



Effect of scarification treatments on seed germination of guava (*Psidium guajava*)

SOURABH, PREETI*, JEET RAM SHARMA and AXAY BHUKAR

CCS Haryana Agricultural University, Hisar, Haryana 125 004, India

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ABSTRACT

The present study was carried out to investigate the effect of pre-sowing and scarification treatments on seed germination and seedling growth of guava (*Psidium guajava* L.) in the laboratories of the Department of Seed Science and Technology, CCS HAU, Hisar during the year 2015. There were three scarification methods with different treatments under study, i.e. water soaking (for 24, 48 and 72 hr), hot water soaking (at 70, 80, 90 and 100°C) and sulphuric acid soaking (at 10, 20, 30, 40 and 50% concentration). Duration for hot water soaking and sulphuric acid soaking was kept as quick dip (5 seconds), 1 min. and 3 min. The study revealed that pre soaking the seeds in hot water and sulphuric acid greatly increased germination, especially soaking of seeds in 20% sulphuric acid solution for 3 min., which showed up to 72% germination. Parameters like germination%, mean germination time, seedling length and seedling vigour were positively affected by all the scarification treatments. Seed viability decreased but the standard germination efficiently increased due to scarification treatments. Hot water soaking at 80°C temperature for 1 min. improved germination and resulted in maximum seedling length (6.03 cm) in laboratory. Seeds treated with higher sulphuric acid concentrations or soaked in hot water at higher temperature showed poor germination as well as poor vigour. Seeds without any scarification treatment showed poor germination.

Key words: Dormancy, Guava, Hot water treatment, Scarification, Sulphuric acid

Guava (*Psidium guajava* L.) is an important fruit crop of tropical and sub-tropical regions of world. Being a good source of Vitamin C, A, B, Iron and Phosphorus, guava is called apple of poor. In India, guava occupies an area of 2.62 lakh ha with production of 36.48 metric tonnes (Saxena and Gandhi 2017). Various propagation methods, viz. veneer grafting, stooling, wedge grafting etc. are employed to obtain quality saplings. However, to fulfill increasing demand of quality rootstocks, seedlings need to be raised from seeds and guava seeds suffer physical dormancy due to hard seed coat and impermeability to water and gases. Different methods like water soaking, scarification and chemical treatments are used for breaking seed dormancy to enhance germination and seedling growth. It has been reported that chemical and mechanical scarification can hasten the imbibition of water by making hard seeds permeable (Harb 2013). Seed imbibition is crucial phase for successful germination as ample water is necessary to rehydrate enzymes and their substrates in preparation for seed germination. Although scarification methods such as pre-soaking in water and different acids (Pandey and Singh 2000) have been previously tested, the effectiveness of different treatment durations and optimum concentrations

for seed germination has not been widely reported.

With increasing market demand of guava by consumers and processing industries; the demand of budded and grafted guava plants by fruit growers has spiked interest. The main hindrance in guava orchard establishment is poor availability of quality planting material due to non-availability of good quality rootstocks. As rootstocks are raised on seedling, it necessitates the study on scarification effects in guava seeds to increase seed germination and facilitate healthy seedling development. The variety chosen (L-49) for the study is commercially important because of its high yield, better fruit quality and ease in processing. This study attempted to break seed coat dormancy in guava using pre-soaking in water, acids and hot water scarification and their effects on seed quality parameters.

MATERIALS AND METHODS

The laboratory experiments were conducted at seed testing laboratory of the Department of Seed Science and Technology, CCS Haryana Agricultural University, Hisar, Haryana, India (29°10' N, 75°46' E) situated at 215.2 m above sea level. Healthy guava seeds were extracted from ripe fruits of Mrig Bahar crop (flowers in June-July, fruiting in November-December) in the month of March 2015. Seeds were separated from fleshy mesocarp, thoroughly washed and shade dried to 15% moisture. They were immersed for

Corresponding author e-mail: preeti.hau@gmail.com

10 min. in 1% w/v NaOCl for surface sterilization, rinsed thoroughly in slow running tap water for about 3 min., surface dried and stored at room temperature.

Sample size of 100 seeds replicated thrice were subjected to different treatments. There were three scarification methods, i.e. water soaking (for 24, 48 and 72 hr), hot water soaking (at 70, 80, 90 and 100°C) and sulphuric acid soaking (at 10, 20, 30, 40 and 50% dilution). Duration for hot water soaking and sulphuric acid soaking was kept as quick dip (5 seconds), 1 min. and 3 min. After treatment, seeds were thoroughly rinsed under tap water to make sure no acid was left on seeds which may hinder the germination process. The methods and formulas applied to measure various parameters are as follows-

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

For standard germination (%) test hundred seeds of each treatment in three replicates were placed in between sufficient moistened rolled towel papers and kept at 27±1°C with 80-85% relative humidity in seed germinator. The final count was taken on 26th day and only normal seedlings were considered for germination % as per the rules of ISTA (2001).

