



In vitro Regeneration of Gerbera (*Gerbera jamesonii*) in SAU Tissue Culture Medium

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Abstract: *In vitro* regeneration potentiality of Gerbera (*Gerbera jamesonii*) flower was evaluated in ammonium nitrate free SAU Plant tissue culture medium. The most population MS (1962) medium and MS powder (Duchefa Biochemie, The Netherland) were used as two check treatments. Data were collected on callus induction, days to shoot initiation, multiple shoot regeneration, shoot length, number of leaves, root number and root length. Among the treatments, MS powder (T1) showed the best shoot performance for producing the highest number of shoots (23.00), longest shoots (7.40 cm) and maximum leaves (5.33) at 42 days after inoculation. The second highest performance showed by the SAU Tissue Culture medium which was very close to MS powder and non significant for most of the traits under study. The SAU TC showed the highest root number (7.33) and length (6.97 cm). Regenerated plantlets showed 95% survival under controlled conditions and 75% in field acclimatization. The findings validate the SAU TC medium as a safe, economical and effective alternative to MS media for large-scale *in vitro* propagation of Gerbera in Bangladesh.

Keywords: Gerbera, regeneration, SAU TC medium, MS (1962) medium.

INTRODUCTION

Gerbera jamesonii Bolus ex Hook. f., an economically important ornamental species of the Asteraceae family and widely cultivated for its diverse color range, attractive inflorescences, and long vase life [1,2]. In Bangladesh, the demand for Gerbera in the cut-flower industry has increased markedly in recent years, driven by expanding urban markets and the growth of commercial floriculture [3]. However, conventional propagation methods remain insufficient to meet industry requirements for large-scale, uniform and disease-free planting materials. Seed propagation is limited by high genetic variability and inconsistent phenotypic expression, which undermine uniformity in flower quality [2]. Vegetative propagation, while genetically stable, suffers from low multiplication rates and increased susceptibility to systemic transmission of viruses and soil-borne pathogens [4]. These limitations highlight the need for a more efficient propagation system. Plant tissue culture offers a precise and rapid alternative for mass multiplication of elite genotypes under aseptic and controlled environmental conditions [5,6]. *In vitro* regeneration of Gerbera has been widely studied using various explants, including shoot tips, leaves, and floral tissues on Murashige and Skoog (MS) medium supplemented with plant growth regulators [7]. In Bangladesh, several studies have optimized hormonal combinations on MS medium with varying success [8,9]. Despite its widespread use, MS medium preparation is constrained by stringent government regulations on ammonium nitrate (NH_4NO_3)—a key nitrogen source—

due to its explosive properties. As laboratories can no longer procure raw NH_4NO_3 , reliance on imported commercial MS powder has increased, significantly elevating production costs. To address this challenge, [10] developed the ammonium nitrate-free SAU Tissue Culture Medium, which has demonstrated successful *in vitro* regeneration in potato. The present study aims to evaluate the effectiveness of this alternative medium for *Gerbera jamesonii* regeneration and assess its potential as a cost-effective and locally adaptable substitute for standard tissue culture medium.

