Indian Journal of Agricultural Sciences **91** (6): 867–71, June 2021/Article
https://doi.org/10.56093/ijas.v91i6.114288

Effect of storage period on different seed viability parameters in rajmash (*Phaseolus vulgaris*)

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Received: 04 November 2020; Accepted: 04 January 2021

ABSTRACT

Seed longevity mainly depends on initial seed quality and storage conditions therefore; farmers and gene bank curators need to be aware of the storage potential of rajmash cultivars that contribute to the production of high quality seed during routine germplasm regeneration/multiplication. Consequently, it is important to generate information on seed longevity of rajmash. In the present investigation, 14 high yielding rajmash cultivars were stored for 4 years and periodical germination percent, time to initiate germination (t_1), time to achieve 50% germination (t_{50}) and uniformity of germination (U_{7525}; time between 25 and 75 % germination) were recorded. The varieties exhibited the mean germination percentage of 93, 93, 73, 3 and 0.9 when stored for 0, 1, 2, 3 and 4 years, respectively. The results demonstrated that, all the 14 varieties could be safely stored for a minimum period of 1 year at ambient condition. Seven varieties (Arka Komal, IPR 98-5, IPR 98-3-1, HUR 137, HUR 203, HPR 35, Gujarat Rajmash 1) could be stored for 2 years as they demonstrated more than the minimum germination requirement (75%) of Indian Minimum Seed Certification Standard. Storing seeds for extended period not only decreased the final germination percentage, but also increased the time for t_1, t_{50} and t_{7525}, suggesting that use of aged seeds could result into poor field establishment coupled with delayed and non-uniform germination.

Keywords: Germination, Longevity, Rajmash, Storage, Viability

Rajmash is an important food legume grown throughout the world, pre-dominantly in America and Africa. In India, it is being mainly grown as *kharif* crop in Hilly regions and as a *rabi* crop in plains (Mishra *et al.* 2019). It is a potential source of high-quality protein (17.5–28.7%), which is a reasonable substitute to animal derived proteins particularly to the resource-poor people of developing countries. It also provides sufficient quantities of important minerals and micro-nutrients including folate, potassium, iron, manganese, copper and zinc (Parihar *et al*. 2016). The average yield of rajmash is quite low in India as compared to other countries owing to many biotic and abiotic factors (Mishra *et al.* 2019). Of them, quality seed plays an instrumental role as it ensures better establishment and yield. In general, after harvesting, the seeds of rajmash are stored until the next cropping season, which could vary from days to months to even years. In addition, the long-term maintenance of seed viability, combined with minimum loss of genetic integrity is a quintessential for effective conservation and use of plant genetic resources. To maintain optimal seed viability, accessions stored in gene banks are in need of frequent regeneration which is an expensive process and involves the risk of losing the genetic integrity due to several factors (Roberts 1992). The initial seed quality and storage conditions are the major factors determining the seed longevity (Kameswara Rao *et al.* 2017). In addition, temperature, nature of seeds, seed moisture, relative humidity during storage influences the seed longevity (Khalequzzaman *et al.* 2012). Rajmash seeds are reported to loose viability rapidly during storage (Pena-Valdivia *et al*. 1999), or completely inhibited after 60 days of storage at 30ºC and 70% RH (Herrera *et al*. 2001). Since, the period of storage of carryover seeds and conservation in a gene bank may vary from a season to number of years; it becomes important to know the exact storage period of different cultivars of rajmash for maximizing production and productivity. Therefore, the present investigation was undertaken to delineate the effect of ambient storage on the seed viability and other related parameters of rajmash.

