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Influence of storage methods and phytochrome activities on seed germination in jackfruit seeds (*Artocarpus heterophyllus* Lam.)

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Abstract

Jackfruit (*Artocarpus heterophyllus* Lam.) is a rainfed tropical crop in coastal warm and humid climate of India. Though the softwood grafting method is standardized for rapid multiplication nevertheless, due to recalcitrant nature of jackfruit seeds obtaining rootstock for grafting throughout the year is the main constraint. Loss of seed viability in very short period during storage imposes a problem in germination. Storage conditions which prevent water loss are useful for prolonging viability of recalcitrant seeds. Hence, an experiment was undertaken during the year 2017-18 to study seed viability under different storage methods at College of Horticulture, Mulde Tal. Kudal, Dist. Sindhudurg. The experiment consists of eight storage methods and six storage period in factorial randomised block design with two replications. Hundred seeds per treatment per replication was the unit.

The study revealed that the process of germination was under the control of phytochrome action and clearly indicated that the conditions of darkness and humidity are essentials for preventing moisture loss and promotion of germination. Storage of seed ether in screw cap bottle or in earthen pot buried in soil was the best for preventing the desiccation and prolonging the seed viability for 150 days. In this context storing the seeds in screw cap bottle appear to be most appropriate where there is no risk of germination of seed within storage container and seeds are kept viable beyond 150 days.

Keywords: Germination, jackfruit seeds, moisture, recalcitrant, seed viability

Introduction

Jackfruit (*Artocarpus heterophyllus* Lam.) is a native fruit of India and grows wild in the rain forest of Western Ghats of India (De Candolle, 1886) [4]. It is preferred in homesteads as a shade tree or a mixed crop. In India, it is widely distributed in Assam, Tripura, Bihar, Uttar Pradesh, Maharashtra and south Indian states of Kerala, Tamil Nadu and Karnataka. It is estimated that about 1.53 lakh hectare area is covered by this crop with production of 17.22 lakh tones in India (www.nhb.gov.in). Availability of genuine planting material is constrained in area expansion of this crop. At present, soft wood grafting technique is being followed for production of jackfruit grafts. However, graft production is limited due to lack of availability of the rootstock year round. A success of a fruit nursery critically depends on continuous supply of quality seeds for raising rootstock for grafting. Loss of viability of jackfruit seeds in very short period due to recalcitrant nature imposes a problem in getting seedlings for grafting to fulfil the demand of genuine planting material. The recalcitrant seed have a characteristic feature that they require the moisture levels above some critical value. If there is loss of water below this level then the seeds become nonviable and loose germinability. It is mostly suggested that storage condition which prevent water loss are useful for prolonging viability of recalcitrant seeds. Such seeds must maintain relatively high moisture content in order to remain viable. However even when these seeds are stored under moist conditions, their span is frequently brief and only occasionally exceed a few months. Hence inability to store seeds of recalcitrant species is a serious problem in almost all such species. The usual method of drying of seed is detrimental for such crop (Bewley and Black, 1985) [2]. The recalcitrant behaviour of jackfruit has been studied by Sheela, (2007) [12], Enny Adelina, (2014) [6] and Fernandez, (1982) [7]. According to Chin *et al.*, (1984) [3] loss of viability may be either due to the moisture

content falling below a certain critical value or simply a general physiological deterioration with time. Jackfruit is such kind of perennial species where storage of seeds is a constraint. Hence research on optimal storage condition for this species needs to be undertaken by conducting logical experiments. The research on physiological and biochemical basis of desiccation intolerance in seed in this species needs to be strengthened. Hence, investigation on seed viability and methods of prolonging it under different storage conditions are of immense value. Keeping in view the present investigation was undertaken.

Materials and Methods

The investigation was undertaken at College of Horticulture, Mulde Tal. Kudal, Dist. Sindhudurg during the year 2017.

The experiment was conducted in factorial randomized block design (FRBD) having two replications. In this experiment eight seed storage methods namely C₁: Screw cap plastic bottle (transparent and 2.5 kg capacity), C₂: Polybag -300 gauge (transparent 25 X 40 cm) + Screw cap plastic bottle (2.5 kg capacity), C₃: Polybag – (transparent 25 X 40 cm and 300 gauge) storing at ambient temp. (32 °C ± 2 °C), C₄: Earthen pot (5.0 liter capacity), C₅: Earthen pot buried in soil (5.0 liter capacity), C₆: Coating with Fresh Cow dung slurry, drying and stored in Poly bag (transparent 25 X 40 cm and 300 gauge), C₇: Coating with mud slurry, drying and stored in Polybag (transparent 25 X 40 cm and 300 gauge) and C₈: Storing at ambient temp. (32 °C ± 2 °C) (Control) and six storage period Viz., 15 days (S₁), 30 days (S₂), 60 days (S₃),

90 days (S₄), 120 days (S₅) and 150 days (S₆) in all possible combinations were taken. Hundred seeds per treatment per replication was the unit.

