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Research Article
Effect of Various Physicochemical Treatments on Seed Germination of *Annona squamosa* L.

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Abstract

Background and Objective: Custard apple (*Annona squamosa* Linn.) is tropical fruit which belongs to genus *Annona* and family Annonaceae. It is native to West Indies and tropical Americas and in India, it mainly grows in parts of Andhra Pradesh, Assam, Bihar, Karnataka, Maharashtra, Madhya Pradesh, Orissa, Rajasthan and Tamil Nadu. Seed dormancy is one the major limitation for natural rejuvenation of this plant. Seeds germinate in 28-30 days after sowing without giving any pre-treatments in field condition. Seeds are implanted in an endosperm which has a slow growing rate and it is due to its degree of maturity, climatic factors during maturation of seeds and its genetic makeup so to get control on poor germination and increase the percentage of seed germination, experiments were carried out at Plant Tissue Culture Laboratory, Pramukh Swami Science College and H.D. Patel Arts College, Kadi, Gujarat, India. **Materials and Methods:** The experiment was conducted in Completely Randomized Block Design Factorial concept and replicated three times. Seeds were treated with different pre-treatments (physical treatments, chemical treatments and combined physical-chemical treatments). Different pre-treatments were significantly affected seed dormancy and It reduced 80% seed dormancy by *in vitro* germination methods and 40% by field condition. **Results:** The results obtained from lab condition (*in vitro* seed germination) (25±2°C), the highest number of seeds germination percentage (91.33±2.08%[a]) in coatless seeds in half strength of MS medium supplemented with GA3 (0.1 mg L^{-1}) was observed within 8-9 days and the field condition (*in vivo* seed germination). **Conclusion:** Seeds showed maximum number of germinations in the treatment, coatless seeds directly sown in soil without any chemical treatment. Germinating percentage (82.22±0.57%[a]) was observed within 20-24 days.

Key words: *Annona squamosa* L., physicochemical treatments, seed germination, MS medium, *in vitro*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Custard apple is tropical fruit which belongs to genus *Annona* and family Annonaceae. This plant is commonly known as Sitafal in Gujarati. It is native to West Indies and tropical Americas and in India, it mainly grows in parts of Andhra Pradesh, Assam, Bihar, Karnataka, Maharashtra, Madhya Pradesh, Orissa, Rajasthan and Tamil Nadu. Custard apple is commonly used in food industry to make ice- creams and other milk products.

Custard apple is tropical fruit which belongs to genus *Annona* and family Annonaceae and are collectively known as annonaceous fruits. There are over 120 species of genus *Annona* and are commonly found in India as a fruit consuming plant[1]. This plant is commonly known as custard apple in English and Sitafal in Gujarati. In India, Custard apple is considered as a vital dry land fruit. *Annona squamosa* L. is native to west Indies and tropical Americas. It mainly grows in parts of Andhra Pradesh, Assam, Bihar, Karnataka, Maharashtra, Madhya Pradesh, Orissa, Rajasthan and Tamil Nadu. The height of the tree reaches up to 10 m and its leaves are 6-18 cm long and 3-7 cm in width.

It is essential to develop the method for cultivation of such an important plant. Cultivation of custard apple is mainly carried out by vegetative methods or by seeds. The tissue culture in *Annona* is mainly used for the micro-propagation, maintains of germplasm collection, embryo rescue technique, preparation of explants for transformation of genes, enhancement of variability by soma clonal variation and production of haploid plant[2]. The success rate is low for doing mass multiplication because of poor culture establishment and poor seed germination, loss of cultures in *in vitro* propagation is mainly due to bacterial[3,4] and fungal[5] contaminants.

MATERIALS AND METHODS

Seeds collection: Custard apple seeds were used for inception of experiments. The seeds were collected from Junagadh district of Gujarat, India.

***In vivo* seed germination:** Various pre-treatments were used to check seed germination. Each treatment was replicated 3 times with 15 seeds in each replicate (Table 1).

In vitro seed germination

Surface sterilization of seeds: Seeds were washed with water 3-4 times and later seeds were surface sterilized by fungicide (Bavistin) for 30 min with continuous shaking. Finally, seeds were washed with 0.1% $HgCl_2$ in LAF for 3 min.

