Original Research Article

Study on *in vitro* seed germination and seedling development of tuberose (*Agave amica* Medik.)

ABSTRACT

Agave amica (Medik.) (Syn. *Polianthes tuberosa* L.) is commercially propagated asexually. For the diversification of the crop, sexual means of propagation is essential hence there is a necessity to study the seed germination for hybrid development. A protocol for *in vitro* germination of tuberose seeds was formulated. Two types of seed were utilized for germination in which the decoated seeds showed early and higher germination percentage than the intact seeds. Seeds inoculated on the basal media composition of half MS with GA_3 (1 mg/l) and BAP (1 mg/l) recorded minimum time for germination (8 days), higher germination percentage (89.33%) and minimum mean germination time (8.88 days). For further growth of the seedlings different composition of shoot and root proliferation media were studied. The MS media with NAA (2 mg/l) and BAP (2 mg/l) ~~induced~~ increased root length (12.33 cm), shoot length (14.80 cm), number of roots (13.23) and number of leaves (6.25). Under *in vivo* condition, the seedling developed from the basal media composition of half MS with GA_3 (1 mg/l) and BAP (1 mg/l) and sub cultured on MS media with NAA (2 mg/l) and BAP (2 mg/l) showed higher survival percentage of 91.53%.

Keywords: *Tuberose; Seed germination; MS media; Asexually; Roots proliferation*

Formatted: Highlight

Comment [Admin1]: Do not use abbreviation in abstrack

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

1. INTRODUCTION

Tuberose is the economically important flower crop widely grown in sub tropical and tropical regions of the world including India [1]. It is botanically called as *Agave amica* (Medik.) (Syn. *Polianthes tuberosa* L.), belonging to the family Asparagaceae [2]. It is native to Mexico from where it spread out to

different parts of the world [3]. In India, tuberose is considered as the major cut and loose flower because of its delightful fragrance and excellent keeping quality. The total production of tuberose in India is 1,97,210 tonnes (2015-2016) [4]. In Tamil Nadu, where tuberose is primarily grown for loose flower production, the total area under tuberose cultivation during 2019-2020 is 7,654 ha and its production is 66,355 tonnes [5].

Tuberose is a bulbous perennial plant which produces waxy creamy white fragrant flowers in spikes. Tuberose has two types of flowers namely single and double. Variegated types of plants are available which also has single type of flowers. Mostly the single types are used as loose flower and for concrete extraction. Double flowers are utilized for preparation of bouquets. Tuberose concrete fetches higher value in the international market. Self incompatibility, limited genetic resources, dichogamy and poor seed setting are the major constraint in hybridization of tuberose [6]. Seed setting is quiet erratic in single type, not observed in double type and high percentage of seed setting is found in variegated types [7]. There are limited varieties available in tuberose especially in Tamil Nadu. Improved hybrids have to be developed with higher yield and concrete recovery percentage.

Tuberose is commercially propagated by bulbs or bulblets but for varietal development seed propagation is applied, hence seed germination is the major factor to consider. Seeds are black in colour, half circular and 3 mm in diameter. As seed setting is difficult in tuberose, hybrid seeds possess low germination percentage [8]. Seeds sown under *in vivo* condition possess dormancy due to hard seed coat and it takes longer time for germination [9]. Considering the importance of hybrid seeds, this study was conducted on germination of seeds under *in vitro* condition. *In vitro* condition can furnish constant moisture content which accelerate seed germination and uniform seedlings [10]. There is no available research on *in vitro* germination of tuberose seeds. This study was conducted to develop a protocol for *in vitro* germination and morphogenesis of tuberose seedlings on various media compositions.

2. MATERIALS AND METHODS

The present research was conducted at Department of Floriculture and Landscape Architecture, Tamil Nadu Agricultural University, Coimbatore during 2019-2020. Six to seven matured pods from open

pollinated spikes of tuberose were harvested and each pod contains 20-30 seeds which were extracted and used for the *in vitro* germination studies (Fig. 1).

