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Studies on Effect of Different Storage Conditions on Viability of Karonda Seeds

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A B S T R A C T

An experiment was conducted to study the storage and viability of karonda seeds (*Carrisa carandas* L.). Studies revealed that the seeds possessed short viability due to recalcitrant nature. This could overcome by finding a suitable storage temperature and container treatment for storage of karonda seeds. This study showed significant differences among the storage conditions and container treatments. Seeds treated with *Trichoderma harzianum* kept in poly bag and stored in refrigerated condition resulted maximum germination 90.00, 80.00, 73.33, 56.66, 50.00 and 43.33 per cent at 15, 30, 45, 60, 75 and 90 days after extraction, respectively. Seed storage under ambient condition is not recommended as seeds losses its viability early. The use of bio- control agents and different leaf powder are recommended to extend seed viability period and germination in karonda seeds.

Introduction

Karonda (*Carissa carandas* Linn.) is native to India and grows wild in Maharashtra, Rajasthan, Uttar Pradesh, West Bengal and Bihar. It is popularly known as "Bengal currant" or "Christ's Thorn". Other names are karamanda, karavanda, kaunda, kalivi, natal plum in India. In Kannada it is called as 'Kavalikayi'. It belongs to family Apocynaceae with chromosome number 2n = 22. Karonda is an important minor indigenous underexploited fruit crop of India. It has recently attained importance as an arid zone horticulture crop because of its hardy nature and its nutritious fruits. Karonda is a woody, evergreen dichotomously branched, spiny shrub grows to height of 10-15ft. Leaves are opposite, small, ovate and shiny. Flowers are white in colour produced in terminal cyme. It is preferred very much as a protective hedge in Gujarat and Punjab. It is sometimes grown as an ornamental plant due to its beautiful cherry like fruits. Karonda is best suited as a live protective fence due to the presence of axillary spines and formation of profuse leaves on crowded branches. It has excellent potential to be used for horticultural plantations in marginal and wastelands, owing to its hardy and xerophytic nature with wide adaptability to saline sodic soils with pH up to 10 (Bankar *et al.*, 1994 and Chundawat.,

1995). Fruits are generally harvested at immature stage for vegetable purpose, while fully ripen fruits are consumed fresh or processed (Malik *et al.,* 2010). The dried fruits are good source of iron (39.10mg/100 g), pectin and contain fair amount of vitamin C.

Fruits are used in preparation of jelly, jam, syrup, murabba and chutney (Kumar *et al.,* 2007). The unripe fruits yield milky white latex which can be used in preparing chewing gum and rubber. The unripe fruits of karonda are medicinally used as an astringent. The ripe fruit is sweet, cooling, appetiser and antiscorbutic and is useful in controlling burning sensation, skin diseases, scabies, pruritus (Imran *et al.,* 2012) and particularly suitable for tarts and puddings. It is also used in curing anaemia.

Seeds are recalcitrant and are relatively high in moisture content and possess a characteristic feature of losing their viability during desiccation. Hence seeds of karonda should be sown just after extraction from fruits (Kumar *et al.,* 2007). Germination is seen within 15 days and exhibited 72 per cent germination. Seeds showed shelf life of maximum five to six months with 50 per cent decline in germination after three months (Kalalbandi and Shinde, 2007) The seeds being recalcitrant, it is much importance to enhance germination per cent and know the viable period so that, the availability of viable seeds can be extended by proper storage in congenial temperature so as to use seeds in multiplication of planting material and for raising of rootstocks. Therefore the objective of this study was to determine the effect of different storage conditions and container treatment on viability of karonda seeds

Materials and Methods

The investigation was carried out during year 2016-2017 at K. R. C. College of Horticulture, Arabhavi. The experiment was laid in Factorial completely randomized design (FCRD) with two factors *viz,* storage condition and container treatment, seeds stored in refrigerated (4-5º C) and seeds kept at room temperature with container treatment T_1- Poly bag (700 gauge thick), T_2- Poly bag + Arappu leaf powder (250 g per kg of seed), T_3- Poly bag + Pongamia leaf powder (250 g per kg of seed), T_4- Poly bag + Bavistin (2g per kg of seed), T_5- Poly bag + *Trichoderma harzianum* (10 g per kg of seed), T_6- Control (cotton bag). Observations on days taken for initiation of germination, days taken for maximum germination, germination percentage, root length, shoot length and seedling vigour index – I were recorded for three month old seedling sown at 15, 30, 45, 60, 75 and 90 days after extraction.