MATERIALS AND METHODS

The experiment was conducted at the Biotechnology Laboratory of Sher-e-Bangla Agricultural University (SAU), Dhaka-1207, Bangladesh, from October 2023 to June 2024. Capitulum of *Gerbera jamesonii* were used as explant. It was collected and washed thoroughly under running tap water, followed by multiple rinses with autoclaved distilled water to remove surface debris. Surface sterilization was performed inside a laminar airflow cabinet, where explants were immersed in 70% ethanol for 1 to 2 minutes with gentle agitation and subsequently rinsed with sterile distilled water. For complete disinfection, explants were treated with 0.2% (w/v) mercuric chloride (HgCl_2) for 2 to 3 minutes, with a few drops of Tween-20 added as a surfactant to enhance sterilant penetration. The HgCl_2 solution was removed through 3 to 4 final rinses with autoclaved distilled water to eliminate all traces of sterilant. All inoculation steps were carried out under aseptic conditions inside the laminar airflow hood. All instruments, glassware, culture bottles and media were sterilized by autoclaving at 121°C for 20 min. The excess outer tissues were trimmed using a sterile scalpel to obtain uniform, healthy explants. Each explant was cut to a length of 0.5-1.0 cm and then inserted vertically into culture bottles containing medium supplemented with plant growth regulators. The necks of culture vessels were flamed before and after inoculation to maintain sterility, and each bottle was sealed and labeled with the treatment code and inoculation date. The inoculated cultures were incubated in a controlled growth chamber at $21 \pm 1^\circ\text{C}$ under a 16-hour light and 8-hour dark photoperiod provided by white fluorescent tubes delivering 4000-5000 lux of light intensity, with relative humidity maintained at approximately 70%-80%. The experiment consisted of three media treatments: T1=commercially formulated MS powder (Duchefa Biochemie, The Netherland) supplemented with plant growth regulators; T2=manually prepared MS medium following Murashige and Skoog (1962) nutrient composition, supplemented with plant growth regulators; and T3=SAU Tissue Culture Medium supplemented with plant growth regulators. The same hormonal concentration were used for all the three treatment. Callus induction media was supplemented with 1.00 mg/L of 2,4-D. For shoot and root induction it was supplemented with 1.00 mg/L KIN + 0.5 mg/L of IAA. Data on *in vitro* developmental parameters were collected through direct measurement and visual observation at 7-day intervals on days 7, 14, 21, 28, 35, and 42 following inoculation. A calibrated scale was used for length-related measurements. Recorded parameters included days to callus induction, days to shoot initiation, shoot length (cm), number of shoots per explant (multiple shoot regeneration), number of leaves per plantlet, number of roots per plantlet, and root length (cm) per plantlet. Mean values were computed for each parameter to represent the average response under each treatment for subsequent analysis.

RESULTS AND DISCUSSION

In vitro regeneration work of gerbera was done in the experiment. The major finding of the research were given in the following sub- heading.

Days to Callus Induction

The treatments showed significant variation in their time for callus induction. T1 (MS Powder) was the most efficient and the shortest time for callus induction. It was 22.67 days followed by T3 (SAU Tissue Culture Medium) at 23.67 days. The longest time was noticed for the treatment T2 (MS, 1962) and it required 26 days for callus induction. T1 (MS Powder) showed highest percentage of callus induction (91.67%) and followed by T3 (SAU TC medium) and it was 90.67%. It was lowest (79.67%) in MS (1962) medium. There is no significant difference among the treatment-1 and treatment-3(Table 1).

Table 1: Days to callus induction and percent of callus induction in Gerbera flower among the three treatments

Treatment	Days to callus initiation	percentage of callus induction
T1= Readymade MS Powder + 1.00 mg/L 2,4 D	22.67b	91.67a
T2=Murashige & Skoog(1962)Medium + 1.00 mg/L 2,4 D	26a	79.67b
T3=SAU Tissue Culture Medium + 1.00 mg/L 2,4 D	23.67b	90.67a
SE (±)	0.6	0.81
LSD (0.05)	1.48	1.99
CV (%)	3.09	1.15

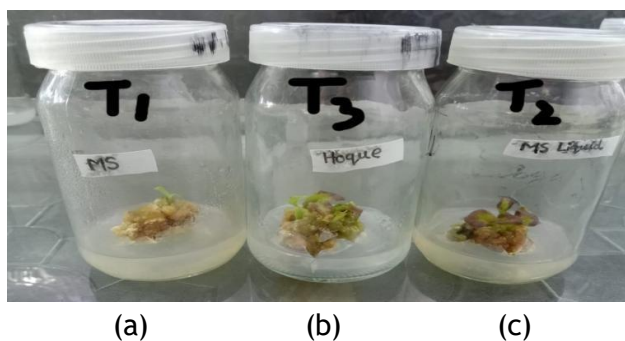


Plate 1: Callus induction of Gerbera at 42 days

(a) T1= Readymade MS Powder + 1.00 mg/L 2,4 D, (b) T2= Murashige & Skoog(1962)Medium + 1.00 mg/L 2,4 D,(C) T3= SAU Tissue Culture Medium + 1.00 mg/L 2,4 D

The strong response observed in T1 can be linked to the use of industrially prepared MS powder medium, which offers a reliable and precisely balanced supply of nutrients and vitamins. In contrast, the slower callus induction in T2 likely resulted from slight modification that can occur when MS medium is prepared manually from individual components, leading to possible nutrient inconsistencies. The performance of T3, which closely matched that of T1, suggests that the SAU Tissue Culture Medium contains a well-adjusted nutrient composition—such as an appropriate ammonium and nitrate ratio or beneficial organic additives that efficiently supports callus development in Gerbera . Using

2,4-D in all treatments is also appropriate, as this auxin is widely recognized for its strong ability to trigger callus formation in many plants, including members of the Asteraceae family like Gerbera [11].