MATERIALS AND METHODS

An experiment was performed at ICAR-Indian Institute of Pulses Research, Kanpur, Uttar Pradesh. The seeds of 14 different varieties of rajmash were produced during *rabi* 2015–16. After observing the initial germination

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percentage, time to initiation of germination (t_1), time to 50% germination (t_{50}) and uniformity of germination (t_{7525}), the seeds were kept inside a cloth bag and were stored at ambient room temperature. Every year and subsequently up to 4 years of storage, the seeds were sampled and tested for its viability, t_1, t_{50} and t_{7525}. Twenty five seeds of each cultivar in four replications were placed in a petri plate containing two wet filter paper and subsequently were kept in germinator maintained at 20ºC for 9 days (ISTA 2015). Seed was considered germinated when the seedling reached an approximate length of 2 mm. Final germination, t_1, t_{50} and t_{7525} were calculated by "Germinator curve-fitting 1.29. xls" microsoft excel model (Joosen *et al*. 2010), using the equation of El Kassaby *et al.* (2008).

RESULTS AND DISCUSSION

Maintenance of seed viability during storage is influenced by many factors, of them storage temperature, RH and moisture are the key determinants. Seed deterioration is an inescapable process; however, the rate of deterioration could be reduced by managing the storage environment (Lamichaney *et al.* 2019). With increase in storage period, not only germination is expected to reduce but could alter the time to initiate germination (t_1), time to achieve 50% germination (t_{50}) and uniformity of germination (U_{7525}) or time between 25 to 75 % germination, which results into heterogeneous plant growth making inter-cultural operation difficult. Therefore, the present investigation was undertaken to understand the longevity of 14 cultivars of rajmash recommended for different agro-climatic zones of India. The seed germination of rajmash varieties before storage varied between 78% (Arka Anoop) to 100% (PDR 14, IPR 98-5 and HUR 203) with a mean value of 93.43%. All the varieties maintained its germination in first year of storage with a mean value of 93.36% and the germination value oscillated between 75% (Arka bold) to 100% (HUR 15, HPR 35, PDR 14 and Gujarat Rajmash-1). The germination percent was significantly reduced when stored for two years with a mean value of 73.29% and ranged between 46% (Arka Bold) to 100% (IPR 98-5). Six out of 14 varieties, namely HUR 15, Arka Komal, IPR 98-5, Arka Anoop, HUR 137 and Arka Bold could not germinated while 7 out of 14 varieties such as Arka Suvidha, IPR 98-3-1, IVFB 1, HUR 203, HPR 35, Gujarat rajmash-1 and Amber exhibited 2-4% germination and PDR 14 demonstrated 28% germination after 3 years of storage. Interestingly, in all the tested varieties of rajmash, germination was completely inhibited after four years of storage except for PDR 14 which recorded 12% germination (Table 1).

There was no difference between seed germination of white seeded (n = 3) and dark seeded (n = 11) varieties before storage. However, the germination significantly reduced (14-26%) in white seeded varieties after 1 and 2 years storage period as compared to dark seeded varieties (Fig 1). The result revealed that significant genotypic variability available in terms of longevity of varieties, where dark seeded varieties had better longevity than white seeded. The pigmentation in seed coat is imparted by tannin (Caldes and Blair 2009), which is reported to have an anti-fungal and anti-microbial properties. Seed coat thickness and its chemical composition are the physical and biochemical defensive mechanism of the seeds (Zhang *et al*. 2016), which are reported to be adequately more in dark seeds as compared to white seeds (Saeidi and Rowland 2000, Segav

Table 1 Effect of storage on seed germination and time taken to initiate germination (t_1) of 14 rajmash varieties