Fresh seeds from ripe soft flesh type fruits were collected. Such seeds were depulped and washed to remove perianth and aril. From this healthy cleaned seeds weighing more than 3 g were treated with Carbendazim (50 % WP @ 2 g/L) for 5 minutes and further kept for surface drying under shade for three hours at room temperature (32 °C) to remove moisture. Such seeds were used to expose treatments (Fig. 2) as suggested by Doijode, (2001) [5]. The seeds were stored in respective containers after taking initial weight by providing sufficient space for air inside and sealed thereafter at this stage seeds were having moisture content of 40.82 per cent (Fig. 3A, Fig. 3B and Fig. 3C).

After completion of storage period of 15, 30, 60, 90, 120 and 150 days seeds stored in different treatments were sown in the black polythene bag of 200 gauges of size 15 X 20 cm containing soil + FYM in 3:1 proportion (Fig.4). Final weight of stored seeds was recorded before sowing. Polybags were kept in Polyhouse for raising the seedlings and watering was done immediately after sowing the seeds. Then after every day light irrigation for each poly bag was given till the seedling emergence.

Germination for all 100 seeds in treatment was observed at every alternate day from the first germination until no further seeds germinated.

Per cent loss in seed weight (Moisture loss) was calculated by using the formula.

$$\text{Per cent loss in seed weight (\%)} = \frac{\text{Fresh weight during storage} - \text{Final weight during sowing}}{\text{Fresh weight during storage}} \times 100$$

Germination percentage was calculated by dividing the total number of germinated seeds to the total number of seeds sown and multiplied by 100 as given below.

$$\text{Germination percentage (\%)} = \frac{\text{No. of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

In the present investigation the constants of various polynomials were worked out on a SPSS statistics 17.0 programme and the level of R² was fixed as 0.80 and fitting was done in such a way that lowest possible degree of polynomial was obtained with R² > 0.80.

Results and Discussion

When germination was started from the first day of germination to last day of germination was observed and the data pertaining to percentage of seeds germination are presented in Table 1 (Fig.5 and Fig.6).

Effect of storage methods on seed germination percentage

Significantly the highest germination was observed in treatment C₁ (79.71%) which was at par with C₅ (76.42%). C₃ recorded 40.38 per cent germination. The lowest germination percentage was recorded in C₆ (17.50%).

Effect of storage period on seed germination percentage

Treatment S₆ (87.58%) gave significantly the highest germination followed by S₄ (82.08%), S₁ (61.72%), S₅ (55.25%) and S₂ (36.04%). Significantly lowest germination was noticed in treatment S₃ (33.27%).

Interaction effect of storage methods and storage period on seed germination percentage

Data indicated that the treatment C₁S₆ (93.00%) had

maximum germination which was significantly superior and was at par with C₂S₆ (90.75%), C₅S₄ (87.00%) and C₁S₁ (84.75%). Treatment C₆S₂ (4.00%) recorded significantly the lowest germination which was par with C₆S₃ (4.50%).

Effect of storage methods on germination percentage of jackfruit over the course of storage is summarized in condensed manner by fitting different kinds of polynomials. The polynomials such fitted on periodic observations on germination percentage under different storage condition are given in Table 2 (Fig. 2).

Keeping the seeds at ambient temperature condition is an obvious practice in any crop. The data in this respect revealed that there was fast deterioration in germinability by keeping seeds under ambient condition. Maximum of 53.25 per cent germination was noticed at first 15 days storage and it declined to 9.5 per cent at 60 days. Thereafter no germination was noticed in this treatment. This phenomenon of loss in germination percentage is predictably explained by quadratic equation given $\hat{Y}_{\text{Ger. \%}} = 65.97 - 1.23X + 0.005X^2$ with coefficient of determination of 0.97. The initial constant value (65.97) declined at a rate of 1.23 and reached almost zero after 60 days storage. Warriar, (2009) [14] explained the storage problem of jackfruit seeds and suggested that ambient (25 ± 2 °C) conditions were not conducive for the storage of jackfruit seeds for more than three weeks. Similarly, Panggabean, (1979) [11] who observed 80 to 86 per cent seed germination of jackfruit seeds after 22 days of storage and

none of the seeds germinated after 38 days of storage.

