Indian Journal of Agricultural Sciences **92** (4): 516–20, April 2022/Article
https://doi.org/ 10.56093/ijas.v92i4.124008

Effect of priming treatments on germination and seedling growth of artificially aged seed of guava (*Psidium guajava*)

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Received: 02 September 2021; Accepted: 25 November 2021

ABSTRACT

An experiment was conducted to study the effect of priming treatments on artificially aged seed germination and seedling growth of guava (*Psidium guajava* L.) at Department of Seed Science and Technology, CCS Haryana Agriculture University, Hisar during 2019–20. It is comprised 21 treatments in complete randomize design with three replications. There were five priming methods with different treatments under study i.e. tap water, GA_3 (at 500, 700 and 1000 ppm concentration), KNO_3 (at 0.5 and 1% concentration), thiourea (at 0.5 and 1% concentration) and HCl (at 5% and 10% for 2 and 4 min). Duration of tap water, GA_3, KNO_3 and thiourea soaking was kept 24 and 48 hours. The study revealed that priming the artificially aged seeds in KNO_3 greatly increased germination, especially soaking of artificially aged seeds in KNO_3 at 1% for 48 h which shows up to 45.0% germination. Parameter like germination%, mean germination time, seedling length, seedling dry weight and seedling vigour were positively affected by all the priming treatments. Artificially aged seed viability decreased but the standard germination efficiently increased due to priming treatments. KNO_3 at 1% for 48 h improved germination and resulted in maximum dry weight of seedling (206.0 mg), seedling length (4.16 cm) and vigour index I and II (187.0 and 9271.0) in laboratory. Artificially aged seeds without any priming treatments showed poor growth and germination.

Keywords: Artificially ageing, Guava, Germination, Priming, Seed

Guava (*Psidium guajava* L.) belongs to the family Myrtaceae is one of the most important commercial fruits. It is indigenous to tropical America, but cultivated in all the tropical and subtropical countries of the world. It was introduces by Portuguese in 17th century and cultived since then. It can be grown up to 1500 m. It is commonly known as Apple of tropics and sub-tropics because of having high vitamin A and B and being exceptionally rich in vitamin C (Rai *et al*. 2010). In India, guava occupies an area of 2.90 lakh hectares with production of 43.59 lakh metric tonnes, respectively (Anonymous 2020). A seed is basically a kernel in which a small embryonic plant is covered by a hard seed coat. It contains some stored food, which promotes the growth of the embryo after receiving the signals under appropriate climatic conditions (Thomas *et al.* 2006). Seed lot having same variety, kind, germination and chronological age do not store equally under same condition. Such type of variation in storage potential of seed lots due to difference in their storage history and exposed condition during post maturation period until storage. Deterioration of seed is associated with ageing phenomenon, which is defined as an irreversible degradation in the quality of seed after it has reached specific stage. Seed deterioration start immediately after attaining the physiological maturity on the plant itself. Accelerated ageing is the most important test among all the seed vigor tests to know the longevity of seed. The common effects of seed ageing on the seeds integrity are explained as function of free radical mediated membrane and chromosomal damage (Khan *et al.* 2005) Many seed priming treatments have been used to reduce the damage of aging and invigorate their performance in various crops (Farooq *et al.* 2009).

MATERIALS AND METHODS

The present study was carried out at research form of CCS Haryana Agricultural University; Hisar situated at 215.2 m amsl with coordinates of 29º10′ N latitude and 75º46′ E longitudes during 2019–20. Accelerated ageing seed sample of 200 seeds were placed in a plastic box over a wire mesh tray above 35 ml of water in the bottom. The seeds were spread out on the wire mesh tray in a uniform layer; the box was closed and transferred to an accelerated ageing chamber maintained at 40–45^{0}C and

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high relative humidity (90–100%) for 72 h. The plastic box was removed after the mentioned time period and guava seed were cooled and tested for normal germination test. The decline in accelerated ageing is related to initial degree of seed deterioration of seed lots. There were in total 21 treatments consisting of various concentration and time of soaking using five different materials i.e. T_1-Control, T_2- GA_3 @500 ppm for 24 h , T_3- GA_3@700 ppm for 24 h, T_4- GA_3 @1000 ppm for 24 h, T_5- GA_3 @500 ppm for 48 h, T_6- GA_3 @700 ppm for 48 h, T_7- GA_3 @1000 ppm for 48 h, T_8- KNO_3 @0.5% for 24 h, T_9- KNO_3 @1.0% for 24 h, T_{10}- KNO_3 @0.5% for 48 h, T_{11}- KNO_3 @1% for 48 h, T_{12}- Thiourea @0.5% for 4 h, T_{13}- Thiourea @1% for 24 h, T_{14}- Thiourea @0.5% for 48 h, T_{15}- Thiourea @1.0% for 48 h, T_{16}- Tap water for 24 h, T_{17}- Tap water for 48 h, T_{18}- HCL @5% for 2 min, T_{19}- HCL @10% for 2 min, T_{20}- HCL @5% for 4 min, T_{21}- HCL @10% for 4 min.