Variety	Germination (%)					Time taken to initiate germination (t_1)				
	Storage period (years)									
	0	1	2	3	4	0	1	2	3	4
HUR-15	98^{aA}	100^{aA}	64^{bcdB}	0^{bC}	0^{bC}	29.0^{bcB}	55.5^{aA}	59.3^{bA}		
Arka Komal	98^{aA}	98^{aA}	80^{abcB}	0^{bC}	0^{bC}	36.9^{bcB}	51.8^{abA}	62.2^{abA}		
PDR-14	100^{aA}	100^{aA}	62^{bcdB}	28^{aC}	12^{aD}	41.1^{abD}	45.7^{abD}	73.0^{abC}	121.35^{cB}	176.3^{A}
IPR 98-5	100^{aA}	98^{aA}	100^{aA}	0^{bB}	0^{bB}	38.3^{abcB}	38.4^{bB}	61.2^{abA}		
Arka Suvidha	90^{bA}	94^{abA}	60^{bcB}	2^{bC}	0^{bC}	29.5^{bcC}	40.3^{abC}	69.9^{abB}	126.6^{cA}	
Arka Anoop	78^{cA}	76^{cA}	62^{bcB}	0^{bC}	0^{bC}	24.1^{cB}	43.8^{abAB}	77.3^{abA}		
IPR 98-3-1	86^{bA}	94^{abA}	98^{aA}	2^{bB}	0^{bB}	33.8^{bcD}	55.6^{aC}	74.6^{abB}	125.6^{cA}	
HUR-137	98^{aA}	96^{aA}	80^{abcB}	0^{bC}	0^{bC}	40.9^{abB}	48.4^{abAB}	66.0^{abA}		
Kashi Param (IVFB 1)	90^{bA}	80^{bcA}	52^{bcB}	2^{bC}	0^{bC}	28.6^{bcD}	38.5^{bC}	63.5^{abB}	148.6^{bA}	
HUR 203	100^{aA}	98^{aA}	78^{abcB}	2^{bC}	0^{bC}	39.3^{abcC}	47.6^{abC}	64.4^{abB}	164.5^{aA}	
Arka Bold	84^{bA}	75^{cA}	46^{dB}	0^{bC}	0^{bC}	42.6^{abB}	40.0^{abB}	79.4^{aA}		
HPR 35	90^{bA}	100^{aA}	80^{abcA}	2^{bB}	0^{bB}	40.3^{abC}	53.7^{abC}	76.7^{abB}	125.6^{cA}	
GR-1	98^{aA}	100^{aA}	90^{abB}	2^{bC}	0^{bC}	27.0^{bcD}	55.6^{aC}	69.6^{abB}	125.6^{cA}	
Amber	98^{aA}	98^{aA}	74^{abcdB}	4^{bC}	0^{bC}	53.4^{aB}	47.5^{abB}	76.2^{abB}	156.3^{abA}	
Average	93.43	93.36	73.29	3.14	0.86	36.06	47.31	69.52	136.77	176.30

Different lowercase and uppercase letters represent significant difference within the row and column values (P<0.05), respectively.