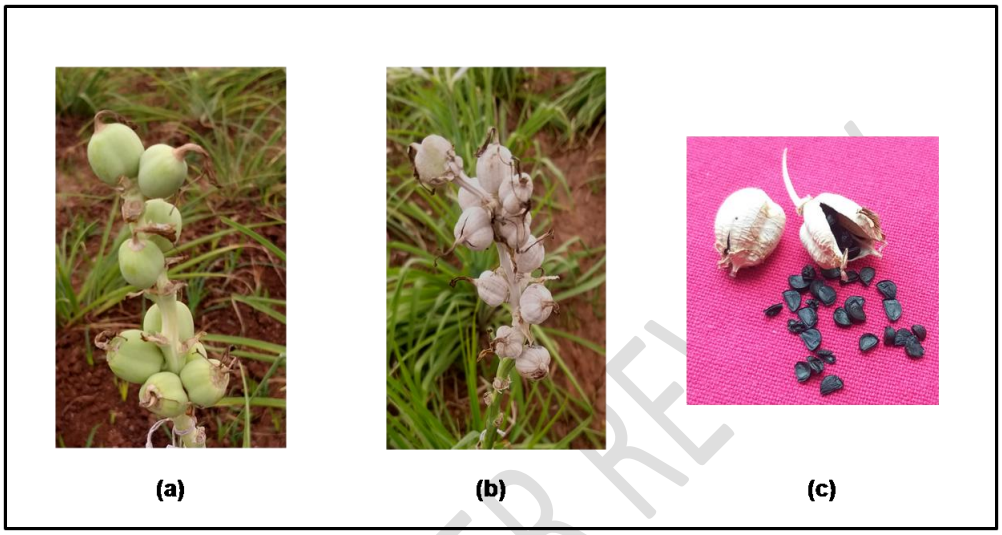


Fig.1. Different stages of pod development in tuberose

(a) Open pollinated fruits (b) Dried pots (c) Tuberose seeds

Matured seeds were pretreated with 250ppm of gibberellic acid (GA_3) for 2 hrs. The seeds are then surface sterilized with 0.1% mercuric chloride for 5 minutes followed by 70% ethanol for 5 minutes. The seed were washed with distilled water for 2- 3 times after each sterilization step to avoid contamination. Two types of seeds were used for *in vitro* seed germination (1) Intact seed (with seed coat) and (2) decoated seed (without seed coat) (Fig 4a). The seeds were inoculated on two strengths MS medium (Murashige and Skoog) namely, Full strength MS salts (FMS) and half strength MS salts (HMS) containing various growth regulators at different concentration for germination. All the *in vitro* seed culture and seedling were incubated under controlled condition 24 ± 2 °C with 24hr photoperiod using fluorescent tubes. All the treatments were repeated thrice with FCRD (Factorial Completely randomized design) as experimental design.

Comment [Admin2]: references???

After four weeks of inoculation, the germinated seedlings were then sub--cultured in MS media with different concentration of growth regulators for morphogenesis. All the treatments were repeated

thrice with CRD (Completely randomized design) as experimental design. The *in vitro* grown seedlings were removed from culture vessel and the roots were washed carefully with tap water and planted in pot filled with perlite and coir pith media. The plants were kept under shade net condition, watered regularly and fertilized with Hoagland's nutrient solution (HS) once a week. Germination was recorded daily and was considered as complete once the radicle protruded about 1cm in length. Mean germination times (MGT), germination percentage (GP) and survival percentage (SP) were calculated using the following formulas [11, 9]

MGT = ∑ (n × d) / N

(where n = number of seeds germinated on each day, d = number of days from the beginning of the test, and N = total number of seeds germinated at the termination of the experiment)

GP= (N/N1) * 100

(where N = total number of seeds germinated and N1=total number of seeds inoculated)

SP= (N/N1) * 100

(where N = total number of seedlings survived after 14 days of hardening and N1=total number of seeds forwarded for hardening)

Data's were analyzed using the SPSS 20.0 statistical software and then subjected to analysis of variance (ANOVA). Means of the treatments were separated by T-test (LSD) with 5% level of significance.

3. RESULT AND DISCUSSION

3.1 Effect of seed type on *in vitro* germination

Pre treated seeds were inoculated on different culture media and then variation in germination time, mean germination time and germination percentage was observed (Table 1).