Inoculation of seeds: Seeds were inoculated in test tubes with one half of MS media with 0.1 mg L^{-1} Gibberellic acid (Table 2). Seeds were allowed to germinate in culture room with temperature (25±2°C).

Sterilized seeds were subjected to 8 different pre-treatments for *in vitro* seed germination of *Annona squamosa* L. Each treatment was replicated 3 times with 50 seeds in each replicate (Table 3).

Seed germination (%): The phenomenon of growth of seeds, which will develop in shoot up to 0.2 mm is termed as seed germination. The ratio of the No. of seeds germinated to the No. of seeds inoculated expressed in percentage, considered as percentage of seed germination[6,7]:

$$\text{Seed germination (\%)} = \frac{\text{No. of seeds germinated}}{\text{No. of inoculated}} \times 100$$

Table 1: Various pre-treatment of seed germination of *Annona squamosa* L.

Treatment number	Inoculated seeds	Pre-treatments
T0	15	Control
Physical treatments		
T1	15	Control+18 h cold treatment
T2	15	Scarified seeds (Coatless seeds)
Chemical treatments		
T3	15	Control+GA3 treatment (30 min)
T4	15	Control+Fungicide wash (30 min)
T5	15	Control+Fungicide wash (30 min)+GA3 treatment (30 min)
Combine treatments: Physical and chemical		
T6	15	Scarified seeds+Fungicide wash (30 min)
T7	15	Scarified seeds+Fungicide wash (30 min)+GA3 treatment (30 min)
T8	15	Control+18 h cold treatment+GA3 treatment (30 min)
T9	15	Control+18 h cold treatment+Fungicide treatment (30 min)

GA3: Gibberellic acid

Statistical software-WASP: The WASP-Web Agri Stat Package was used to analyze experimental data. The WASP is web-based software used for doing statistical analysis. Completely randomized experimental design was used to find the significance of data.

RESULTS AND DISCUSSION

***In vivo* seed germination:** Seeds inoculated in soil mixed with different treatments. Seeds were pre-treated with various chemicals like fungicides, GA3 and seed coat was removed to check whether the seed dormancy could be reduced, out of these treatments. Seeds showed maximum number of germination in T2 treatment i.e., seed coat removed and sown in soil without any chemical treatment. Germinating percentage of (82.22 ± 0.57^{a}) was observed. Lowest germinating percentage was observed (13.33 ± 1.0^{f}) in T8 i.e., control seeds with 18 h of cold treatment with GA3 (Table 4, 5 and Fig. 1).

***In vitro* seed germination:** Seeds were treated with fungicide for 30 min followed by $HgCl_2$ (0.1 mg L^{-1}) for 1-2 min. Two types of seeds were inoculated i.e., control seeds and coatless seeds. Seeds were placed in two types of treatments i.e., half strength of MS and half strength of basal MS medium with GA3 (0.1 mg L^{-1}). The highest No. of seeds germination (91.33 ± 2.08^{a}) was observed in coatless seeds in half strength of MS medium supplemented with GA3 (0.1 mg L^{-1}) which is shown in S4. Lowest No. of seeds germination (21.33 ± 2.08^{ef}) was observed in control seeds inoculated in half the strength of MS medium as shown in S5 (Table 6, 7 and Fig. 2, 3).

Table 2: Seed germination media of *Annona squamosa* L.

Inoculation media	Concentration
MS 0 (Control)	½ MS
MS 1 (without growth regulator)	½ MS+0.1 mg L^{-1} gibberellic acid

MS: Murashige and Skoog medium

Table 3: Seed inoculated for germination in media of *Annona squamosa* L.