Coating the seeds with mud is conventional practice of seed preservation. In current investigation, this practice noticed 40.25 per cent germination at 30 days storage period. In further course of storage the germination was not at all seen. This phenomena could be explained by cubic equation $\hat{Y}_{Ger. \%} = 69.02 - 2.78X + 0.032X^2 + 0.0003X^3$ with a high coefficient of determination i.e. $R^2 = 0.82$.

Coating seeds with cow dung is another conventional practice. In this investigation it was found to be unsuccessful for maintaining germinability. At 15 day storage there was 37.50 per cent germination under this condition. However, at 30 and 60 days it remained 4 to 4.5 per cent. This declined germinability is also well explained by a cubic equation $\hat{Y}_{Ger. \%} = 59.51 - 2.15X + 0.024X^2 - 0.00008X^3$ with a high value of coefficient of determination ($R^2 = 0.83$)

Preserving seeds in earthen pot is another common practice among the farmers. In current investigation, this technique was found to be better in comparison with cow dung coating, mud coating and storage under ambient temperature. The jackfruit seeds could be stored with a germination of 32.75 per cent up to 30 days which declined rapidly reaching 9.75 per cent by 60 days of storage. In further course of storage no germination was noticed. This loss in germinability can be highly predicted by a quadratic equation $\hat{Y}_{Ger. \%} = 78.71 - 1.49X + 0.007X^2$ having R^2 value of 0.96. Girija and Srinivasan, (2000)^[8] observed no germination of mango stones after six weeks of storage when stored in mud pot.

Keeping the seeds in polybag can be useful for prolonging the viability Padma and Reddy, (2000)^[10]. However, in current investigation the germination percentage went down sharply from 54.75 per cent at 15 days to 11.50 per cent at 60 days. This was 99 per cent explainable phenomenon as revealed from quadratic equation $\hat{Y}_{Ger. \%} = 73.03 - 1.30X + 0.006X^2$. Results of the investigation are in line with the findings of Sheela, (2007)^[12] who got short longevity for 110 days of jackfruit seeds under storage in polythene bags at room temperature. Krishnasamy, (1990)^[9] observed 100 per cent germination after 6 weeks when jackfruit seeds were stored in 700 gauge thick polythene containers. Anandalakshmi *et al.*, (2005)^[11] stored non-desiccated seeds in various containers, namely, polybag (150, 200 and 250 gauge), polybag with two pores (200 gauge), cloth bag, paper bag and plastic container, and stored at 20 °C and after five months observed that irrespective of thickness and presence of pores, storage of seeds in polybags was able to prolong seed viability seeds of *Syzigium cuminii*.

From the above results, it is apparent that the usual practice of storing the seed is ineffective in maintaining the germination beyond 60 days.

In an attempt to store the seeds in screw cap plastic bottle with a good success was noticed in maintaining germinability of seeds even after 150 days. The value of germination percentage was 84.75 per cent at 15 days. It declined slightly and remained in the range of 66 to 79 per cent up to 120 days. Importantly seeds under such condition showed as high as 93 per cent germination at 150 days. This could be attributed to slower rate of seed deterioration in screw cap bottle due to impervious nature of container (Fernandez, 1982)^[7]. The stable germination percentage over a period of 150 days storage could not be explained by any of the polynomials like linear, quadratic or cubic equation. However, a cubic equation $\hat{Y}_{Ger. \%} = 87.38 - 0.59X + 0.007X^2 - 0.000017X^3$ can be suggested to explain the phenomena with coefficient of determination of 0.62.

Keeping the seed in polybag and putting it into screw cap bottled showed a uncertain picture as 79.50 per cent at 15 day period which was brought down to 4.50, 7.0 and 10.0 per cent at 30, 60 and 120 days respectively. However, high germination percentage of 81.75 per cent and 90.75 at 90 and 150 days warrants a need of physiological investigation. It must be noted that no polynomial equation could explained satisfactory changes in germination percentage over storage period under this storage condition.

The seeds when stored in earthen pot and pot buried in soil (C₅) gave very encouraging picture. The seed germination in this practice remained in the range of 62 to 87 per cent throughout course of storage. Thus the germination percentage was quite stable over entire storage condition. There was no any polynomials equation to explain this phenomena as there was no declined or deterioration in seed germination due to storage.

From above results, it is clear that preserving seeds in screw cap bottle or in earthen pot buried in soil are the reliable techniques for obtaining a successful germination over a period of even five months. Storing the seeds in earthen pot buried in soil derived a great attention because it not only kept the seed viable but also favoured the germination within the storage itself right form 90 days storage period. This appears to be due to temperature, humidity and light conditions prevailing in storage method. Such sprouting needs to be understood by physiological investigation. Some factors in maternal tissues which otherwise prevent the germination by desiccation must have become ineffective. Similarly storage in polybag which were kept in screw cap bottle also prevented the water loss to a great extent and could maintain more than 90 per cent germination even at 150 days.