Results and Discussion

The investigation revealed that, seeds stored under refrigerated conditions took less number of days 11.17, 11.78, 13.56, 16.06, 16.61 and 16.88 for initiation of germination at 15 days after extraction than compared with the seeds stored in ambient condition (Table 1).

Among container treatments minimum number of days (9.17) taken for initiation of germination at 15 days after extraction was found in seeds treated with *Trichoderma harzian*um and kept in poly bag, same treatment took less number of days 10.67, 14.00, 15.50, 16.33 and 17.16 for initiation of germination at 30, 45, 60, 75 and 90 days after extraction, respectively.

In case of interaction effects seed treated with *Trichoderma harzian*um kept in poly bag and stored under refrigerated condition showed less number of days (8.33, 9.33, 11.33, 14.00, 15.66 and 16.33 for initiation of germination at 15, 30, 45, 60, 75 and 90 days after extraction, respectively.

Table.1 Effect of different container treatments and storage conditions on initiation of germination of karonda seeds sown at different Days after extraction (DAE)

Container (T)	Days taken for Initiation of germination								
	15 DAE			30 DAE			45 DAE		
	Storage 1	Storage 2	Mean (Container)	Storage 1	Storage 2	Mean (Container)	Storage 1	Storage 2	Mean (Container)
T_1	13.30	12.33	12.83	13.33	14.00	13.67	14.33	17.00	15.67
T_2	10.33	11.33	10.83	10.67	13.00	11.83	12.33	17.33	14.83
T_3	11.00	10.00	10.50	12.00	12.00	12.00	12.67	16.33	14.50
T_4	12.00	13.33	12.67	12.33	13.33	12.83	14.00	18.33	16.17
T_5	8.33	10.00	9.17	9.33	12.00	10.67	11.33	16.67	14.00
T_6	12.00	16.00	14.00	13.00	16.33	14.67	16.67	19.00	17.83
Mean (Storage)	11.17	12.17	-	11.78	13.44	-	13.56	17.44	-
	SEm±	**CD@5%**	-	**SEm±**	**CD@5%**	-	**SEm±**	**CD@5%**	-
Storage (S)	0.13	0.42	-	0.11	0.34	-	0.14	0.39	-
Container (T)	0.24	0.72	-	0.20	0.60	-	0.23	0.69	-
Interaction(SxT)	0.34	1.01	-	0.28	0.85	-	0.33	0.98	-

Container treatment details:

T_1: Poly bag (700 gauge)
T_2: Poly bag + Arappu leaf powder (250 g per kg of seed)
T_3: Poly bag + Pongamia leaf powder (250 g per kg of seed)
T_4: Poly bag + Bavistin (2 g per kg of seed)
T_5: Poly bag + *Trichoderma harzianum* (10 g per kg of seed)
T_6: Control (cotton bag)

Storage conditions:

Storage 1: Refrigerated (4-5°C)
Storage 2: Ambient (25-30^{0}C)

Table.1 Continued…

Container (T)	Days taken for Initiation of germination								
	60 DAE			75 DAE			90 DAE		
	Storage 1	Storage 2	Mean (Container)	Storage 1	Storage 2	Mean (Container)	Storage 1	Storage 2	Mean (Container)
T_1	17.00	18.00	17.50	17.33 (4.22)**	19.33 (4.45)	18.33 (4.33)	18.33 (4.33)**	19.66 (4.49)	19.00 (4.41)
T_2	14.67	18.33	16.50	15.66 (4.02)	18.66 (4.37)	17.16 (4.19)	16.33 (4.11)	19.00 (4.41)	17.66 (4.25)
T_3	17.00	17.67	17.33	16.66 (4.14)	18.00 (4.30)	17.33 (4.22)	17.00 (4.18)	18.66 (4.37)	17.83 (4.28)
T_4	16.00	20.00	18.00	17.00 (4.18)	0.00 (0.70)	8.50 (2.44)	16.00 (4.06)	0.00 (0.70)	8.00 (2.38)
T_5	14.00	17.00	15.50	15.00 (3.93)	17.66 (4.26)	16.33 (4.09)	15.66 (4.02)	18.66 (4.37)	17.16 (4.19)
T_6	17.67	20.00	18.83	18.00 (4.30)	0.00 (0.70)	9.00 (2.50)	18.00 (4.30)	0.00 (0.70)	9.00 (2.50)
Mean (Storage)	16.06	18.50	-	16.61 (4.13)	12.27 (3.13)	-	16.88 (4.16)	12.66 (3.17)	-
	SEm±	**CD@5%**	**-**	**SEm±**	**CD@5%**	**-**	**SEm±**	**CD@5%**	**-**
Storage (S)	0.15	0.46	-	0.11 (0.01)	0.33 (0.04)	-	0.08 (0.01)	0.25 (0.03)	-
Container (T)	0.27	0.79	-	0.19 (0.02)	0.56 (0.06)	-	0.15 (0.02)	0.45 (0.06)	-
Interaction(SxT)	0.38	1.11	-	0.28 (0.03)	0.83 (0.09)	-	0.21 (0.03)	0.63 (0.08)	-