Table 1. Effect of different culture media composition on germination of tuberose seeds under *in vitro* condition

Media composition	Seed type	Days taken for germination	Germination percentage	Mean germination time (days)

Formatted: Highlight

Formatted: Highlight

Comment [Admin3]: Media named as M1-M10

Half MS + GA_3 (1mg/l)	IS	17.67 ± 2.33^{ghi}	24.00 ± 6.11^{cd}	18.00 ± 0.10^{f}
	DCS	9.67 ± 0.33^{abc}	34.67 ± 3.53^{c}	13.00 ± 0.33^{b}
Half MS + BAP (1mg/l)	IS	21.67 ± 2.03^{j}	13.33 ± 5.81^{d}	22.17 ± 0.09^{i}
	DCS	14.00 ± 0.58^{fg}	24.00 ± 2.31^{cd}	16.59 ± 0.07^{e}
Half MS + GA_3 (1 mg/l) + BAP (1 mg/l)	IS	12.67 ± 0.33^{de}	60.00 ± 2.31^{b}	14.41 ± 0.16^{c}
	DCS	8.00 ± 0.58^{a}	89.33 ± 4.81^{a}	8.88 ± 0.06^{a}
Half MS + GA_3 (0.5 mg/l) + BAP (0.5 mg/l)	IS	14.33 ± 0.33^{ef}	29.30 ± 7.06^{c}	15.75 ± 0.25^{d}
	DCS	9.33 ± 0.33^{ab}	86.67 ± 3.53^{a}	9.32 ± 0.02^{a}
Half MS	IS	21.67 ± 0.33^{j}	53.33 ± 5.33^{b}	24.60 ± 0.23^{k}
	DCS	19.00 ± 0.58^{ij}	13.33 ± 3.53^{d}	21.50 ± 0.47^{i}
Full MS + GA_3 (1mg/l)	IS	18.33 ± 0.88^{ghi}	22.67 ± 11.39^{cd}	20.73 ± 0.29^{h}
	DCS	11.33 ± 0.33^{bcd}	30.67 ± 5.81^{c}	14.79 ± 0.02^{c}
Full MS + BAP (1mg/l)	IS	25.00 ± 1.15^{k}	9.33 ± 9.33^{d}	25.71 ± 0.25^{l}
	DCS	16.00 ±0.58^{fg}	13.33 ± 6.67^{d}	17.79 ± 0.19^{f}
Full MS + GA_3 (1 mg/l) + BAP (1 mg/l)	IS	16.33 ± 0.33^{fgh}	37.33 ± 2.67^{c}	18.10 ± 0.18^{f}
	DCS	10.33 ± 0.33^{abcd}	78.67 ± 2.67^{a}	12.67 ± 0.14^{b}
Full MS + GA_3 (0.5 mg/l) + BAP (0.5 mg/l)	IS	18.67 ±0.67^{hi}	32.00 ± 4.62^{c}	19.63 ± 0.49^{g}
	DCS	12.00 ±0.58^{cde}	61.33 ± 2.67^{b}	14.94 ± 0.30^{c}
Full MS	IS	21.67 ± 0.33^{j}	9.33 ± 1.33^{d}	23.00 ± 0.13^{j}
	DCS	19.33 ± 0.88^{ij}	12.00 ± 4.00^{d}	23.20 ±0.29^{j}

In each columns means, numbers followed by the same letter are not different significantly ($p \leq 0.05$) (mean ± standard error) (IS-intact seed; DCS-decoated seed; GA_3 - gibberellic acid; BAP-b*enzylaminopurine*)

Source Of Variance	Days taken for germination		Germination percentage		Mean germination time (days)	
	SEd	CD (0.05)	SEd	CD (0.05)	SEd	CD (0.05)
Media	0.88	1.78	5.21	10.52	0.24	0.48

Comment [Admin4]: Why these concentrations of GA3 and BAP were used? Which references?

Seed Type	0.39	0.79	2.32	4.7	0.1	0.21
Media × Seed Type	1.24	2.52	7.36	14.88	0.34	0.69

Both the seed types and basal media composition significantly influenced the seed germination. While comparing the seed type used for germination, decoated seed showed early germination within 8-9 days followed by intact seeds which takes 19-20 days for germination. Early germination leads to minimum mean germination time in decoated seeds of 8.88 to 23.20 days and the intact seeds with mean germination time of 14.41 to 25.71 days. Different seed types had high impact on the germination percentage where the decoated seed had higher germination percentage in range from 12 to 89 percent followed by intact seed in the range from 9 to 53 percent (Fig. 2).