Role of phytochromes in seed germination

The whole process of germination is believed to be predominantly under control of phytochrome activities (Doijode, 2001)^[5]. Phytochrome is a blue coloured pigment exists in pearl tissue which renders various photo biological activities in the plants. The process of germination is believed to be under the control of phytochrome action. Phytochemical reactions occur between 290 to 800 nanometers (nm) radiation. The molecule absorbs the light particles, which stimulates the germination process. According to Smith, (1975)^[13] the phytochrome exists in two forms; Pr (red light absorbing form) and Pfr (Far- red absorbing form). The red light has 660 to 700 nm wavelengths which promote germination whereas Far-red has 730 to 760 nm wavelengths which are inhibitory. Usually when the Pr form absorbs red light (peak absorption at 670 nm) it is slowly converted in to Pfr form and when Pfr absorbs far- red light (peak absorption at 720 to 730 nm) it is slowly converted in to Pr form.

The dormancy of seed is also explained on the basis of exposure of seeds to light. If seed acquires dormancy by keeping it long time in dark then it is termed as Skotodormancy; whereas, if the seed acquire dormancy when exposed to light for longer time then it is termed as photodormant seeds (Smith, 1975)^[13]. In current investigation the seeds kept at ambient temperature in open environment have lost viability as they are exposed to light for 15 days. Thus light has inhibitory effect on germination of jackfruit. This phenomenon is explained with the help of speculative model (Fig. 7) given by Smith, (1975)^[13] for the action of phytochrome in regulating rapid metabolic processes and long term developmental process for seeds stored in ambient condition under open environment.

Coating the seeds with mud or cow dung or keeping them in earthen pot could not prevent from effect of light. The light not only induced dormancy but also caused loss of germinability in this crop. Interestingly storage in screw cap bottle prevented inhibition. This warrants that not only light but some other factors come in operation in maintaining viability of jackfruit seed and that could be the moisture loss which is prevented by air tight bottle used for investigation.

The most interesting phenomena noticed in this investigation is germination in earthen pot which was buried in soil. In this case seeds are protected from exposure to light and also prevented from desiccation loss. Under such condition the Pfr form of phytochrome could be accumulated in the tissue of the seed which promote the hormonal activities for germination and humid condition with in the storage pot must have favoured the germination. This clearly indicated that the conditions of darkness and humidity are essentials for preventing water loss and promotions of germination. Speculative model (Fig. 8) explained the action of phytochrome in regulating rapid metabolic processes and long term developmental process for seeds stored in earthen pot which was buried in soil.

The prime objective of this investigation was to find a method

of storage for keeping the seed viable for a long time. In this context storing in screw cap bottle appear to be most appropriate. Here there is no risk of germination of seed within storage container and seeds are kept viable till 150 days.

Speculative model for the action of phytochrome for seeds stored in screw cap bottle (Fig. 9) explained that seed stored in polybag which was further kept in screw cap bottle showed inconsistency in various attributes related to germination. High germination percentage under 15, 90 and 150 day storage as against very low percentage of germination under 30, 60 and 120 days suggested unpredictable behaviour of this storage condition. The seeds in polybag and further kept in screw cap bottle could have created the complex situation like accumulation of CO₂, penetration of light and unevenness in humidity caused by water escaping from seeds within container. The seeds in resting stage released CO₂ through respiration. Accumulation of CO₂ further can cause adverse effect on germination (Enny Adelina *et al.*, 2014)^[6]. Even the small changes in humidity, light penetration, CO₂ accumulation and temperature can reflect in big changes in respect of seed germination. This could be a possible cause of unpredictable germination of seed in the treatment of polybag in screw cap bottle.