The mean germination time (MGT) was calculated by taking the germination counts every 24 hr from replicates used for standard germination test and calculated by using the formula proposed by Moradi Dezfuli *et al.* (2008).

Seedling length was measured on 10 randomly selected normal seedlings and recorded in cm. At last, average of 10 seedlings was taken for final calculations.

Dry weight per seedling (mg) was measured by hot air oven method. Samples were dried in oven at 65 ± 2°C temperature for 48 hr and the seedling dry weight was recorded in milligram. At last average weight of 10 seedlings was taken for further calculation.

For calculation of seedling vigour, the root and shoot vigour 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 vigour was expressed as the sum of mean of the root and shoot vigour (Srinivasan and Saxena 2007). Seedling vigour index I and II were calculated with the help of method given by Abdul-Baki and Anderson (1973).

Statistical analysis- The experiment was conducted in a Completely Randomized Design suitable for laboratory parameters. The data were subjected to the statistical analysis on the basis of the model described by Ostle and Mensing (1975) and Gomez and Gomez (1984). The data

presented in this research paper are the mean values of different parameters.

RESULTS AND DISCUSSION

Effect on seed viability: Different scarification treatments affected seed viability depending on their duration and concentration. Seed viability ranged from 31.1 to 65.6%. The maximum seed viability (82.7%) was found in seeds which were devoid of any treatment (control) which was statistically at par with 24 hr soaking in water and quick dip in 70°C hot water treatment (77.3%). Except these two treatments, seed viability decreased significantly as compared to control treatment. The minimum seed viability (26.7%) was recorded when seeds were soaked in 50% sulphuric acid solution for 3 min. The decrease in viability by various scarification treatments may be due to over exposure of the seeds to scarification treatments. Sulphuric acid have a negative effect as it can end up damaging the seed, when the acid can penetrate into the seed via its exposed micropyle. The results are in accordance with the findings of Musara *et al.* (2015) in okra. Hot water soaking at higher temperature (90°C and 100°C) for longer duration may also damage the seed embryo and viability is reduced.

Standard germination (%): Under different scarification treatments standard germination (%) of guava ranged from 20.7 to 72%. The maximum standard germination (72.0%) was observed with 3 min. soaking of seeds in 20% sulphuric acid solution that was statistically at par with quick dip and 1 min. soaking in 30% sulphuric acid solution (70.0 and 71.3%, respectively) while the minimum standard germination (20.7%) was observed with 3 min. soaking in 50% sulphuric acid solution (Table 1). Water, hot water soaking at 70°C, 80°C and 90°C, 10, 20 and 30% sulphuric acid soaking and 40% sulphuric acid soaking (quick dip) significantly improved the standard germination of guava seed. Whereas, treatment of seeds with 50% sulphuric acid, significantly decreased the standard germination.

The effectiveness of sulphuric acid could be attributed to successful removal of several lignified layers in the testae, which are packed tightly together and contain water repelling compounds (Baskin 2003). Acid scarification causes softening of hard seed coat which in turn makes the seed permeable to water and gases and induces germination. These results are in close conformity with the findings of Brijwal *et al.* (2013) as they reported that soaking of guava seeds in sulphuric acid for 3 minutes improved the germination counts over control. Ali *et al.* (2007) also reported higher guava seed germination (85%) using 50% sulphuric acid for 12 hr. They reported negative effect of increased acid concentration on seed germination which explains the lower germination percentage at higher sulphuric acid concentration. The effectiveness of boiling water treatment in enhancing germination has been attributed to the release of physical dormancy from hard seeded species by causing ruptures in the seed wall thereby allowing imbibition, for diffusion and germination to occur (Maslin and McDonald 2004, Sedbrook 2006). The decline

Table 1 Effect of scarification treatments on guava seed viability and germination parameters