Days to Shoot Initiation and Percent of Shoot Regeneration

Distinct differences were observed among the treatments in terms of the time required for shoot initiation. The earliest response occurred in T1 (Readymade MS Powder), where shoots developed in 19.33 days. The T3 (SAU Tissue Culture Medium) treatment required slightly more time (21.33). It was non significant with T1 and remained considerably faster than T2. The longest duration for shoot initiation was recorded in T2 (MS, 1962), with an average of 23.33 days (Table 2). Regarding regeneration efficiency, T1 performed the best and the highest shoot regeneration rate (90.00%). This was followed by T3, which produced a regeneration rate of 88.66%. The lowest value was observed in T2 (79.66%) (Plate 2). Overall, all three media supported shoot regeneration in Gerbera.

Table 2: Days to shoot initiation and percent of regeneration in Gerbera flower among the three media

Treatments	Days to shoot initiation	Percent of regeneration (%)
T1= Readymade MS Powder + 1 mg/L KIN+0.5 mg/L IAA	19.33c	90.00a
T2= Murashige & Skoog(1962)Medium + 1 mg/L KIN+0.5 mg/L IAA	23.33a	79.66c
T3=SAU Tissue Culture Medium + 1 mg/L KIN+0.5 mg/L IAA	21.33b	88.66b
SE (\pm)	0.47	2.06
LSD (0.05)	1.15	2.12
CV (%)	2.71	6.27

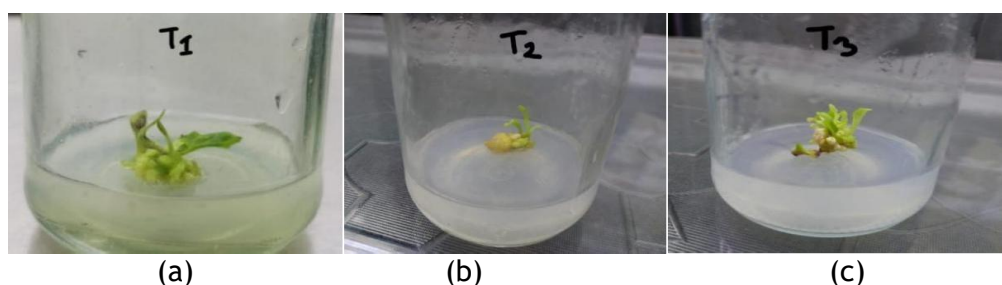


Plate 2: Shoot initiation of Gerbera at 21 days

(a)T1= (Readymade MS Powder + 1 mg/L KIN+0.5 mg/L IAA), (b)T2= Murashige & Skoog(1962)Medium + 1 mg/L KIN+0.5 mg/L IAA, (c)T3= SAU Tissue Culture Medium + 1 mg/L KIN+0.5 mg/L IAA

Previous studies shown that adventitious shoots can be successfully induced from callus originating from different explants, including leaf and petiole tissues [12]. Similarly, capitulum-based cultures also been widely documented and highly responsive sources for shoot regeneration in Gerbera [7,12,13]. The use of KIN and IAA is well established for promoting shoot bud differentiation. The superior results in T1 suggest that its nutrient consistency may enhance the plant's hormonal response. The efficient response in T3 further indicates that the SAU medium is well balanced and suitable for both callus induction and

organogenesis. The use of kinetin (KIN) in combination with IAA proved highly effective. Kinetin stimulates cell division and shoot meristem activation, while IAA supports organized tissue growth.

Multiple Shoot Regeneration

The number of shoots generally increased over time for all treatments. The highest multiple shoot regeneration was occurred at 42 DAI was 23.00 in T1(Readymade MS Powder), while T2(MS (1962) medium) had the lowest yielder (Table 3). Treatments T1 and T3 consistently outperformed than T2, suggesting that these media provide a more favorable cytokinin-supported biochemical environment for shoot development. This strong performance highlights kinetin's effectiveness in activating shoot meristems and promoting organized shoot proliferation.