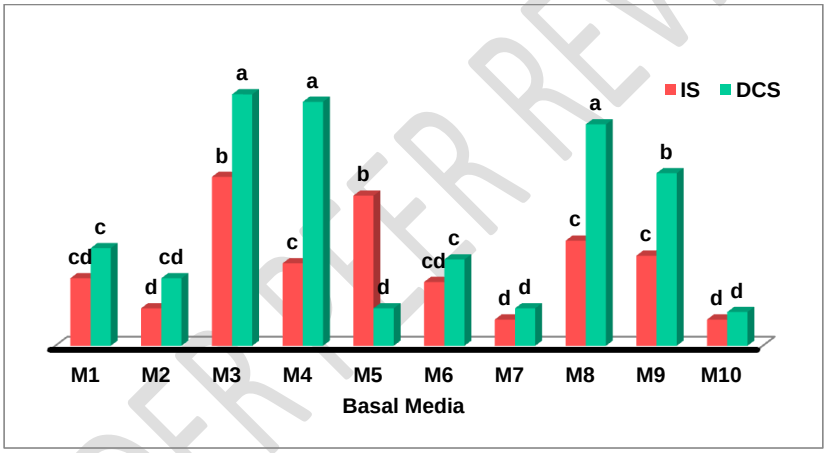


Fig. 2: Comparing the germination percentage of seed type on various basal media

M1	Half MS + GA_3 (1 mg/l)	M6	Full MS + GA_3 (1mg/l)
M2	Half MS + BAP (1 mg/l)	M7	Full MS + BAP (1mg/l)
M3	Half MS + GA_3 (0.5 mg/l) + BAP (0.5 mg/l)	M8	Full MS + GA_3 (0.5mg/l) + BAP (0.5 mg/l)
M4	Half MS + GA_3 (1 mg/l) + BAP (1 mg/l)	M9	Full MS + GA_3 (1 mg/l) + BAP (1 mg/l)
M5	Half MS	M10	Full MS

Comment [Admin5]: This table maybe use as Table 1 and transfer to the Method section.

The physiological process of imbibitions of water which leads to the germination of seeds occurs rapid in decoated seed but in intact seeds, seed coat act as a barrier for imbibitions. Similarly, in bambara

groundnut the seed without seed coat showed rapid and highest germination percentage [12]. In *Ardisia crenata* also the seed coat removal increased the germination frequency by enhanced water uptake [13].

3.2 Effect of basal media on *in vitro* germination of seeds

The decoated seeds and intact seeds were inoculated on the basal media with different concentration of growth regulators. Media type significantly affects the germination of seeds. Half MS induced higher germination frequency (89.33%), minimum days for germination (8 days) and mean germination time (8.88) compared to the full MS (Fig .4b). This indicates that higher concentration of MS affects the germination than half MS which is permeable for seed germination. Plessis *et al.* [14] reported higher germination in *Hibiscus coddii subsp. Barnardii* seed cultured on lower strengths (¼, ½, ¾) of MS medium than full MS. Basal media composition of half MS with GA_3 (1 mg/l) and BAP (1 mg/l) induced minimum number of days for seed germination (8 -12 days) and took more days for germination in the basal media without any growth regulator. Minimum mean germination time was recorded in the basal media composition of GA_3 (1 mg/l) and BAP (1 mg/l) and higher in full MS with BAP (1mg/l).High germination percentage (89.33%) was recorded in half MS with GA_3 (1 mg/l) and BAP (1 mg/l) followed by half MS with GA_3 (0.5 mg/l) and BAP (0.5 mg/l) of 86.67% (Table 1).

Gibberellic acid (GA) is one of the factors for seed germination including the light, temperature and moisture. GA influences the germination by inducing the production of proteolytic enzyme i.e, α-amylase, which involved in germination [15]. Ranchana *et al*. [9] reported that *in vivo* treatment of tuberose seeds with GA_3 enhanced the germination to 63.68 percent than control seeds. With this as reference, in this study GA_3 was used as growth regulator in the *in vitro* culture of tuberose seeds. Gibberellic acid used for *in vitro* germination of berg seeds regardless its concentration promoted an increase of 10% in germination of seeds (guavira) [16]. Similarly, Kim [17] suggested that seed coat removal and treatment with GA_3 improved the germination percentage and mean germination time of *Prunus yedoensis*. BAP is the synthetic cytokinin which promotes cell division; stimulate initiation and growth of shoots under *in vitro*. According to Sukmadjaja and Widhiastuti [18], BAP treatment increased the plantlet percentage to grow normally and increased the height of the plantlet.

Comment [Admin6]: ???

Comment [Admin7]: ???