() ** Values in parentheses are square root transformed data

Container treatment details:

T_1: Poly bag (700 gauge)
T_2: Poly bag + Arappu leaf powder (250 g per kg of seed)
T_3: Poly bag + Pongamia leaf powder (250 g per kg of seed)
T_4: Poly bag + Bavistin (2 g per kg of seed)
T_5: Poly bag + *Trichoderma harzianum* (10 g per kg of seed)
T_6: Control (cotton bag)

Storage conditions:

Storage 1: Refrigerated (4-5°C)
Storage 2: Ambient (25-30^{0}C)

Table.2 Effect of different container treatments and storage conditions on germination percentage of karonda seeds sown at different Days after extraction (DAE)

Container (T)	Germination percentage								
	15 DAE			30 DAE			45 DAE		
	Storage 1	Storage 2	Mean (Container)	Storage 1	Storage 2	Mean (Container)	Storage 1	Storage 2	Mean (Container)
T_1	73.33 (59.00)*	70.00 (56.78)	71.66 (57.89)	66.66 (54.78)*	56.66 (48.84)	61.66 (51.81)	56.66 (48.84)*	46.66 (43.07)	51.66 (45.96)
T_2	83.33 (66.14)	76.66 (61.21)	80.00 (63.43)	76.66 (61.28)	60.00 (50.76)	68.33 (55.99)	66.66 (54.78)	53.33 (46.92)	60.00 (50.85)
T_3	80.00 (63.43)	80.00 (63.42)	80.00 (63.43)	73.33 (59.00)	66.66 (54.78)	70.00 (56.89)	63.33 (52.73)	56.66 (48.84)	60.00 (50.81)
T_4	76.66 (61.21)	73.33 (59.00)	75.00 (60.11)	73.33 (59.00)	63.33 (52.77)	68.33 (55.88)	60.00 (50.76)	40.00 (39.23)	50.00 (45.00)
T_5	90.00 (71.56)	80.00 (63.43)	85.00 (67.50)	80.00 (63.43)	70.00 (56.78)	75.00 (60.11)	73.33 (59.00)	56.66 (48.84)	65.00 (53.92)
T_6	66.66 (54.78)	60.00 (50.76)	63.33 (52.80)	63.33 (52.77)	53.33 (46.92)	58.33 (49.84)	53.33 (46.92)	33.33 (35.21)	43.33 (41.07)
Mean (Storage)	78.33 (62.69)	73.33 (59.10)	-	72.22 (58.37)	61.66 (51.81)	-	62.22 (52.18)	47.77 (43.69)	-
	S.Em±	**CD@5%**	-	**SEm±**	**CD@5%**	-	**SEm±**	**CD@5%**	-
Storage (S)	0.93 (0.64)	2.75 (1.89)	-	1.13 (0.70)	3.33 (2.07)	-	1.16 (0.68)	3.40 (1.99)	-
Container (T)	1.62 (1.12)	4.77 (3.28)	-	1.96 (1.21)	5.77 (3.58)	-	2.00 (1.18)	5.89 (3.45)	-
Interaction(SxT)	2.30 (1.58)	6.75 (4.66)	-	2.78 (1.72)	8.16 (5.05)	-	2.84 (1.66)	8.33 (4.88)	-