Table 1: Germination percentage (%) under different storage conditions

Sr. No.	Storage Methods (C)	Storage Period (S) Days						Mean (C)
		15	30	60	90	120	150	
1	Screw Cap bottle C1	84.75 (67.71)	66.00 (54.38)	77.50 (61.84)	77.50 (61.85)	79.50 (63.21)	93.00 (74.78)	79.71 (63.96)
2	Polybag in Screw cap C2	79.50 (63.22)	4.50 (12.23)	7.00 (15.31)	81.75 (64.88)	10.00 (18.14)	90.75 (72.35)	53.55 (46.47)
3	Poly Bag C3	54.75 (47.76)	40.50 (39.53)	11.50 (19.72)	-	-	-	40.38 (38.69)
4	Earthen Pot C4	64.25 (53.42)	32.75 (34.85)	9.75 (18.15)	-	-	-	35.58 (35.47)
5	Earthen Pot in soil C5	79.50 (63.48)	62.00 (52.04)	74.75 (60.19)	87.00 (69.45)	76.25 (60.84)	79.00 (62.83)	76.42 (61.47)
6	Cow Dung C6	37.50 (37.67)	4.00 (11.10)	4.50 (12.08)	-	-	-	17.50 (21.92)
7	Mud Coating C7	40.25 (39.30)	-	-	-	-	-	40.25 (39.30)
8	Ambient Temp. C8	53.25 (46.87)	29.00 (32.46)	9.50 (17.95)	-	-	-	30.58 (32.42)
	Mean (S)	61.72 (52.43)	36.04 (35.12)	33.27 (33.03)	82.08 (65.39)	55.25 (47.40)	87.58 (69.99)	
		Period (S)		Method (C)		Interaction		
	S. Em ±	1.03		1.30		2.94		
	CD at 5 %	2.92		3.67		8.34		

Figures in parenthesis are arcsine transform values

Table 2: Polynomials for germination percentage ($\hat{Y}_{Ger. \%}$) against period of storage (X) for jackfruit seeds

Sr. No.	Storage Methods	Polynomial Equations for Germination percentage	R ²
1	Screw Cap bottle (C ₁)	$\hat{Y}_{Ger. \%} = 72.34 + 0.095X$	0.31
		$\hat{Y}_{Ger. \%} = 82.89 - 0.303X + 0.002X^2$	0.60
		$\hat{Y}_{Ger. \%} = 87.38 - 0.59X + 0.007X^2 - 0.000017X^3$	0.62
2	Polybag + Screw Cap bottle (C ₂)	$\hat{Y}_{Ger. \%} = 30.15 + 0.199X$	0.06
		$\hat{Y}_{Ger. \%} = 71.74 - 1.37X + 0.01X^2$	0.26
		$\hat{Y}_{Ger. \%} = 100.36 - 3.21X + 0.04X^2 + 0.0001X^3$	0.29
3	Polybag (C ₃)	$\hat{Y}_{Ger. \%} = 49.20 - 0.405X$	0.78
		$\hat{Y}_{Ger. \%} = 73.03 - 1.30X + 0.006X^2$	0.99
4	Earthen pot (C ₄)	$\hat{Y}_{Ger. \%} = 50.30 - 0.42X$	0.71
		$\hat{Y}_{Ger. \%} = 78.71 - 1.49X + 0.007X^2$	0.96
5	Earthen pot buried in soil (C ₅)	$\hat{Y}_{Ger. \%} = 71.70 + 0.06X$	0.15
		$\hat{Y}_{Ger. \%} = 68.95 + 0.17X + 0.0001X^2$	0.17
		$\hat{Y}_{Ger. \%} = 82.25 - 0.69X + 0.012X^2 - 0.00005X^3$	0.34
6	Cow dung coating (C ₆)	$\hat{Y}_{Ger. \%} = 22.46 - 0.191X$	0.46
		$\hat{Y}_{Ger. \%} = 38.41 - 0.79X - 0.004X^2$	0.70
		$\hat{Y}_{Ger. \%} = 59.51 - 2.15X + 0.024X^2 - 0.00008X^3$	0.83
7	Mud coating (C ₇)	$\hat{Y}_{Ger. \%} = 20.95 - 0.18X$	0.34
		$\hat{Y}_{Ger. \%} = 39.73 - 0.89X + 0.004X^2$	0.62
		$\hat{Y}_{Ger. \%} = 69.02 - 2.78X + 0.032X^2 + 0.0003X^3$	0.82
8	Ambient temp. (C ₈)	$\hat{Y}_{Ger. \%} = 42.88 - 0.33X$	0.73
		$\hat{Y}_{Ger. \%} = 65.97 - 1.23X + 0.005X^2$	0.97