The seed quality parameters:

Seed viability (%): 25 seeds of each treatment replicated thrice were soaked in 50 ml water for 16 h at 25ºC to activate dehydrogenase enzymes. After longitudinal sectioning of guava seeds with sharp blade, the seeds were stained in 0.01% tetrazolium solution (2, 3, 5-triphenyl tetrazolium chloride) for 5 h at 38ºC, in petri plates. After that, solution was poured off and seeds were washed in tap water and examined under magnifying glass. The completely red stained seeds were considered as normal viable seeds and expressed in percentage.

Standard germination (%): 25 seeds of each treatment in three replicates were placed on the top of paper and kept at 27 ± 1^0C with 80–85% relative humidity in seed germinator.

The mean germination time (MGT): It was calculated by taking the germination counts from replicates used for standard germination test. The germination counts were taken every 24 h and MGT was calculated according to the formula proposed by Dezfuli *et al*. (2008).

Seedling length (cm): Seedling length was measured on 10 randomly selected normal seedlings in centimeter. At last, average of 10 seedlings was taken for final calculations.

Dry weight per seedling (mg): The ten seedlings selected for measuring seedling length were further kept in hot air oven for taking dry weight. Seedlings were dried in oven at 55 ± 2ºC temperature for 48 h and the seedling dry weight was recorded in milligram. At last, average weight of 10 seedlings was taken for further calculation.

Seedling vigour (cm): For calculation of seedling vigor, the root and shoot vigor were calculated as the sum of total root length (cm) and shoot length (cm) of all the seedlings of a replicate divided by 10. The seedling vigor was expressed as the sum of mean of the root and shoot vigour (Srinivasan and Saxena 2007).

Vigour index (seedling vigour index I and II): It was calculated with the help of method given by Abdul-Baki and Anderson (1973);

Vigour index-I = Standard germination (%) × Average seedling length (cm),

Vigour index-II = Standard germination (%) × Average seedling dry weight (mg)

The data were analyzed with the help of windows based computer package OPSTAT (Sheoran *et al*. 1998), which calculate the critical difference (CD) between the treatments at 5% level of significance.

RESULTS AND DISCUSSION

Different Priming treatments of artificially aged guava seed significantly affected seed viability (Table 1). The data revealed that the un-primed artificially aged guava seed resulted in significantly higher seed viability (51.1%) over other seed priming treatments except KNO_3 @1.0% solution for 48 h, while the minimum seed viability (16.3%) was observed in seed dipped with GA_3 @500 ppm for 24 h. This might be due to cause losing in membrane integrity of artificially aged seed ultimately resulted into less vigour and viability of seed (Singhal *et al*. 2017). The results are in accordance with the findings of (Windaurer *et al*. 2007)

Table 1 Effect of different priming treatments on seed viability (%), germination (%) and days taken for germination (days) of artificially aged guava seedling L-49 under laboratory condition

Treatment	Seed viability (%)	Germination (%)	Days taken for germination (days)
T_1-Control	51.1	3.2	93.0
T_2- GA_3 500 ppm, 24 h	16.3	7.5	88.3
T_3- GA_3 700 ppm, 24 h	22.1	11.5	88.5
T_4- $GA_3$1000 ppm, 24 h	38.1	13.4	86.0
T_5- GA_3 500 ppm, 48 h	22.5	14.7	83.2
T_6- $GA_3$700 ppm, 48 h	37.3	17.4	81.2
T_7- $GA_3$1000 ppm, 48 h	39.9	22.5	77.8
T_8- KNO_3 0.5%, 24 h	38.8	27.7	61.8
T_9- $KNO_3$1.0%, 24 h	44.6	32.2	55.0
T_{10}- KNO_3 0.5%, 48 h	46.1	37.2	54.3
T_{11}- KNO_3 1.0%, 48 h	47.2	45.0	48.7
T_{12}- Thiourea 0.5%, 24 h	26.8	24.5	84.0
T_{13}- Thiourea 1.0%, 24 h	29.0	24.9	85.0
T_{14}- Thiourea 0.5% , 48 h	32.4	32.9	64.3
T_{15}- Thiourea 1.0% , 48 h	22.8	22.2	73.0
T_{16}- Tap water, 24 h	35.5	33.3	66.5
T_{17}- Tap water, 48 h	34.3	26.8	74.8
T_{18}- HCL 5%, 2 min	19.2	15.7	81.8
T_{19}- HCL 10%, 2 min	21.1	17.2	76.3
T_{20}- HCL 5%, 4 min	30.5	17.7	73.8
T_{21}- HCL 10%, 4 min	36.2	21.5	68.7
CD	4.7	3.6	2.8