()*Values in parentheses are arc sign transformed data

Container treatment details:

T_1: Poly bag (700 gauge)
T_2: Poly bag + Arappu leaf powder (250 g per kg of seed)
T_3: Poly bag + Pongamia leaf powder (250 g per kg of seed)
T_4: Poly bag + Bavistin (2 g per kg of seed)
T_5: Poly bag + *Trichoderma harzianum* (10 g per kg of seed)
T_6: Control (cotton bag)

Storage conditions:

Storage 1: Refrigerated (4-5°C)
Storage 2: Ambient (25-30^{0}C)

Table.2 Continued…

Container (T)	Germination percentage								
	60 DAE			75 DAE			90 DAE		
	Storage 1	Storage 2	Mean (Container)	Storage 1	Storage 2	Mean (Container)	Storage 1	Storage 2	Mean (Container)
T_1	43.33 (41.15)*	33.33 (35.21)	38.33 (38.18)	40.00 (39.14)*	16.66 (23.85)	28.33 (31.50)	26.66 (30.99)*	10.00 (18.43)	18.33 (24.71)
T_2	53.33 (46.92)	36.66 (37.22)	45.00 (42.07)	43.33 (41.15)	20.00 (26.07)	31.66 (33.61)	40.00 (39.23)	16.66 (23.85)	28.33 (31.54)
T_3	53.33 (46.92)	40.00 (39.23)	46.66 (43.07)	33.33 (35.21)	23.33 (28.78)	28.33 (31.99)	23.33 (28.78)	23.33 (28.78)	23.33 (28.78)
T_4	46.66 (43.07)	13.333 (21.14)	30.00 (32.11)	42.33 (40.15)	0.00 (0.28)	21.16 (20.72)	33.33 (35.22)	0.00 (0.28)	16.66 (17.75)
T_5	56.66 (48.84)	40.00 (39.23)	48.33 (44.03)	50.00 (45.00)	30.00 (33.21)	40.00 (39.10)	43.33 (41.15)	23.33 (28.78)	33.33 (34.96)
T_6	33.33 (35.21)	10.00 (18.43)	21.66 (26.82)	20.00 (26.07)	0.00 (0.28)	10.00 (13.73)	16.66 (23.85)	0.00 (0.28)	8.33 (12.07)
Mean (Storage)	47.77 (43.69)	28.88 (31.74)	-	38.33 (37.95)	15.00 (18.74)	-	30.55 (33.20)	12.22 (16.73)	-
	SEm±	**CD@5%**	**-**	**S.Em±**	**CD@5%**	-	**SEm±**	**CD@5%**	-
Storage (S)	1.21 (0.75)	3.55 (2.20)	-	1.10 (0.77)	3.24 (2.28)	-	0.81 (0.56)	2.38 (1.66)	-
Container (T)	2.10 (1.30)	6.16 (3.81)	-	1.91 (1.35)	5.61 (3.95)	-	1.40 (0.97)	4.12 (2.88)	-
Interaction(SxT)	1.83 (1.83)	8.71 (5.40)	-	2.60 (1.90)	7.93 (5.59)	-	1.98 (1.38)	5.83 (4.07)	-

()*Values in parentheses are arc sign transformed data

Container treatment details:

T_1: Poly bag (700 gauge)
T_2: Poly bag + Arappu leaf powder (250 g per kg of seed)
T_3: Poly bag + Pongamia leaf powder (250 g per kg of seed)
T_4: Poly bag + Bavistin (2 g per kg of seed)
T_5: Poly bag + *Trichoderma harzianum* (10 g per kg of seed)
T_6: Control (cotton bag)

Storage conditions:

Storage 1: Refrigerated (4-5°C)
Storage 2: Ambient (25-30^{0}C)

Table.3 Effect of different container treatments and storage conditions on seedling vigour index – I of karonda seeds sown at different Days after extraction (DAE)