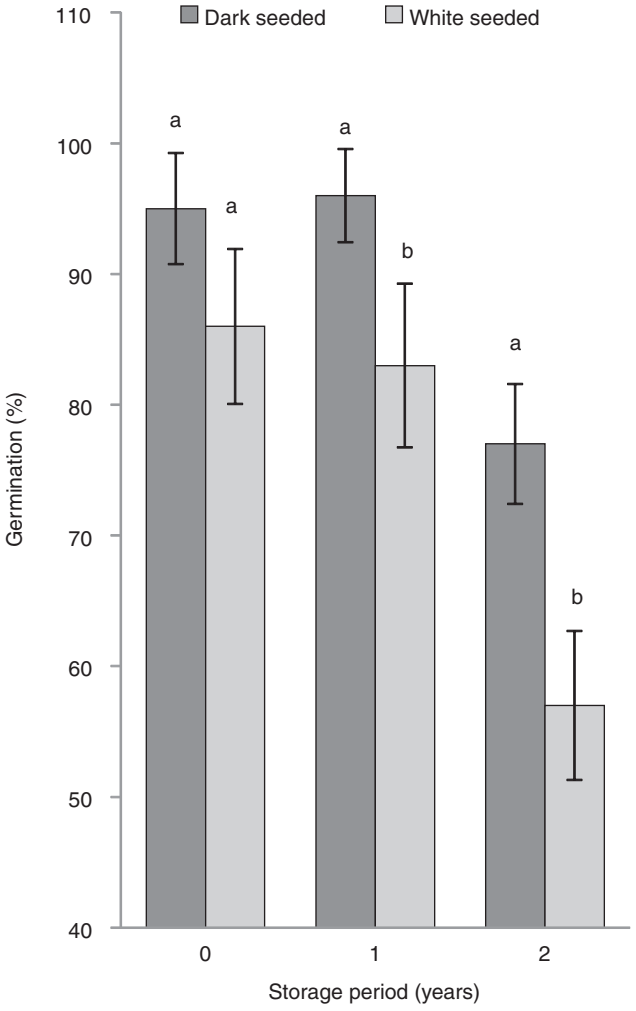

Fig 1 Germination differences between dark seed coloured (n = 11) and white seed coloured (n = 3) varieties during storage.

et al. 2010). This could be the reason for better longevity of dark seeds as noticed in the present investigation. Similar reports of association of seed colour with vigour differences have also been reported in other legumes (Lamichaney *et al*. 2016, Lamichaney *et al*. 2017a, Lamichaney *et al*. 2017b, Sooganna *et al*. 2016, Borji 2007). Free radical mediated or enzymatic peroxidation of poly unsaturated fatty acids was reported to play an important role in seed deterioration. Production and accumulation of free radicals or reactive oxygen species due to lipid peroxidation causes rapid loss of seed viability and vigour by disturbing cellular integrity (Corbineau *et al*. 2002). By scavenging the free radicals, cell protects itself and maintains cellular integrity. The most important mechanism of scavenging free radicals is via antioxidants (vitamin E or tocopherols, isoflavones) or antioxidant enzymes (SOD, CAT, POX, GR), which lowers the content of free radicals and maintain seed viability and vigour during storage. However, during storage, loss of antioxidant enzymes such as catalase, superoxide dismutase and peroxidase are reported (Lehner *et al*. 2008). Loss of seed germination in the present study might be attributed to reduction in the antioxidant enzymes.

There was a significant difference among the varieties in terms of time taken for initiation of germination (t_1). The time for t_1 before storage varied between 24.1 h (Arka Anoop) to 53.4 h (Amber) with a mean value of 36.06 h. The time to t_1 significantly increased with storage duration and took 47.31 h, 69.52 h, 136.77 h and 176.30 h after 1, 2, 3 and 4 years of storage, respectively. The seeds of 6 out of 14 varieties failed to germinate when stored for 3 years and all the varieties failed to germinate after 4 years, therefore no value of t_1 are mentioned in table (Table 1).

Since, none of the varieties registered 50% germination (t_{50}) when stored for 3 and 4 years, therefore, time to

Table 2 Effect of storage on time taken to achieve 50% germination (t_{50}) and uniformity of germination (t_{7525}) of 14 rajmash varieties