Formatted: Highlight

Comment [Admin8]: proteolytic enzyme don't include amylase

3.3 Effect of root and shoot proliferation media on *in vitro* grown tuberose seedlings

After four weeks days of inoculation, the germinated seeds were sub cultured on to the root and shoot proliferation media of MS with different concentration of growth regulators. Seedlings sub cultured on the MS with NAA (2 mg/l) and BAP (2 mg/l) showed increased root length and number of roots of 12.33 cm and 13.23 respectively followed by MS with NAA (1 mg/l) and BAP (1 mg/l) (Table 2).

Table 2. Growth performance of *in vitro* tuberose seedling on different root and shoot proliferation media after five weeks of incubation

Media composition	Root length (cm)	Shoot length (cm)	Number of Roots	Number of leaves
MS + NAA (1 mg/l)	9.53 ± 0.18^{b}	6.73 ± 0.45^{d}	10.30 ± 0.24^{a}	2.50 ± 0.29^{c}
MS + BAP (1 mg/l)	8.45 ± 0.13^{c}	11.00 ± 0.38^{c}	6.90 ± 0.42^{b}	4.25 ± 0.48^{b}
MS + NAA (1 mg/l) + BAP (1 mg/l)	9.98 ± 0.36^{b}	12.68 ± 0.70^{b}	11.35 ± 1.85^{a}	5.75 ± 0.25^{a}
MS + NAA (2 mg/l) + BAP (2 mg/l)	12.33 ± 0.09^{a}	14.80 ± 0.23^{a}	13.23 ± 0.76^{a}	6.25 ± 0.48^{a}
MS	6.28 ± 0.33^{d}	5.10 ± 0.21^{e}	4.43 ± 0.59^{b}	2.25 ± 0.25^{c}
SEd	0.421	0.749	1.659	0.632
CD (P=0.05)	0.897	1.596	3.535	1.347

In each columns means, numbers followed by the same letter are not different significantly ($p \leq 0.05$) (mean ± standard error) (BAP- b*enzylaminopurine;* NAA- 1-*naphthaleneacetic acid*)

The root length was improved in the media with NAA comparing to the BAP in the medium. Likewise the maximum shoot length was observed in the media with high BAP concentration. Growth regulators play the major role in the root and shoot proliferation of micro propagated plantlets. The concentration of NAA significantly influences the rizhogenesis (root growth). The seedling sub cultured on media with NAA showed improved root length and root number compared to the media without growth regulator. This was supported by the findings of Abdulmalik *et al.* [19] on groundnut in which the micro shoots sub cultured in the media with NAA results in highest number of roots per plantlet and root-induction frequency. Fukaki

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

and Tasaka [20] reported that the auxin induces root initiation by activating quiescent pericycle cells to initiate division and then expansion. Auxin is mainly involved in cell division, cell elongation and differentiation which leads to increased root frequency. But auxin at higher concentration inhibits root growth by inducing ethylene biosynthesis [21].

Increased shoot length (14.80 cm) and number of leaves (6.25) was observed in the seedlings sub cultured on media MS with NAA (2 mg/l) and BAP (2 mg/l) followed by MS with NAA (1 mg/l) and BAP (1 mg/l) (Fig 4c). The concentration of BAP significantly influenced the shoot organogenesis (shoot growth). This result was in accordance with *in vitro* shoot growth of curcumas which showed increased shoot height in BAP treatment than the control [22]. Similary, in micropropagation of *Azadirachta indica* longest shoot and increased number of leaves was obtained in MS media with BAP [23]. Cytokinins encourage the growth of shoot apical meristem and provide sufficient stem cells for the protrusion of leaf primordium. It is involved in the synthesis and transport of auxin which promote the leaf primordial emergence. Cytokinins increases the number of leaf cells in a short period and represses the transition of leaf cells into the expansion stage by promoting cell proliferation [24]. The combination of NAA and BAP in MS media induces improved seedling growth (root and shoot growth) in tuberose. This was supported by *in vitro* regeneration of *Nelumbo nucifera* [25] and *in vitro* culture of dahlia [26].