that seed storage caused a decrease in the protein content, which may be related to oxidation of the amino acid, due to the increase in the respiratory activity and advance in the deterioration process of the stored seeds. Germination per cent data (Table 1) reveals that the seed priming of artificially aged guava seed dipped with KNO_3 @1% for 48 h had significantly higher germination (45%) as compare to all other seed priming treatments. It is due to effectiveness of KNO_3 which could be attributed to successful removal of several lignified layers in the testa, are packed tightly together and contain water-repelling compounds (Baskin 2003). Priming treatment is responsible for repairing the age related cellular and subcellular damage of low vigour seeds that may accumulated during seed development (Bray 1995). Raj *et al.* (2013) studied improvement in germination characteristics in artificially aged seeds of okra (*Abelmoschus esculentus*) by osmo-conditioning. The minimum standard germination percentage of artificially aged seed was observed in control treatment. Among the various reasons, the main are external (irradiation, fungal attack) and internal factors (accumulation of toxic compounds, loss of growth hormones, degradation of genetic material, and proteins or membranes) Priestley (1986). Singh *et al*. (2014) studies osmo-priming with KNO_3 @1% for 6 h resulted into greater seed germination and seedling length in cowpea. These results are in close conformity with the finding of Shim *et al.* (2008) who reported that 0.2% or 0.5% solution of KNO_3 for 72 h is a recommended method that can be practically applied for increasing germination of paspalum Tomato seed, primed with KNO_3 solution and benefits in removing dormancy and promoting faster germination (Lara 2014).

Data pertaining to days taken to seed germination of artificially aged guava seed (Table 1) reveal that seed priming with KNO_3 @1% solution for 48 h (48.7 days) significantly reduced the germination time as compared to all other seed priming treatments. While the maximum days taken for germination (93 days) was observed in control. It might due to priming of seed promote germination by repair of damage proteins, genetic material (Koehler *et al*. 1997) and increase in oxidation of nicotinamide adenine dinucleotide phosphate and increase in oxidation of NADPH (Kumar 2019). A perusal of data (Table 2) exhibited that different seed priming treatments significantly affected seedling length over the control. Results showed that the seed priming with KNO_3 @1% solution for 48 h (4.16 cm) resulted into significantly increase the seedling length over other priming treatments excepts it was statistically at par with KNO_3 @0.5% solution for 48 h. However, the minimum germination percentage of artificially aged seed was in control treatment. Many seed priming treatment have been used to decrease the damage of aging and invigorates their performance in many crops (Farooq *et al*. 1998). The hike in vigor might be due to the direct influence on extensive growth of seedlings probably in increasing mobilization of reserve foods to growing seedlings (lay *et al*. 2013). Similar trend was followed by Demer and Mavi

Table 2 Effect of different priming treatments on seedling length (cm), dry weight of seedling (mg), seedling vigor index I and II of artificially aged guava seedling L-49 under laboratory condition