Treatment No.	Inoculated seeds	Pre-treatments	Inoculation media
S1	50	Control	MS 0
S2	50	Control	MS 1
S3	50	Scarified seeds (Coatless seeds)	MS 0
S4	50	Scarified seeds (Coatless seeds)	MS 1
S5	50	Control+18 h cold treatment	MS 0
S6	50	Control+18 h cold treatment	MS 1
S7	50	Scarified seeds+18 h cold treatment	MS 0
S8	50	Scarified seeds+18 h cold treatment	MS 1

Seeds were daily observed to check for germination, MS: Murashige and Skoog medium

Table 4: Seed germination (%) of *Annona squamosa* L. (*in vivo*)

		No. of seeds germinated (10 days)			
		No. of replication			
Treatment code	Showed seeds	1	2	3	Mean seeds germination (%)±SD
T0	15	5	4	5	31.11 ± 0.57^{cde}
T1	15	5	4	3	26.66 ± 1.00^{de}
T2	15	13	12	12	82.22 ± 0.57^{a}
T3	15	8	8	7	51.11 ± 0.57^{b}
T4	15	6	5	5	35.55 ± 0.57^{cd}
T5	15	8	7	8	51.11 ± 0.57^{b}
T6	15	6	6	5	37.37 ± 0.57^{c}
T7	15	10	9	7	57.77 ± 1.52^{b}
T8	15	3	2	1	13.33 ± 1.15^{f}
T9	15	4	4	2	22.22 ± 1.15^{ef}

Table 5: Analysis of variance (ANOVA) on the effect of pre-treatment on seed germination of *Annona squamosa* L. in field condition (*in vivo*)

Source of variation	Degree of freedom	Sum squares	Mean sum of squares	F-cal	F-Prob
Treatments	9	236.033	26.229	34.209	3.195
Error	20	15.33	0.766	-	-
Total	29	-	-	-	-
Coefficient of variation = 14.049					
Treatments found significant at 1 and 5% level of significance					
	CD (0.01) = 2.039		CD (0.05) = 1.493		

F-cal: F-calculated, F-prob: F-probability

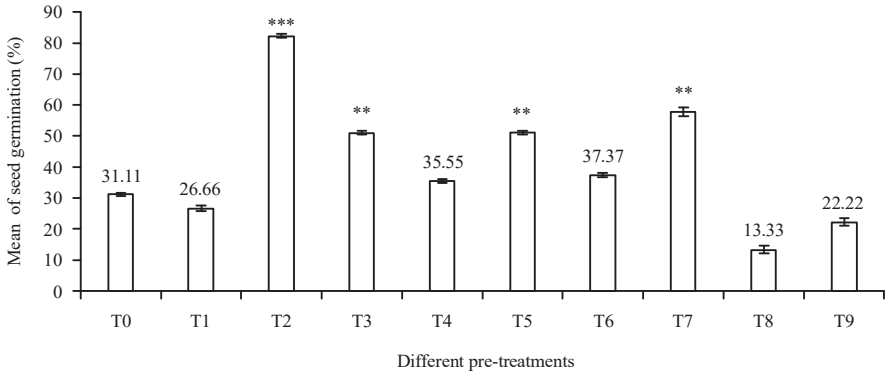

Fig. 1: Seed germination (%) of *Annona squamosa* L. (*in vivo*)

**p<0.01, *** p<0.001 when compared to control

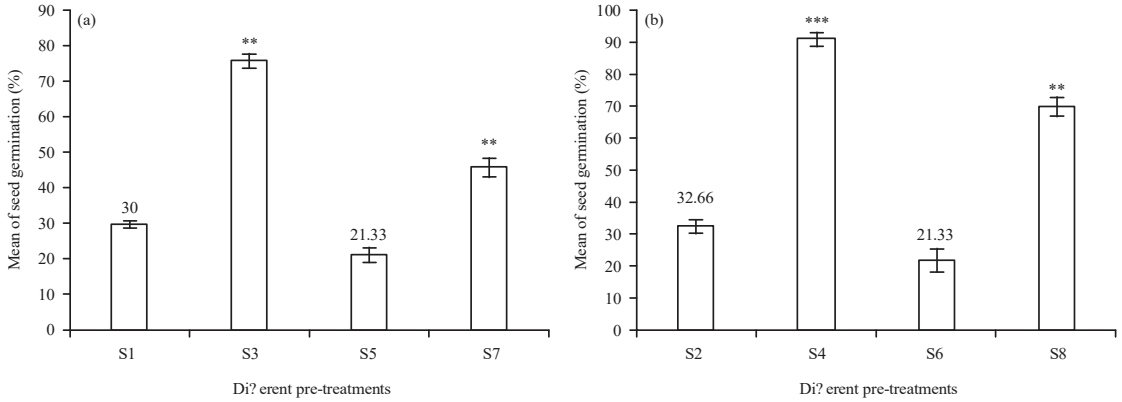

Fig. 2(a-b): Seed germination (%) of *Annona squamosa* L. (*in vitro*) (a) Seed inoculated in half MS media and (b) Seed inoculated in half MS media +0.1 mg L^{-1} GA3