3.4 Survival of the *in vitro* grown plantlets

~~Seeds germinated on the basal media were sub cultured on the root and shoot proliferation media. Both intact seed and decoated seed germinated well in the basal media of half MS with GA_3 (1 mg/l) and BAP (1 mg/l) with the germination percentage of 60 % and 89.33 % respectively (Fig 4d). Seedling from this media was further sub cultured on the different root and shoot proliferation media. After sub culturing the plantlets were hardened under *in vivo* condition.~~ The *in vivo* survival percentage of the plantlets was significantly influenced by the basal and sub cultured media. Plantlets from basal media of half MS with GA_3 (1 mg/l) and BAP (1 mg/l) and sub cultured on MS with NAA (2 mg/l) and BAP (2 mg/l) recorded higher survival rate of 91.53 % (decoated seed) and 89.29 % (intact seed) under *in vivo* condition (Fig 3). Lowest survival rate was observed in the basal media of half MS with GA_3 (1 mg/l) and BAP (1 mg/l) and sub cultured in MS. Survival percentage was mainly influenced by the root and shoot

Comment [Admin9]: ???

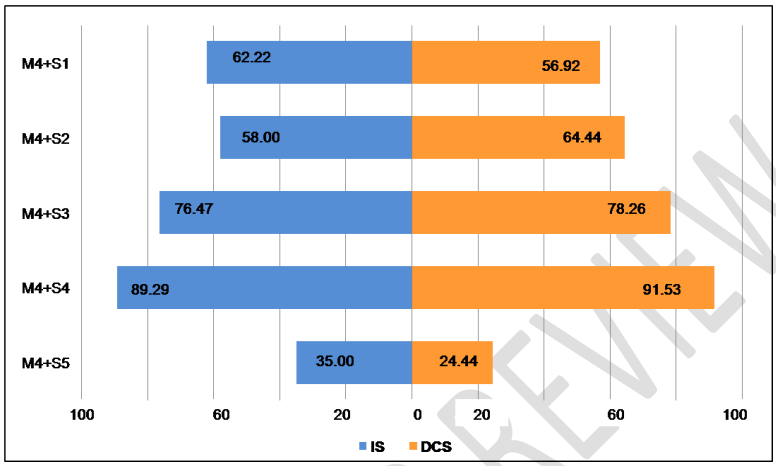
Formatted: Highlight

Formatted: Strikethrough

Formatted: Strikethrough, Highlight

Formatted: Strikethrough

proliferation media because the plantlets with higher rate of root and shoot growth leads to a strong base for the plantlets to grow under *in vivo* condition.



S1	MS + NAA (1 mg/l)
S2	MS + BAP (1 mg/l)
S3	MS + NAA (1 mg/l) + BAP (1 mg/l)
S4	MS + NAA (2 mg/l) + BAP (2 mg/l)
S5	MS
M4	Half MS + GA_3 (1 mg/l) + BAP (1 mg/l)

Fig. 3. Survival percentage of the *in vitro* grown tuberose seedlings on different media composition