Table 3: Number of shoots per explant at different days after initiation in Gerbera at three different media

Treatment	14 Days	28 Days	42 Days
T1= Readymade MS Powder + 1 mg/L KIN+0.5 mg/L IAA	3.67a	14.67a	23a
T2= Murashige & Skoog(1962)Medium + 1 mg/L KIN+0.5 mg/L IAA	2.33b	9.67b	15.67b
T3=SAU Tissue Culture Medium + 1 mg/L KIN+0.5 mg/L IAA	2.67ab	12b	21a
SE (\pm)	1.48	1.15	1.15
LSD (0.05)	1.15	2.49	3.32
CV (%)	9.19	10.3	8.38



Plate 3: Multiple Shoot regeneration of Gerbera at 42 days

(a) T1= Readymade MS Powder + 1 mg/L KIN+0.5 mg/L IAA, (b) T2= Murashige & Skoog(1962)Medium + 1 mg/L KIN+0.5 mg/L IAA, (c) T3= SAU Tissue Culture Medium + 1 mg/L KIN+0.5 mg/L IAA

These observations align closely with the findings of [11], who reported that kinetin-rich media induce rapid and efficient shoot regeneration in Gerbera. Their study demonstrated that MS medium supplemented with 1 mg/L kinetin produced approximately six shoots per callus within five days of culture, accompanied by shoot lengths averaging 5.2 cm.

Length of Shoot (cm) per Explant

The shoot length of T1 (Readymade MS Powder) was 7.4 cm at 42 DAI, while T3 (SAU Tissue Culture Medium) had a slightly shorter shoot at 7.00 cm. The T2 (Murashige & Skoog medium) treatment showed the shortest result, measuring 6.13 cm (Fig 1, plate 4). The relatively similar shoots obtained in T1 and T3 indicate that these media create a favorable biochemical environment for auxin-mediated elongation. This effect may be linked to an optimal balance of key nutrients such as calcium and boron, which play essential roles in maintaining cell wall integrity, membrane stability, and overall cell expansion. These findings align well with the results reported by [11], who observed that MS medium supplemented with 1 mg/L kinetin produced shoots averaging 4.5 ± 0.1 cm, while the highest shoot length (5.2 ± 0.1 cm) was achieved with 1.5 mg/L kinetin. This demonstrates that kinetin not only promotes shoot initiation but also enhances shoot elongation in a concentration-dependent manner.

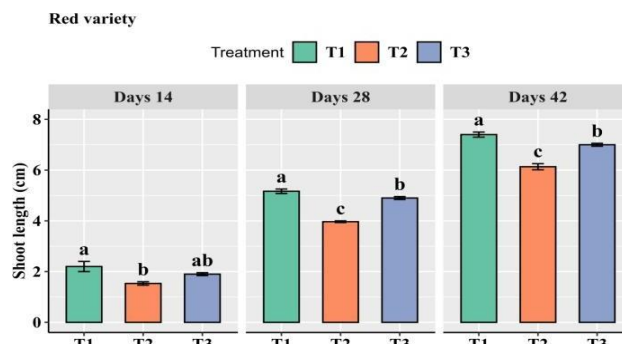


Figure 1: Effect of different tissue culture media on length of shoot (cm) at different days after initiation in Gerbera

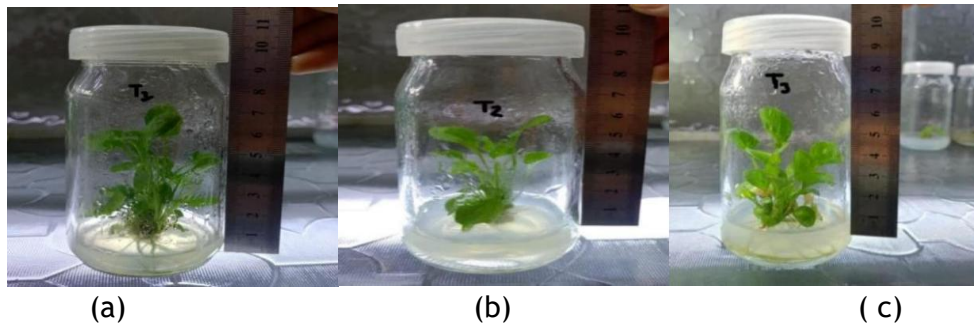


Plate 4: Multiple Shoot regeneration of Gerbera at 42 days

(a) T1= Readymade MS Powder + 1 mg/L KIN+0.5 mg/L IAA, (b) T2= Murashige & Skoog(1962)Medium + 1 mg/L KIN+0.5 mg/L IAA, (c) T3= SAU Tissue Culture Medium + 1 mg/L KIN+0.5 mg/L IAA