Variety	Time taken to achieve 50% germination (t50)			Uniformity of germination (t_{7525})		
	0	1	2	0	1	2
	Storage period (years)					
HUR-15	45.1^{cC}	62.2^{bB}	112.9^{abA}	10.2^{cdeB}	3.4^{deB}	38.4^{abcA}
Arka Komal	51.8^{abcB}	63.9^{abB}	131.9^{abA}	8.5^{deB}	6.3^{cdB}	39.2^{abcA}
PDR-14	60.4^{abB}	65.2^{abB}	193.7^{aA}	11.0^{cdeB}	11.0^{bcB}	51.8^{aA}
IPR 98-5	48.2^{bcB}	68.8^{abB}	122.1^{abA}	5.3^{eB}	20.5^{aB}	40.5^{abA}
Arka Suvidha	62.7^{abB}	66.2^{abB}	131.0^{abA}	16.0^{bcB}	14.5^{abB}	21.4^{bcdA}
Arka Anoop	57.8^{abcB}	71.2^{aB}	124.7^{abA}	19.0^{abA}	12.9^{bcA}	15.9^{cdA}
IPR 98-3-1	53.0^{abcB}	62.0^{bB}	99.0^{cA}	10.3^{cdeA}	3.2^{eB}	13.3^{dA}
HUR-137	54.5^{abcB}	67.2^{abB}	122.7^{abA}	7.4^{deB}	10.2^{bcAB}	30.9^{abcdA}
Kashi Param (IVFB 1)	62.0^{abB}	68.6^{abB}	119.6^{abA}	22.3^{aAB}	14.6^{abB}	32.2^{abcdA}
HUR 203	49.4^{bcB}	67.1^{abB}	122.9^{abA}	5.4^{eA}	10.9^{bcA}	29.9^{abcdA}
Arka Bold	62.8^{abB}	72.7^{aB}	138.1^{bA}	10.5^{cdeA}	15.5^{abA}	16.4^{cdA}
HPR 35	62.0^{abB}	69.4^{abB}	119.6^{abA}	11.9^{cdAB}	8.5^{bcdA}	20.5^{bcdA}
GR-1	54.5^{abcB}	67.5^{abB}	113.6^{abA}	18.1^{abAB}	6.3^{cdB}	25.0^{bcdA}
Amber	65.5^{aB}	68.3^{abB}	146.8^{bA}	6.3^{deB}	11.6^{bceB}	36.2^{abcdA}
Average	56.41	67.16	128.47	11.58	10.67	29.40

Different lowercase and uppercase letters represent significant difference within the row and column values (P<0.05), respectively.

t_{50} was calculated up to 2 years of storage. There was a significant difference among the varieties in terms of time taken to achieve t_{50}. The time for t_{50} before storage varied between 45.1 h (HUR 15) to 65.5 h (Amber) with a mean value of 56.41 h. The time to t_{50} significantly increased with storage duration. In first year of storage, the time to t_{50} varied between 62 h (IPR 98-3-1) to 72.7 h (Arka Bold) with a mean of 67.16 h. Likewise, in second year of storage, the time to t_{50} varied between 99 h (IPR 98-3-1) to 146.8 h (Amber) with a mean of 128.47 h (Table 2).

The uniformity of germination (t_{7525}) was only calculated up to 2 years of storage, as at 3 and 4 years of storage, the germination recorded for 14 varieties was not enough for calculating t_{7525}. Significant varietal and storage period differences were observed for t_{7525}. The time for t_{7525} varied between 5.3-22.3 h; 3.2-20.5 h; 13.3-51.8 h for fresh, one and two year stored seeds, respectively (Table 2).

The reduction in germination value and increase in the time taken for t_1, t_{50} and t_{7525} with an increase in storage period in rajmash may be due to decrease in the activity of hydrolytic enzymes like amylase and protease (Marques *et al*. 2014), which are essential for breaking down the reserve metabolites and making it available as a food material for germination to occur. The reduction in the activity of catalase enzyme during storage is reported to reduce respiratory capacity, decreasing the supply of energy (ATP) and resulting into loss of germination (Demirkaya *et al*. 2010). According to Indian Minimum Seed Certification Standards, the seeds of rajmash should have a minimum germination of 75%. All the varieties recorded a germination percent of >75% after 1 year of storage. Seeds of Arka Komal, IPR 98-5, IPR 98-3-1, HUR 137, HUR 203, HPR 35 and Gujarat Rajmash-1 recorded >75% seed germination even after 2 years of storage. Therefore, it is recommended to store the seeds of these varieties up to 2 years. On the contrary, seeds of none of the varieties studied could be stored for more than 2 years. Finally, these findings would be helpful for farmers and germplasm curator to take appropriate decision regarding storage of seeds and their regenerations strategies, respectively.

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