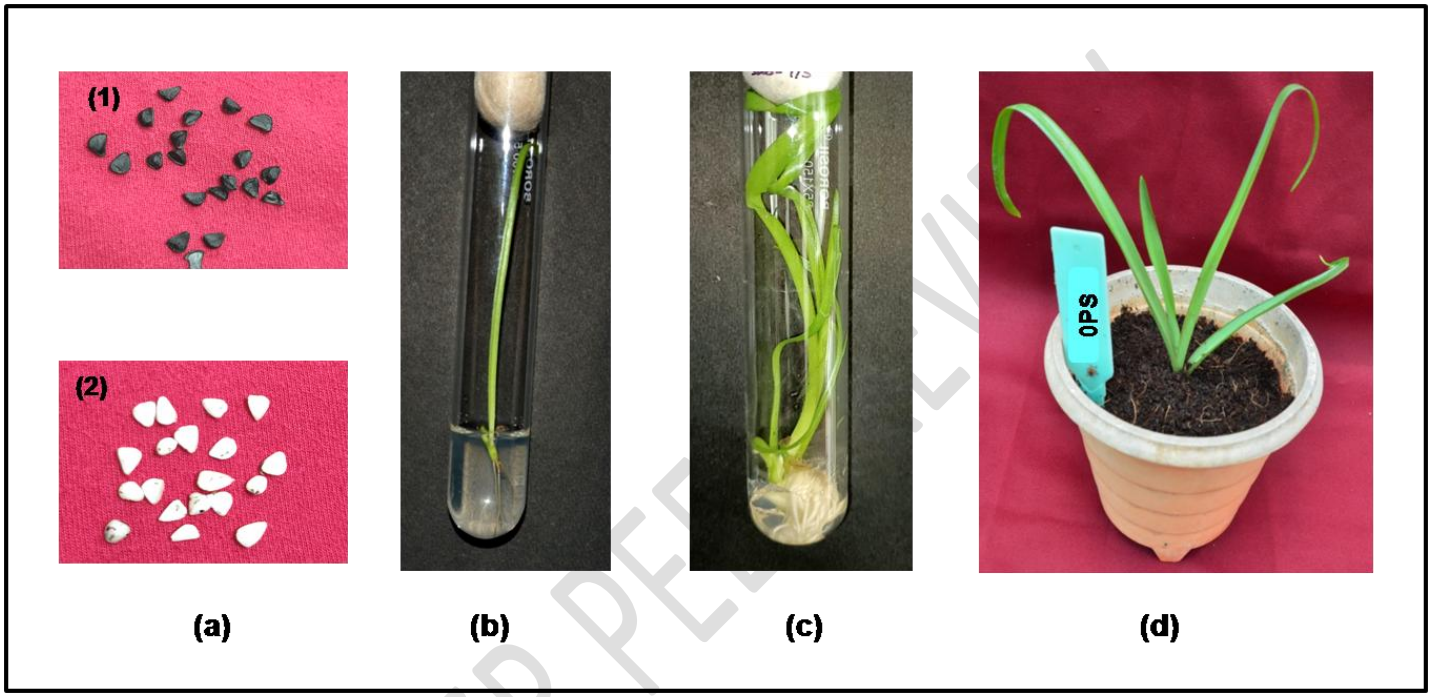


Fig 4: Overall steps *in vitro* seed germination of tuberose

a) 1. Intact seeds and 2. Decoated seeds

b) Seed germinated in the basal media of Half MS + GA_3 (1 mg/l) + BAP (1 mg/l) (four weeks after inoculation)

c) Seedling after sub culturing in media MS + NAA (2 mg/l) + BAP (2 mg/l) (five weeks after incubation)

d) Hardening of *in vitro* grown seedlings

Formatted: Highlight

4. CONCLUSION

~~*In vitro* study of tuberose seeds was conducted to maintain the purity of hybrids as well as for the early germination. Main aim of this study was to develop the protocol for *in vitro* germination of tuberose seeds.~~ Among the seed type used, decoated seed showed early and highest germination percentage compared to the intact seeds. Both basal and sub culture media significantly influenced the seedling emergence and its growth. Basal media composition of half MS with GA_3 (1mg/l) and BAP (1mg/l) recorded early germination and higher germination frequency. Improved root and shoot characters were observed in root and shoot proliferation media composition of MS with NAA (2 mg/l) and BAP (2 mg/l). *In vitro* produced seedlings were maintained well under *in vivo* condition with higher survival percentage of 91.53%. With this preliminary study hybrid seeds can also be grown under in vitro condition for its development.

Formatted: Strikethrough

Formatted: Highlight

REFERENCE

1. Benschop M. Polianthes. In: De Hertogh, A., Le Nard, M. (Eds.), The physiology of flower bulbs. Elsevier, Amsterdam, The Netherlands. 1993; pp. 589-601.
2. Chase MW, Christenhusz MJM, Fay MF, Byng JW, Judd WS, Soltis DE, et al. An update of the angiosperm phylogeny group classification for the orders and families of flowering plants: APG IV. Bot. J. Linn. Soc. 2016; 181(1): 1–20.
3. Bailey LH. The standard cyclopedia of horticulture: a discussion, for the amateur, and the professional and commercial grower, of the kinds, characteristics and methods of cultivation of the species of plants grown in the regions of the United States and Canada for ornament, for fancy, for fruit and for vegetables; with keys to the natural families and genera, descriptions of the horticultural capabilities of the states and provinces and dependent islands, and sketches of eminent horticulturists. 1919; Vol. 3: Macmillan.
4. National Horticulture Board, Ministry of Agriculture and Farmers Welfare, Government of India. website-http://nhb.gov.in/
5. Salient Statistics on Agriculture, Agriculture Department, Govt. of Tamil Nadu, 2021.website-http://www.tnagriculture.in/dashboard/book.