Number of Leaves per Plantlet

Maximum leaves per plantlet (5.33) at 42 DAI was found in the T1(Readymade MS Powder) and it was followed by T3 (SAU Tissue Culture Medium). The lowest (3.67) leaves were produced by the T2 (Murashige & Skoog medium,1962). T1 consistently demonstrated superior performance in promoting leaf growth across all time period. The treatments T1 and T3 consistently yielded a greater number of leaves, suggesting that these media compositions effectively stimulated both apical and lateral meristematic activity. This trend

is in agreement with the observations of [11], who reported 8.7 ± 0.3 leaves in MS medium containing 0.5 mg/L kinetin and 12.0 ± 1.0 leaves at 1.5 mg/L kinetin, demonstrating a positive relationship between kinetin concentration and leaf initiation in Gerbera. Studies have shown that kinetin significantly boosts leaf initiation compared to auxin-dominant or hormone-free media [15].

Table 4: Number of leaves per explant at different days after initiation in Gerbera flower at three different media

Treatment	14 Days	28 Days	42 Days
T1= Readymade MS Powder + 1 mg/L KIN+0.5 mg/L IAA	2.33a	4a	5.33a
T2= Murashige & Skoog(1962)Medium + 1 mg/L KIN+0.5 mg/L IAA	2a	2.33b	3.67b
T3=SAU Tissue Culture Medium + 1 mg/L KIN+0.5 mg/L IAA	1.33a	3.33ab	5ab
SE (\pm)	0.6	0.6	0.6
LSD (0.05)	1.48	1.48	1.48
CV (%)	9.46	13.13	15.97

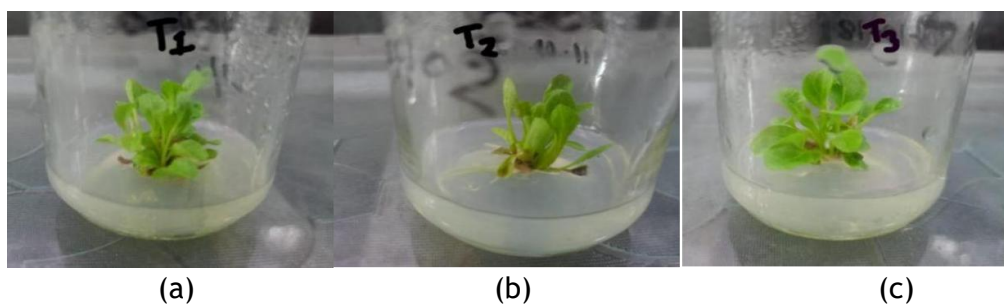


Plate 5: Number of leaves per explant of Gerbera at 42 days

(a)T1= Readymade MS Powder + 1 mg/L KIN+0.5 mg/L IAA, (b)T2= Murashige & Skoog(1962)Medium + 1 mg/L KIN+0.5 mg/L IAA, (c)T3= SAU Tissue Culture Medium + 1 mg/L KIN+0.5 mg/L IAA

Length of Root (cm)

Highest root length (6.97) was observed in T3 (SAU Tissue Culture Medium)at 42 DAI.

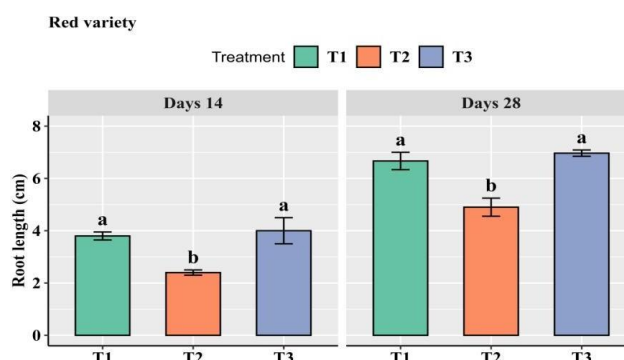


Figure 2: Length of root (cm) at different days after root initiation in Gerbera

T1= Readymade MS Powder + 1 mg/L KIN+0.5 mg/L IAA, T2= Murashige & Skoog(1962) Medium + 1 mg/L KIN+0.5 mg/L IAA, T3= SAU Tissue Culture Medium + 1 mg/L KIN+0.5 mg/L IAA

It was lowest (4.90) in T2 (Murashige & Skoog medium, 1962). The T1 (Readymade MS Powder) showed second highest (6.67 cm) root length at 42 DAI (Fig 2, plate 6)



(a) (b) (c)
Plate 6: Length of root (cm) of Gerbera at 28 days

(a) T1= Readymade MS Powder + 1 mg/L KIN+0.5 mg/L IAA, (b) T2= Murashige & Skoog(1962)Medium + 1 mg/L KIN+0.5 mg/L IAA, (c) T3= SAU Tissue Culture Medium + 1 mg/L KIN+0.5 mg/L IAA

Significant variation in root length was observed among the treatments. The longest roots (7.33 cm) were produced with IAA at 1.5 mg L⁻¹ (T7), whereas NAA at 1.5 mg L⁻¹ (T3) resulted in shorter roots (4.67 cm), which were significantly lower than all IAA treatments [15]. These results highlight the superior efficacy of IAA in promoting not only root initiation but also elongation.