Treatment		Seed viability (%)	Standard germination (%)	Mean germination time (days)
<i>Water soaking</i>				
	24 h	77.3 (61.6)	46.7 (43.1)	21.4 (4.73)
	48 h	73.7 (59.1)	50.0 (45.0)	17.3 (4.28)
	72 h	71.3 (57.6)	42.0 (40.4)	16.9 (4.23)
<i>Hot water soaking</i>				
70°C	QD (quick dip)	77.3 (61.6)	54.0 (47.3)	19.0 (4.47)
	1 min	72.0 (58.1)	52.0 (46.1)	17.9 (4.34)
	3 min	69.3 (56.4)	42.0 (40.4)	18.9 (4.46)
80°C	QD	71.7 (57.8)	58.0 (49.6)	14.5 (3.93)
	1 min	68.0 (55.5)	62.0 (51.9)	15.1 (4.02)
	3 min	56.0 (48.4)	64.0 (53.1)	14.2 (3.89)
90°C	QD	64.0 (53.1)	60.7 (51.1)	14.5 (3.93)
	1 min	60.0 (50.8)	46.0 (42.7)	16.1 (4.14)
	3 min	49.3 (44.6)	42.7 (40.8)	18.7 (4.44)
100°C	QD	49.3 (44.6)	40.0 (39.2)	18.8 (4.45)
	1 min	45.0 (42.1)	37.7 (37.8)	20.0 (4.59)
	3 min	40.0 (39.2)	32.7 (34.8)	20.1 (4.59)
<i>Sulphuric acid soaking</i>				
10%	QD	76.0 (60.7)	40.0 (39.2)	19.7 (4.55)
	1 min	69.7 (56.6)	48.0 (43.8)	18.6 (4.43)
	3 min	66.3 (54.5)	52.0 (46.1)	15.3 (4.04)
20%	QD	75.3 (60.2)	52.0 (46.1)	17.3 (4.28)
	1 min	69.7 (56.6)	56.0 (48.4)	16.6 (4.20)
	3 min	66.7 (54.7)	72.0 (58.0)	14.1 (3.89)
30%	QD	74.7 (59.8)	70.0 (56.8)	11.5 (3.53)
	1 min	66.7 (54.7)	71.3 (57.6)	14.9 (3.99)
	3 min	58.0 (49.6)	60.7 (51.1)	15.2 (4.03)
40%	QD	49.3 (44.6)	38.7 (38.4)	16.0 (4.13)
	1 min	38.7 (38.4)	32.7 (34.8)	18.9 (4.46)
	3 min	33.0 (35.0)	30.7 (33.6)	18.5 (4.42)
50%	QD	44.0 (41.5)	28.7 (32.4)	19.4 (4.52)
	1 min	33.3 (35.2)	24.7 (29.8)	20.1 (4.60)
	3 min	26.7 (31.1)	20.7 (27.0)	20.5 (4.64)
Control		82.7 (65.6)	34.7 (36.1)	24.9 (5.09)
CD (P=0.05)		6.4 (4.0)	3.4 (2.05)	1.3 (0.15)

of germination could be attributed to embryo damage caused by wet heating or probably due to low oxygen availability at high temperature which resulting in destruction of certain enzymatic components (Teketay 1998).

Mean germination time (days): The minimum mean germination time (11.5 days) was recorded with quick dip soaking in 30% sulphuric acid solution treatment. The maximum mean germination time (24.9 days) was observed in control (Table 1). Mean germination time decreased significantly with each scarification treatment

and it varied from minimum in seeds treated with quick dip 30% sulphuric acid and maximum in control. This was possibly due to injuring the hard seed coat by sulphuric acid to accelerate the water absorption and to improve the gaseous exchange for hastening the process of germination (Nayak and Sen 1999).

Seedling length (cm): Results showed that water soaking of seeds significantly increased the seedling length over control (Fig 1). Length of guava seedlings ranged from 1.45 cm to 6.03 cm. Maximum seedling length (6.03 cm) was recorded in seeds treated with 80°C hot water soaking for 1 min. duration and minimum seedling length (1.45 cm) was recorded in seeds treated with 50% sulphuric acid soaking for 3 min. duration. The maximum seedling length with 80°C hot water soaking for 1 min. may be attributed to stimulated sub-subsequent germination. The results are in accordance with the findings of David and Midcap (2007) in woody ornamentals.

Dry weight per seedling (mg): The maximum dry weight per seedling (320.0 mg) was observed with quick dip in 30% sulphuric acid while the minimum dry weight per seedling (90.7 mg) was observed with 3 min. soaking in 50% sulphuric acid solution (Fig. 1). Quick dip, 1 min. and 3 min. soaking in 20 and 30% and quick dip in 40 and 50% sulphuric acid solution significantly increased the dry weight per seedling as compared to control. The reason behind this may be the corresponding seedling height with corresponding treatments.