Treatment	Seedling length	Dry weight of seedling (mg)	Vigor index I	Vigor index II
T_1-Control	0.97	26.3	3.0	84.0
T_2- GA_3 500 ppm, 24 h	1.91	37.1	14.2	279
T_3- GA_3 700 ppm, 24 h	2.14	42.1	24.5	484
T_4- $GA_3$1000 ppm, 24 h	1.98	82.0	26.4	1091
T_5- GA_3 500 ppm, 48 h	1.50	41.7	22.0	611
T_6- $GA_3$700 ppm, 48 h	2.14	60.0	37.0	1037
T_7- $GA_3$1000 ppm, 48	2.90	153.7	65.3	3465
T_8- KNO_3 0.5%, 24 h	3.19	131.2	88.4	3631
T_9- KNO_3 1.0%, 24 h	3.53	185.6	113.6	5951
T_{10}- KNO_3 0.5%, 48 h	3.93	164.1	146.1	6098
T_{11}- KNO_3 1.0%, 48 h	4.16	206.0	187.2	9271
T_{12}- Thiourea 0.5%, 24 h	2.02	94.7	49.3	2324
T_{13}- Thiourea 1.0%, 24 h	1.67	108.8	41.0	2705
T_{14}- Thiourea 0.5%, 48 h	2.95	205.0	96.8	6797
T_{15}- Thiourea 1.0%, 48 h	3.42	152.0	76.0	3365
T_{16}- Tap water, 24 h	3.24	102.0	102.8	3397
T_{17}- Tap water, 48 h	3.12	121.9	83.7	3261
T_{18}- HCL 5%, 2 min	1.50	69.9	23.4	1104
T_{19}- HCL 10%, 2 min	2.04	89.9	34.9	1545
T_{20}- HCL 5%, 4 min	2.21	81.9	39.0	1435
T_{21}- HCL 10%, 4 min	1.28	108.8	27.9	2334
CD	0.34	9.4	13.9	556

(2004) halo primed watermelon seed treated with KNO_3 3% for 6 days found that increase in seedling weight and hypocotyl length. Different Priming treatments significantly affected dry weight per seedling (Table 2) under laboratory conditions. Seed priming treatments significantly influenced dry weight of seedling as compared to control treatment. The data reveals that the seed priming of fresh guava seed with KNO_3 @1% concentration for 48 h (206.0 mg) resulted into significantly increase in the dry weight of seedling as compared to all other seed priming treatments except thiourea @0.5% solution for 48 h. Ageing of seed would increase metabolic activity and consequently reduce the reserve substance content and ultimately reduce the dry material weight of the seeds (Bewley and Black 1982). However, minimum dry weight per seedling (41.7 mg) was observed in control treatment. This suggested that under nutrient deficient conditions the leakage of the membrane is more due to which metabolites and biomolecules leached out and the seed quality deteriorates over the storage interval. Abdelgadir *et al.* (2012) reported that seeds treated with KNO_3 for 24 h produced heavier and longer seedlings which resulted in increased vigour indices of *Jatropha curcas* seeds. Similar trend was followed by Vazirimehr *et al*. (2014) stated that KNO_3 @1% decrease the time of seed emergence and increase the dry matter in corn. Data (Table 2) reveals that the seed priming with KNO_3 @1% solution for 48 h resulted into significantly highest vigor index-I (187.0) and vigor index-II (9271.0) as compare to all other priming treatments. The lowest vigour index I (3.0) and vigour index II (84.0) was recorded in control. Seedling vigour is not a single measurable entity, but it is a sum of many growth parameters, such as seedling fresh weight, seedling dry weight and seedling length. Deshraj (2002) also reported the vigour index-I and vigor index-II were positively and significantly correlated with germination per cent, seedling length, seedling dry weight in coriander. Khan *et al.* (2005) founded that the turnip seed showed a decrease in seedling vigour as accelerated ageing duration increased. Similar results were also reported in papaya by Tokuhisa *et al.* (2007) as they reported the seedling vigour index II to be significantly highest in seed treated with KNO_3 at 0.5% concentration for 48 hours. Similar findings were also reported by Akbudak and Bolkan (2010) in tomato crop. Golizadeh *et al*. (2015) reported that seed vigour index was higher in Cannabis seed (*Cannabis Sativa* L.) primed with KNO_3 over the non-primed seed. As seedling vigour is dependent on germination percentage and seedling length, the seeds treated with potassium nitrate had highest per cent of germination and also recorded the highest height. Abdelgadir *et al.* (2012) reported that seeds treated with KNO_3 for 24 h produced heavier and longer seedlings which resulted in increased vigour indices of seeds of *Jatropha curcas*.

On the basis of current experimental findings, it can be concluded that seed priming can play a vital role in improving germination in artificially aged guava seeds of L-49. Various priming treatments were found effective and had a significant effect on promoting the growth and development of guava artificially aged seeds. Priming of artificially aged guava seeds with Potassium Nitrate (KNO_3) gave best results, especially soaking of artificially aged seed in KNO_3 @1% for 48 h with respect to seed germination percentage, days to emergence, mean germination time, seed vigour index I, II and growth of seedlings.

ACKNOWLEDGEMENT

Chaudhary Charan Singh Haryana Agricultural University, Hisar is duly acknowledged to provide the financial support and technical staff along with the labour during the experiment.

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