Number of Roots per Explants

The maximum number of roots per plantlet (7.33) at 28 DAI was observed in T3, while T1 produced a slightly lower count of 7.00 roots. The minimum number of roots (3.67) was recorded in T2 (MS, 1962) (Plate 6). Across the entire observation period, T3 consistently maintained the highest root numbers, indicating its strong ability to support root development. T1 showed a performance similar to T3, whereas T2 repeatedly exhibited comparatively reduced root formation.

Table 5: Number of roots per explant at different days after initiation in Gerbera flower at three different media

Treatment	14 Days	28 Days
T1= Readymade MS Powder + 1 mg/L KIN+0.5 mg/L IAA	3ab	7a
T2=Murashige & Skoog(1962)Medium + 1 mg/L KIN+0.5 mg/L IAA	1.67b	3.67b
T3=SAU Tissue Culture Medium + 1 mg/L KIN+0.5 mg/L IAA	3.67a	7.33a
SE (±)	1.99	1.15
LSD (0.05)	1.48	1.48
CV (%)	6.83	12.42

Exogenous application of IAA and NAA significantly influenced root formation in *Gerbera*. No roots were observed in auxin-free MS medium, highlighting the essential role of auxins in adventitious root induction [15]. Auxins promote dedifferentiation of

pericycle and parenchyma cells, initiating mitotic activity and root primordium formation [16]. Overall, IAA was more effective than NAA in promoting root proliferation under in vitro conditions.

Acclimatization of Plantlets

Plantlets were first grown in a controlled growth chamber for 14 days, then moved to field conditions for an additional 28 days. The experiment utilized a total of 20 plantlets for each of three culture media.

Table 22: Acclimatization of Gerbera in growth chamber & in the field condition

Treatments	In growth chamber (14 days)			In field condition (28 days)		
	Plantlets transferred	Seedlings established	Survival rate (%)	Plantlets transferred	Seedlings established	Survival rate (%)
T1= Readymade MS Powder	20	20	100	20	16	80
T2=Murashige & Skoog(1962)Medium	20	18	90	20	14	70
T3=SAU Tissue Culture Medium	20	19	95	20	15	75

In the controlled growth chamber, survival rates ranged from 90% to 100%. The highest survival rates were observed in treatments T1 and T3, suggesting that these plantlets successfully adapted to the new conditions. After being transplanted to the field, the survival rate ranged from 75% to 80%. Acclimatization is a crucial stage in micropropagation, as tissue culture-derived plantlets are often fragile and physiologically unprepared for external environmental conditions [18]. A period of hardening allows plantlets to gradually adapt, restoring normal leaf structure, water balance, and photosynthetic function [19]. Well-developed shoots in T1 and robust roots in T3 enabled plantlets to better withstand environmental stresses, while less vigorous T2 plantlets showed lower survival.



Plate 7: Acclimatization of Gerbera in field condition at 30 days

CONCLUSION

The experiment was conducted to evaluate the regeneration potential of Gerbera (*Gerbera jamesonii*) using a new SAU tissue culture medium. Multiple-shoot formation was highest in the MS Powder medium supplemented with hormones. The MS Powder medium produced

the maximum number of leaves (5.33 per explant) and the longest shoots (7.4 cm) at 42 days, whereas the standard MS (1962) medium consistently exhibited the lowest performance across these parameters. In contrast, root development was most pronounced in the SAU Tissue Culture Medium, which supported the maximum root length (6.97 cm), compared with the shortest roots in MS (1962) (4.90 cm). Acclimatized plantlets exhibited high survival rate and average 95% in the culture chamber and 75% under open-field conditions. Collectively, these findings indicate that the SAU Tissue Culture Medium is most suitable for root induction, whereas MS Powder medium is optimal for shoot and leaf proliferation. Further refinement of media composition and growth regulator ratios may enhance the efficiency and cost-effectiveness of *Gerbera* micropropagation.

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