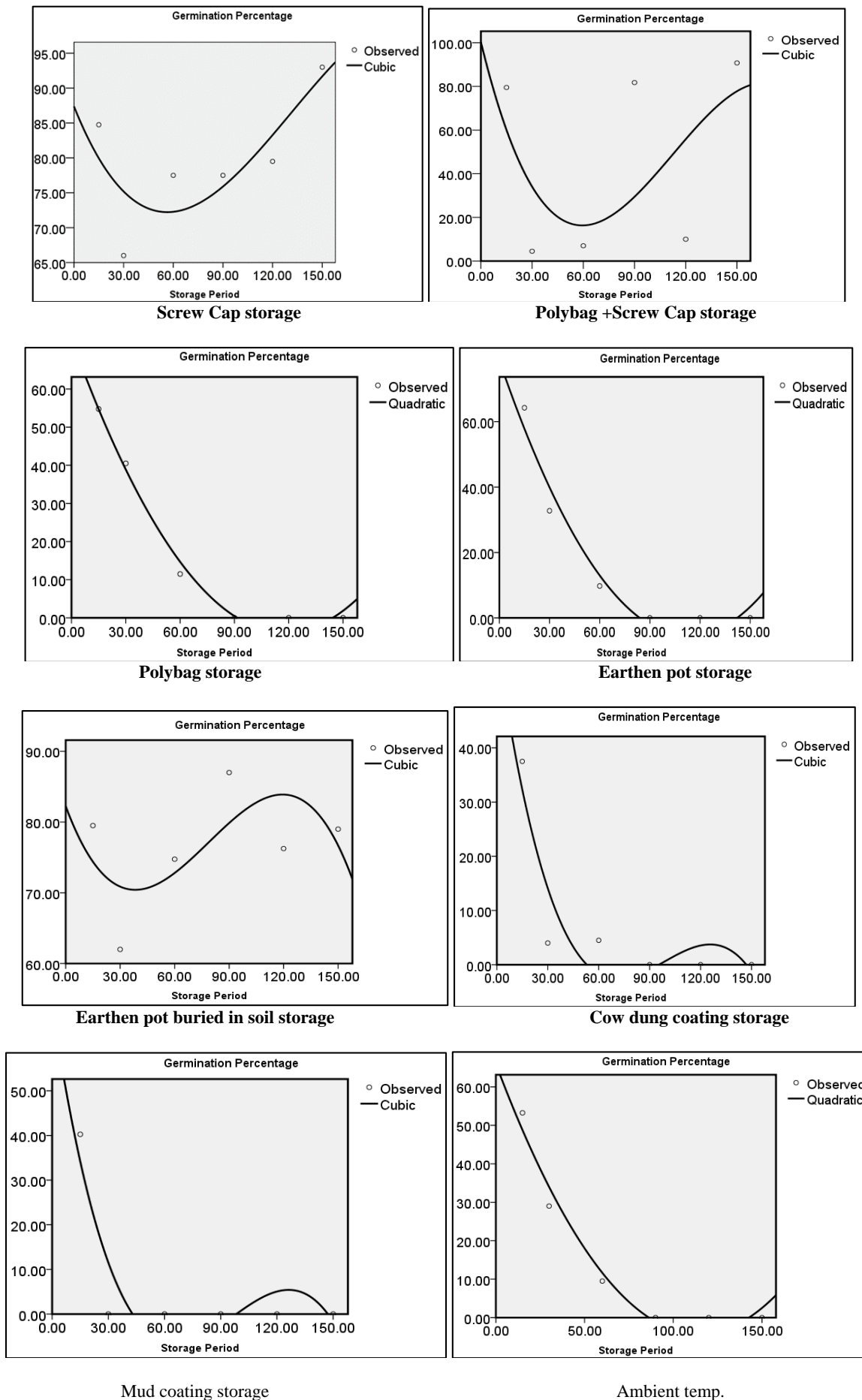


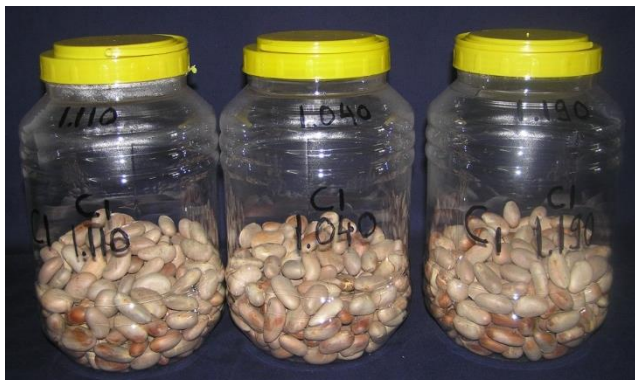
Fig 1: Polynomial observed on germination percentage of jackfruit seed against storage period



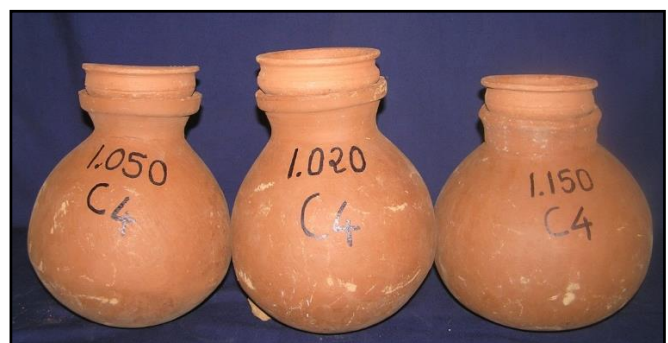
Fig 2: Shade dried seeds ready for storage under different treatments