6. Hemanta L, Srivastava R, Kumar A, Chand S. Studies on Seed Set by Open Pollination in Tuberose (*Polianthes tuberosa* L.) Under Tarai Regions of Uttarakhand. Journal of Agriculture and Technology. 2016; *3*(1), 71.
7. Datta SK. Breeding of ornamentals: tuberose (*Polianthes tuberosa* L.). Current Science. 2017; 113 (7):1255–1263.
8. Hemanta L. Studies on floral biology, pollination and crossability in tuberose (*Polianthes tuberosa* L.). PhD. Thesis, GB Pant Univ. Agri. Technol, Pantnagar, India. 2015.
9. Ranchana P, Kannan M, Jawaharlal M. Methods for breaking dormancy and germination of tuberose (*Polianthes tuberosa*) seeds. Asian Journal of Horticulture 9.2.2014; 334-337.
10. Roni, Md Zohurul Kadir, Md Saiful Islam, and Kazuhiko Shimasaki. *In vitro* seed germination and tracking the seedling growth of eustoma. New Zealand Journal of Crop and Horticultural Science. 2018; 46.3, 224-242.
11. Ellis RH, Roberts EH. The quantification of ageing and survival in orthodox seeds. Seed Science and Technology. 1981; 9(2):373–409.
12. Koné M, Koné T, Silué N, Soumahoro AB, Kouakou TH. *In vitro* seeds germination and seedling growth of Bambara groundnut (*Vigna subterranea* (L.) Verdc.(Fabaceae)). The Scientific World Journal. 2015.
13. Tezuka T, Yokoyama H, Tanaka H, Shiozaki S, Oda M. Seed and embryo germination in *Ardisia crenata*. Journal of Botany, 2012.
14. Plessis HJD, Nikolova RV, Kleynhans R, Egan BA. *In vitro* seed germination and seedling performance of *Hibiscus coddii* subsp. barnardii. Ornamental Horticulture-26. 2020; 598-606.
15. Gupta R, Chakrabarty SK. Gibberellic acid in plant: still a mystery unresolved. Plant signaling & behavior. 2013; 8(9).
16. Damiani CR, da Silva LD, Goelzer A, Déo TG. Seed origin, storage conditions, and gibberellic acid on *in vitro* germination of *Campomanesia adamantium* (Cambess.) O. Berg. African Journal of Biotechnology. 2016; 15(32), 1731-1737.
17. Kim D. Practical methods for rapid seed germination from seed coat-imposed dormancy of *Prunus yedoensis*. Scientia Horticulturae. 2019; 243, 451-456.

18. Sukmadjaja D, Widhiastuti H. Effects of plant growth regulators on shoot multiplication and root induction of cassava varieties culture *in vitro*. BIOTROPIA-The Southeast Asian Journal of Tropical Biology. 2011; 18(1).
19. Abdulmalik MM., Usman IS, Olarewaju JD, Aba DA. Effect of naphthalene acetic acid (NAA) on in vitro rooting of regenerated microshoots of groundnut (*Arachis hypogaea* L.). Bayero Journal of Pure and Applied Sciences. 2012; 5(2), 128-131.
20. Fukaki H, Tasaka M. Hormone interactions during lateral root formation. Plant molecular biology. 2009; 69(4), 437-449.
21. Taiz L, Zeiger E. Auxins. In: Plant Physiology. Macmillan Publishing Co., New York. 2003; 623pp.
22. Rustikawati R, Herison C, Inoriah E, Dwisari V. Effect of BAP (6-Benzyl Aminopurine) on *In Vitro* Shoot Growth of Curcumas. AGRITROPICA: Journal of Agricultural Sciences. 2021; 4(1), 82-92.
23. Bello AM, Kamali Aliabad K, Saravi AT, Sodaei Zade H. Determination of the Best Culture Medium and Plant Growth Regulators for Micropropagation of Neem Tree (*Azadirachta indica* A. Juss). International Journal of Horticultural Science and Technology. 2022; 9(2), 237-245.
24. Wu W, Du K, Kang X, Wei H. The diverse roles of cytokinins in regulating leaf development. Horticulture Research, vol-8. 2021.
25. Mahmad N, Mat Taha R, Othman R, Saleh A, Hasbullah NA, Elias H. Effects of NAA and BAP, double-layered media, and light distance on *in vitro* regeneration of *Nelumbo nucifera* Gaertn.(lotus), an aquatic edible plant. The Scientific World Journal. 2014.
26. Ibrahim MA, Daraj IA. Effect of some treatments on seeds germination, shoots multiplication and rooting of dahlia plants via *in vitro* culture. Swed. J. Plant Biotechnol. 2015; 13-21.