Container (T)	Seedling vigour index - I								
	15 DAE			30 DAE			45 DAE		
	Storage 1	Storage 2	Mean (Container)	Storage 1	Storage 2	Mean (Container)	Storage 1	Storage 2	Mean (Container)
T_1	2000.00	1913.00	1957.00	1698.00	1465.00	1582.00	1378.00	1090.00	1234.00
T_2	2503.00	2140.00	2322.00	2107.00	1580.00	1843.00	1803.00	1312.00	1557.00
T_3	2400.00	2293.30	2347.00	2158.00	1765.00	1962.00	1667.00	1397.00	1532.00
T_4	2297.00	1930.00	2113.00	2038.00	1525.00	1782.00	1560.00	887.00	1223.00
T_5	2940.00	2373.00	2657.00	2467.00	1960.00	2213.00	2153.00	1478.00	1816.00
T_6	1870.00	1460.00	1665.00	1733.00	1202.00	1467.00	1307.00	657.00	982.00
Mean (Storage)	2335.00	2018.00	-	2034.00	1583.00	-	1645.00	1137.00	-
	SEm±	**CD@5%**	-	**SEm±**	**CD@5%**	-	**SEm±**	**CD@5%**	-
Storage (S)	29.00	85.18	-	29.11	85.39	-	27.87	81.76	-
Container (T)	50.30	147.53	-	50.40	147.91	-	48.28	141.61	-
Interaction(SxT)	71.10	208.64	-	71.31	209.17	-	68.28	200.27	-

() ** Values in parentheses are square root transformed data

Container treatment details:

T_1: Poly bag (700 gauge)
T_2: Poly bag + Arappu leaf powder (250 g per kg of seed)
T_3: Poly bag + Pongamia leaf powder (250 g per kg of seed)
T_4: Poly bag + Bavistin (2 g per kg of seed)
T_5: Poly bag + *Trichoderma harzianum* (10 g per kg of seed)
T_6: Control (cotton bag)

Storage conditions:

Storage 1: Refrigerated (4-5°C)
Storage 2: Ambient (25-30^{0}C)

Table.3 Continued…

Container (T)	Seedling vigour index - I								
	60 DAE			75 DAE			90 DAE		
	Storage 1	Storage 2	Mean (Container)	Storage 1	Storage 2	Mean (Container)	Storage 1	Storage 2	Mean (Container)
T_1	990.00	702.00	846.00	873.00 (29.34)**	302.00 (17.23)	587.00 (23.28)	547.00 (23.28)**	163.00 (12.78)	355.00 (18.03)
T_2	1373.00	833.00	1103.00	1058.00 (32.45)	508.00 (22.35)	783.00 (27.40)	933.00 (30.55)	340.00 (18.14)	636.00 (24.34)
T_3	1328.00	907.00	1117.00	793.00 (28.11)	640.00 (25.30)	717.00 (26.70)	527.00 (22.86)	465.00 (21.43)	496.00 (22.15)
T_4	1133.00	272.00	702.00	1013.00 (31.77)	0.00 (0.70)	507.00 (16.23)	740.00 (27.13)	0.00 (0.70)	370.00 (13.91)
T_5	1550.00	980.00	1265.00	1292.00 (35.94)	700.00 (26.45)	996.00 (31.19)	1080.00 (32.78)	442.00 (20.60)	761.00 (26.69)
T_6	753.00	182.00	467.00	422.00 (20.13)	0.00 (0.70)	211.00 (10.42)	333.00 (18.06)	0.00 (0.70)	167.00 (9.38)
Mean (Storage)	1188.00	646.00	-	909.00 (29.62)	358.00 (15.45)	-	693.00 (25.78)	235.00 (12.39)	-
	SEm ±	**CD@5%**	-	**SEm±**	**CD@5%**	-	**SEm±**	**CD@5%**	-
Storage (S)	33.18	97.31	-	25.5 (0.51)	74.82 (1.51)	-	21.50 (0.51)	63.17 (1.51)	-
Container (T)	57.47	168.56	-	44.18 (0.89)	129.60 (2.62)	-	37.30 (0.89)	109.42 (2.61)	-
Interaction(SxT)	81.27	238.38	-	62.48 (1.26)	183.28 (3.71)	-	52.70 (1.26)	154.75 (3.70)	-

()** Values in parentheses are square root transformed data

Container treatment details:

T_1: Poly bag (700 gauge)
T_2: Poly bag + Arappu leaf powder (250 g per kg of seed)
T_3: Poly bag + Pongamia leaf powder (250 g per kg of seed)
T_4: Poly bag + Bavistin (2 g per kg of seed)
T_5: Poly bag + *Trichoderma harzianum* (10 g per kg of seed)
T_6: Control (cotton bag)