Treatment C₃ - Storing the seeds in Polybag - 300 gauge at ambient temperature



Treatment C₁ - Storing the seeds in Screw Cap plastic bottle



Treatment C₄ - Storing the seeds in Earthen pot at ambient temperature



Treatment C₂ - Storing the seeds in Polybag - 300 gauge + Screw Cap plastic bottle



Treatment C₅ - Storing the seeds in Earthen pot buried in soil

Fig 3B: Storing the seeds under different storage methods

Fig 3A: Storing the seeds under different storage methods

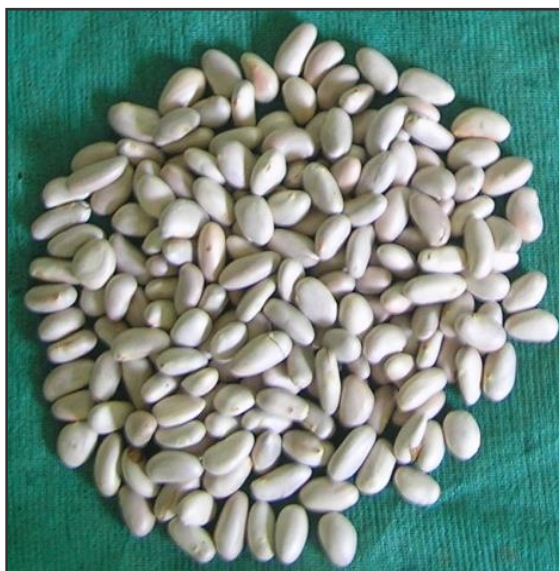


Treatment C₆ - Coating with Fresh Cow dung slurry, drying and stored in Poly bag





Treatment C₇ - Coating with mud slurry, drying and stored in Poly bag



Treatment C₈ - Storing at ambient temperature (Control)

Fig 3C: Storing the seeds under different storage methods





Fig 4: Seeds under different treatments after 120 days storage period



At 90 days storage

At 120 days storage



Germination during storage

At 150 days storage (S6)



Growth of Seedlings under treatment C₅S₆ after transplanting in polybag

Fig 5: Germination observed in treatment C₅ i.e. Storing the seeds in earthen pot buried in soil at various storage periods



Fig 6: Germination under treatment C₁S₆ i.e. Storing the seeds in screw cap plastic bottle at 150 days storage

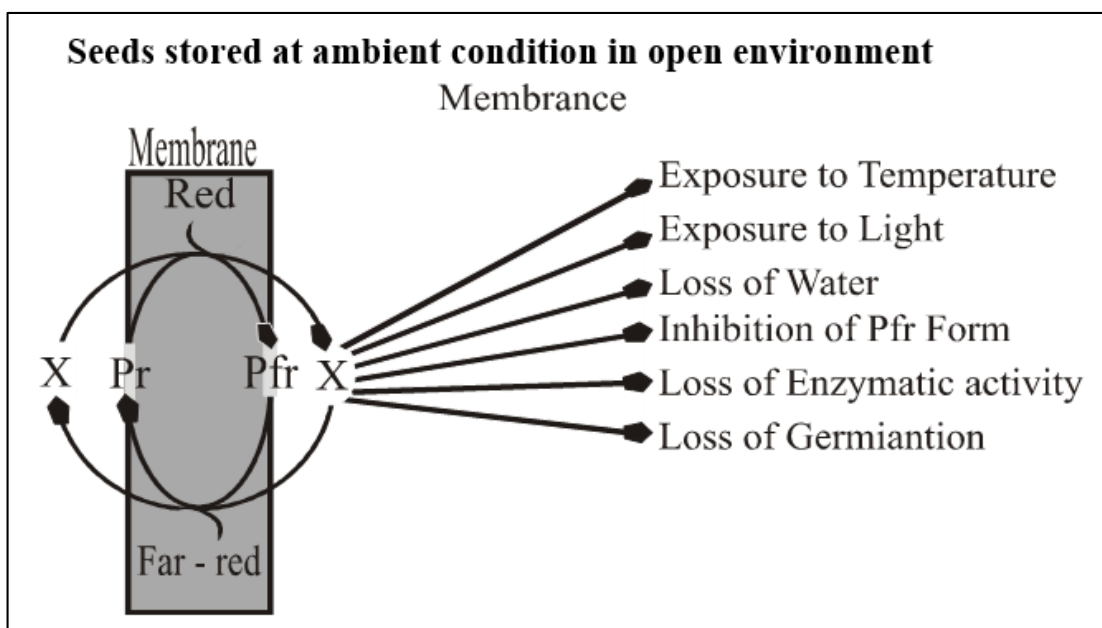


Fig 7: Speculative model for the action of phytochrome for seeds stored at ambient temperature