**p<0.01, *** p<0.001 when compared to control

Table 6: Seed germination (%) of *Annona squamosa* L. (*in vitro*)

		No. of seeds germinated (10 days)			
		No. of replication			
Treatment code	Showed seeds	1	2	3	Mean seeds germination (%)±SD
S1	50	15	16	14	30.00±1.00^{de}
S2	50	17	18	14	32.66±2.08^{d}
S3	50	40	36	38	76.00±2.00^{b}
S4	50	48	45	44	91.33±2.08^{a}
S5	50	13	10	9	21.33±2.08^{ef}
S6	50	15	10	8	22.00±3.60^{f}
S7	50	25	24	20	46.00±2.64^{c}
S8	50	35	38	32	70.00±3.00^{b}

Table 7: Analysis of variance table (ANOVA) on the effect of pre-treatment on seed germination of *Annona squamosa* L. in Laboratory condition (*in vitro*)

Source of variation	Degree of freedom	Sum squares	Mean sum of squares	F-cal	F-Prob
Treatments	7	3819.33	545.610	92.87	9.320
Error	16	94.00	5.87	-	-
Total	23	-	-	-	-
Coefficient of variation = 9.96					
Treatments found significant at 1 and 5% level of significance					
	CD (0.01) = 5.78		CD (0.05) = 4.19		

F-cal: F-calculated, F-prob: F-probability

Fig. 3: Seed germination of *Annona squamosa* L. (*in vitro*)

CONCLUSION

Different physical and chemical treatments are used to improve seed germination of *Annona squamosa* L. In field study (*in vivo*), it have seen maximum seed germination by physical scarification of seed without giving any chemical treatments so after doing this experiment we assured that seed dormancy in custard apple seed is mainly due to hard seed coat. In lab condition (*in vitro*) after giving fungicide treatment followed by surface sterilization scarified seeds were placed in half MS media containing 0.1 mg L^{-1} GA3 shown maximum seed germination. After applying various pre-treatments on seeds, it concluded that scarified seed directly sowed in soil and scarified seed placed in half MS media containing 0.1 mg L^{-1} GA3 are best and cost-effective method compare to others.

Research is to decrease the seed dormancy period and increase the percentage of seed germination. It is stated that the dormancy in custard apple seeds is due to its degree of maturity, climatic factors during maturation of seeds and its genetic makeup. Seeds are implanted in an endosperm which has a slow growing rate.

REFERENCES

1. Hiwale, S., 2015. Sustainable Horticulture in Semiarid Dry Lands. Springer, India, ISBN: 978-81-322-2243-9, Pages: 393.
2. Pinto, A.D.C.Q., M.C.R. Cordeiro, S.R.M. de Andrade, F.R. Ferreira, D.A.D.C. Filgueiras, R.E. Alves and D.I. Kinpara, 2005. *Annona* species. International Centre for Underutilized Crops, University of Southampton, UK., ISBN: 0854327851.
3. Mathias, P.J., P.G. Alderson and R.R.B. Leakey, 1987. Bacterial contamination in tropical hardwood cultures. Acta Hortic., 212: 43-48.
4. Leifert, C., 1990. Contaminants of plant tissue cultures. Ph.D. Thesis, Nottingham University, School of Agriculture, England.
5. Enjalric, F., M.P. Carron and L. Lardet, 1988. Contamination of primary cultures in tropical areas: The case of hevea brasiliensis. Acta Hortic., 225: 57-66.
6. Nayak, R., D. Patel and R. Suthar, 2015. *In vitro* seed germination and propagation of kagdi lemon (*Citrus limon* L.). J. Global Agric. Ecol., 2: 130-134.
7. Suthar, R.S., D.P. Bhatt and P.N. Bhatt, 2014. Effect of culture filtrate of *Fusarium equiseti* on seed germination and seedling growth of cumin (*Cuminum cyminum*). Pathology, 67: 193-194.