Storage conditions:

Storage 1: Refrigerated (4-5°C)
Storage 2: Ambient (25-30^{0}C)

The observations from table 1 clearly established the fact that there was consequential increase in germination time with advancement of seed storage period from 15 to 90 days after extraction. This could be attributed to the seed deterioration during storage, leading to reduction in vigour, germination rate, enzymatic activity, respiration, increase in permeability and susceptibility in stresses, decrease in seedling growth rate, reproductive processes and yield. Whereas, due to different treatment effect seeds treated with *Trichoderma harzianum* took less number of days for initiation germination. This may be due to *Trichoderma* strains that produce cytokinin-like molecules or GA_3. The controlled production of these compounds could improve biofertilization (Osiewacz, 2002). *Trichoderma* produces growth factors that increased the rate of seed germination and also compete with other microorganisms for key exudates from seeds that stimulate the germination of propagules of plant-pathogenic fungi in soil (Howell, 2002) and more generally, compete with soil microorganisms for nutrients and space, thus helps in early germination by reducing the risk of pathogen attack.

Germination percentage in case of storage condition, seeds stored in refrigerated conditions showed maximum germination 78.33 to 30.55 per cent from 15 days to 90 days after extraction (Table 2). In case of container treatment effect germination percentage was significantly high in seeds treated with *Trichoderma harzianum* kept in poly bag showed 85.00, 75.00, 65.00, 48.33, 40.00 and 33.33 per cent germination at 15, 30, 45, 60, 75 and 90 days after extraction, respectively.

Among interaction *Trichoderma* treated seeds kept in poly bag and stored in refrigerated condition resulted maximum germination 90.00, 80.00, 73.33, 56.66, 50.00 and 43.33 per cent at 15, 30, 45, 60, 75 and 90 days after extraction, respectively. There was decreasing trend in seed germination as storage period increased. The decline in per cent germination with advance in storage period may be attributed to the phenomenon of aging, depletion of food reserves, and decline in synthetic activity. Whereas, in every sowing at 15 days intervals, seeds treated with *Trichoderma harzianum* showed highest germination. This may be due to *Trichoderma harzianum* might have released growth promoting substance which help in rapid germination. Together with the synthesis or stimulation of phyto-hormone production, most *Trichoderma* strains acidify their surrounding environment by secreting organic acids, such as gluconic, citric or fumaric acid. These organic acids result from the metabolism of other carbon sources mainly glucose and in turn, are able to solubilize phosphates, micronutrients and mineral cautions including iron, manganese and magnesium (Harman, 2006).

Seedling vigour index – I decreased as storage period increased. Vigour index of seedling was found high in seeds stored in refrigerated condition at 15 days after extraction 2335.00 compared with seeds stored in ambient condition (Table 3).

In case of different container treatment seeds treated with *Trichoderma harzianum* kept in poly bag recorded highest (2657.00, 2213.00, 1816.00, 1265.00, 996.00 and 761.00 vigour index compared with the rest of treatments at 15, 30, 45, 60, 75 and 90 days after extraction, respectively. Seeds treated with *Trichoderma harzianum* kept in poly bag and stored in refrigerated condition gave highest (2940.00, 2467.00, 2153.00, 1550.00, 1292.00 and 1080.00 vigour index at 15, 30, 45, 60, 75 and 90 days after extraction, respectively. Highest growth parameters were recorded in seeds treated with *Trichoderma*. This may be

due to *Trichoderma* strains are always associated with plant roots and root ecosystems that increases uptake of nitrates and other ions that frequently enhances root growth and development, crop productivity, resistance to abiotic stresses (Arora *et al.*, 1992). *Trichoderma* strains as plant symbiont opportunistic avirulent organisms, able to colonize plant roots by mechanisms similar to those of mycorrhizal fungi and to produce compounds that stimulate growth and plant defence mechanisms. As the germination percentage, seedling root and shoot length were found highest in seeds treated with *Trichoderma harzianum*, hence seedlings vigour index -I were recorded highest.

Seed viability can be maintained up to 120 days after extraction when seeds of karonda are treated with *Trichoderma harzianum* kept in polybag and stored in refrigerated conditions compared with seeds kept in cotton bag and stored in ambient conditions. Viability can be maintained twice the period compared with the control treatment.

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