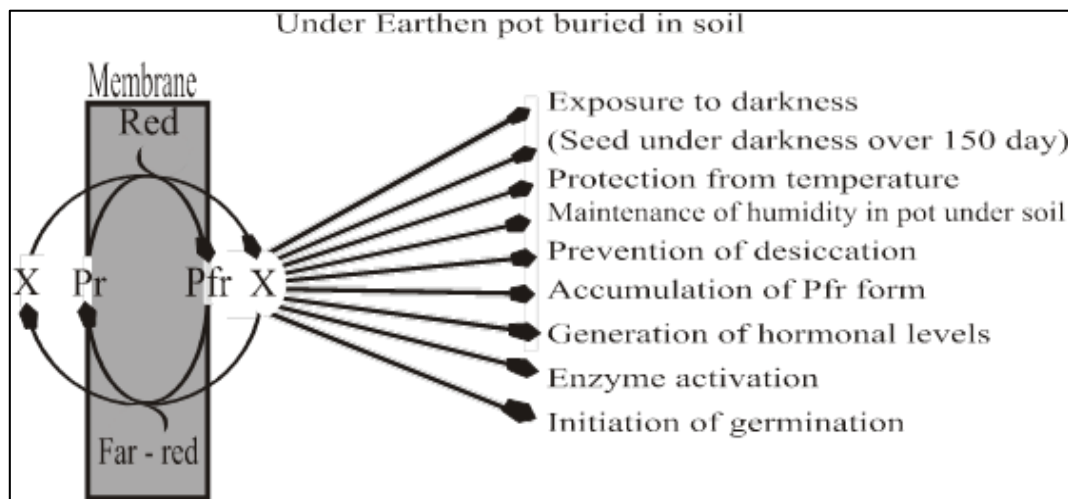


Fig 8: Speculative model for the action of phytochrome for seeds stored at ambient temperature in earthen pot buried in soil

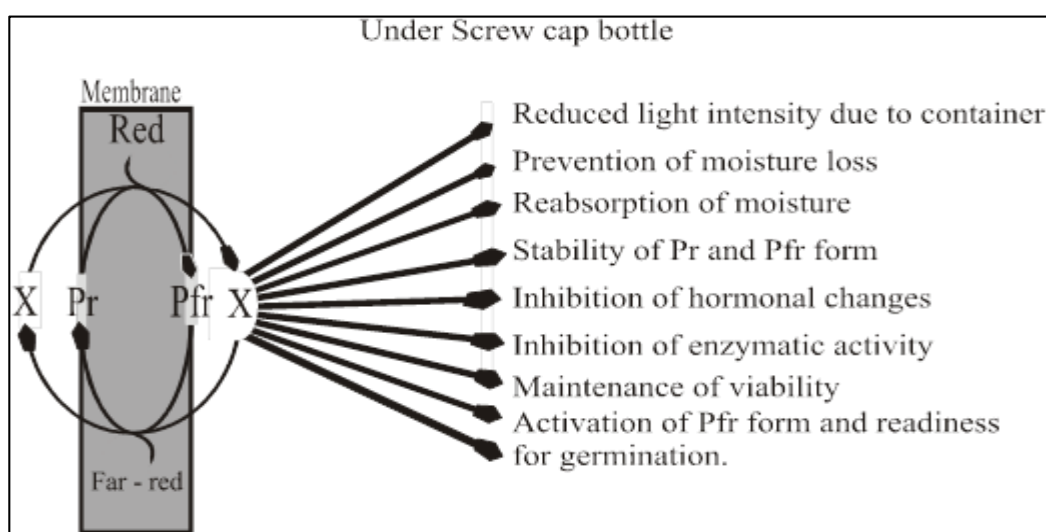


Fig 9: Speculative model for the action of phytochrome for seeds stored in screw cap bottle

Conclusion

Jackfruit seeds could be effectively stored for period of 150 days in screw cap plastic bottle or in Polybag 300 gauge + Screw Cap plastic bottle. Seeds stored in earthen pot buried in soil were already germinated at 90, 120 and 150 days storage hence not effective for storing the seeds in viable conditions. In this context storing the seeds in screw cap bottle appear to be most appropriate where there is no risk of germination of seed within storage container and seeds are kept viable beyond 150 days.

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