61	1.67	4.33	7.00	4.33
Mean duration (days)	26.33	23.54	27.17		13.88	14.92	15.54		2.50	3.25	4.58	
Control			15.45				10.45				3.00	
C.D. (0.05)												
Storage temperature			3.21			0.65					0.61	
Storage duration			2.78			0.56					0.53	
Interaction			5.56			1.12					1.06	

The combination of storage temperature and duration indicated that seeds stored at 8°C for 60 days (17.67 cm; 7) showed maximum plant spread and number of branches followed by 4°C for 60 days (16.83 cm; 5.33 branches) while minimum was seen in control (10.45 cm; 3). Low temperatures during seed storage likely preserved seed vigour and viability, contributing to enhanced plant development post-germination (Sysoeva *et al.*, 2010). The synergistic effects of temperature and duration suggest that precise control of storage variables can enhance phenotypic traits, such as canopy architecture and branching. These attributes are essential for ornamental plants like snapdragon, where aesthetic value is critical.

**Flowering time:** The significant ( $P < 0.05$ ) influence of storage temperature on the timing of bud initiation (Table 3) and flowering in snapdragon highlights the critical role of storage of seed treatments in modulating physiological processes. The minimum days to bud formation at 4°C (86.22 days) followed by 8°C (87.78 days) align with the concept of cold stratification, which helps overcome seed dormancy by mimicking natural winter conditions (Baskin, 1988). Seeds stored at this temperature likely experienced enhanced metabolic activity and hormonal balance, including increased gibberellin levels, which is essential for early developmental stages. The slightly longer time to bud formation in the control group (110 days) compared to 4°C suggests a threshold beyond which the efficiency of vernalization diminishes.

For flowering, the combination of storage temperature and duration was significant (Table 3), with seeds stored at 4°C for 60 days taking the fewest days to flower (95.33 days). This finding is consistent with a study in salvia, indicating that a combination of cold storage and extended duration optimizes the internal readiness of seeds for germination and subsequent growth phases (Demir *et al.*, 2020). The reduction in flowering time with 4°C storage aligns with findings in other ornamental species, such as in *Brunonia*, *Calandrinia* sp. (Cave and Jhonston 2010), *Thellungiella halophila* (Guo *et al.*, 2012) and in marigold (Sysoeva *et al.*, 2010), where cold stratification accelerated flowering. The relatively efficient performance at 0°C (103 days) further underscores the viability of cold storage, although slightly less effective than 4°C, likely due to the metabolic slowdown at temperatures approaching freezing (Ren *et al.*, 2023).

While longer storage durations (60 days) facilitated earlier flowering (103.25 days), they showed diminishing returns after a threshold period, suggesting that prolonged cold exposure stabilizes the physiological benefits without additional gains. The significantly delayed flowering in the control group (128 days) confirms the inadequacy of ambient temperatures (26.5°C) for 60 days, as seeds at this temperature lack

the physiological cues necessary for a rapid transition to flowering. In Punjab, due to a lack of planting material from state nurseries, the flowering stock of snapdragon in November is supplied by nurseries from other states like West Bengal and Karnataka, while local sowing starts in November and results in flowering by late February. Thus, the technique of giving pre-sowing cold treatment has enabled early seed germination in October with early flowering by late December hence benefitting the timely production of local planting material.

This study is novel in demonstrating how controlled storage conditions can be utilized as a non-chemical approach to enhance seed viability, vigour and flowering manipulation in snapdragon, which is particularly beneficial for regions with fluctuating climatic conditions. Adopting pre-sowing cold treatments at 4°C for 60 days serve as a practical approach to enhance seed germination, vegetative quality and coordinated flowering. Commercial growers and floriculturists can directly apply the findings to optimize nursery production, flowering synchronization and off-season cultivation. By refining storage temperature and duration, this study provides an efficient, cost-effective and sustainable strategy to improve seed quality parameters and flowering in snapdragon, making it a valuable tool for modern floriculture practices. Further studies examining hormonal changes and genetic markers during cold storage would provide deeper insights into the mechanisms driving these responses.

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**Author contribution:** Dr. Tanya Thakur supervised and planned the layout of work. Ms. Arushi Garg did data collection and analysis. The first draft of the manuscript was written by (Ms. Arushi Garg) and both authors commented on previous versions.

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Table 3. Effect of storage temperature (°C) and duration (days) on flowering time

Storage temperature	Days to bud initiation				Days to flowering			
	30	45	60	Mean	30	45	60	Mean
0	90.00	90.67	90.00	90.22	102.33	105.33	104.00	103.89
4	87.00	86.00	85.67	86.22	99.67	99.00	95.33	98.00
6	88.00	88.33	89.00	88.44	105.0	110.0	104.0	106.33
8	87.00	87.67	88.67	87.78	110.6	111.6	109.6	110.67
Mean duration	88.00	88.17	88.33		104.4	106.5	103.2	
Control			110.0				128.0	
C.D. (0.05)								
Storage Temp.		0.88				0.77		
Storage duration		NS				0.66		
Interaction		NS				1.33		

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