Seedling vigour: The influence of different scarification treatments on relative seedling vigour reveals that under different scarification treatments seedling vigour in laboratory varied from 2.63 to 8.12. Maximum seedling vigour (8.12) was observed with quick dip in 30% sulphuric acid solution (Fig 1). Minimum seedling vigour (2.63) was observed with 3 min. soaking in 50% sulphuric acid solution. Results showed that water soaking of seeds significantly increased the seedling vigour over control. Quick dip and soaking for 3 min. in hot water at 70°C non-significantly decreased the seedling vigour, while, 1 min. soaking increased seedling vigour significantly as compared to control. Soaking in 80°C and 90°C hot water significantly increased the seedling vigour except 3 min. soaking in 90°C hot water. Quick dip, 1 and 3 min. soaking in 100°C hot water significantly decreased seedling vigour as compared to control. Quick dip in 10% sulphuric acid significantly increased the seedling vigour, while, effect was non-significant in 1 and 3 min. soaking. Quick dip and soaking in 20, 30 and 40% sulphuric acid solution significantly increased the seedling vigour except 3 min. soaking in 40% sulphuric acid solution. Quick dip and soaking in 50% sulphuric acid solution significantly decreased the seedling vigour as compared to control. Similar results were reported in aonla by Nayaka (2006) as he reported that acid scarified seeds gave maximum germination and had very high seedling vigour as compared to control.

Vigour index I and II: Both the vigour indices followed the same trend of maximum and minimum in all the

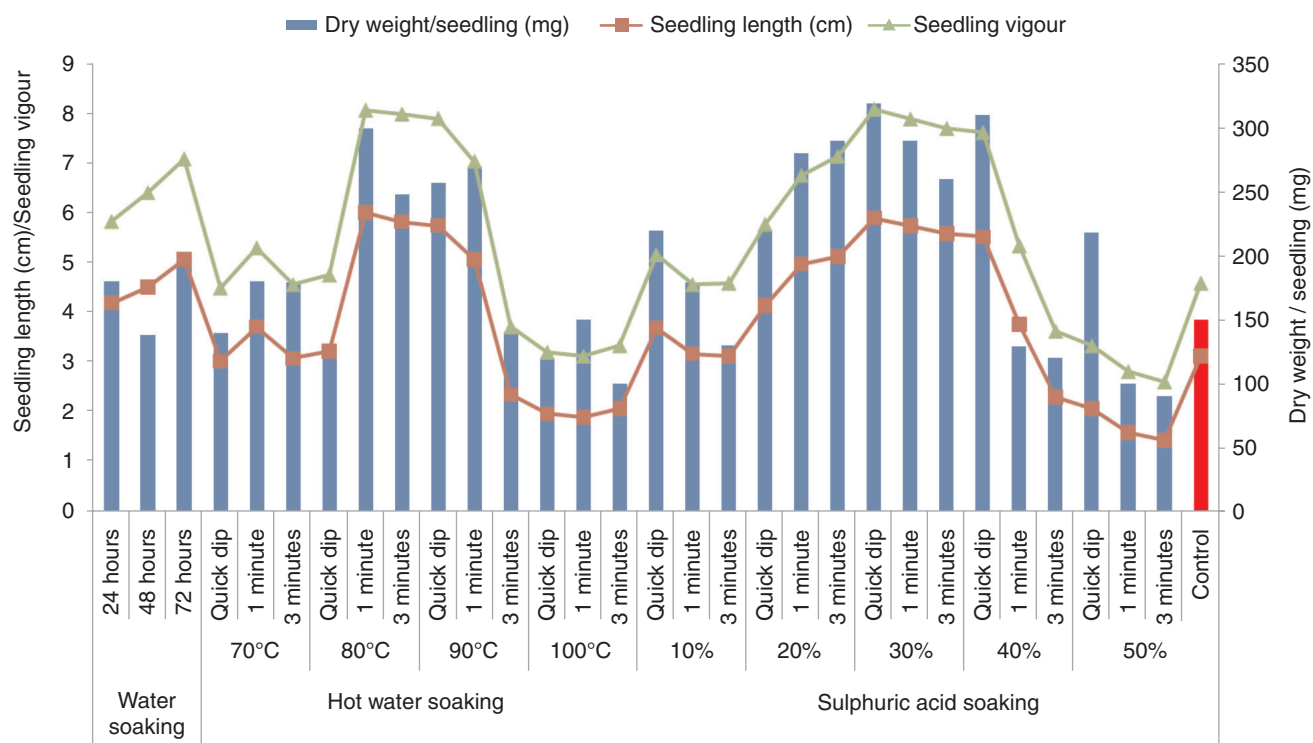


Fig 1 Effect of different scarification treatments on dry weight per seedling, shoot length and seedling vigour.

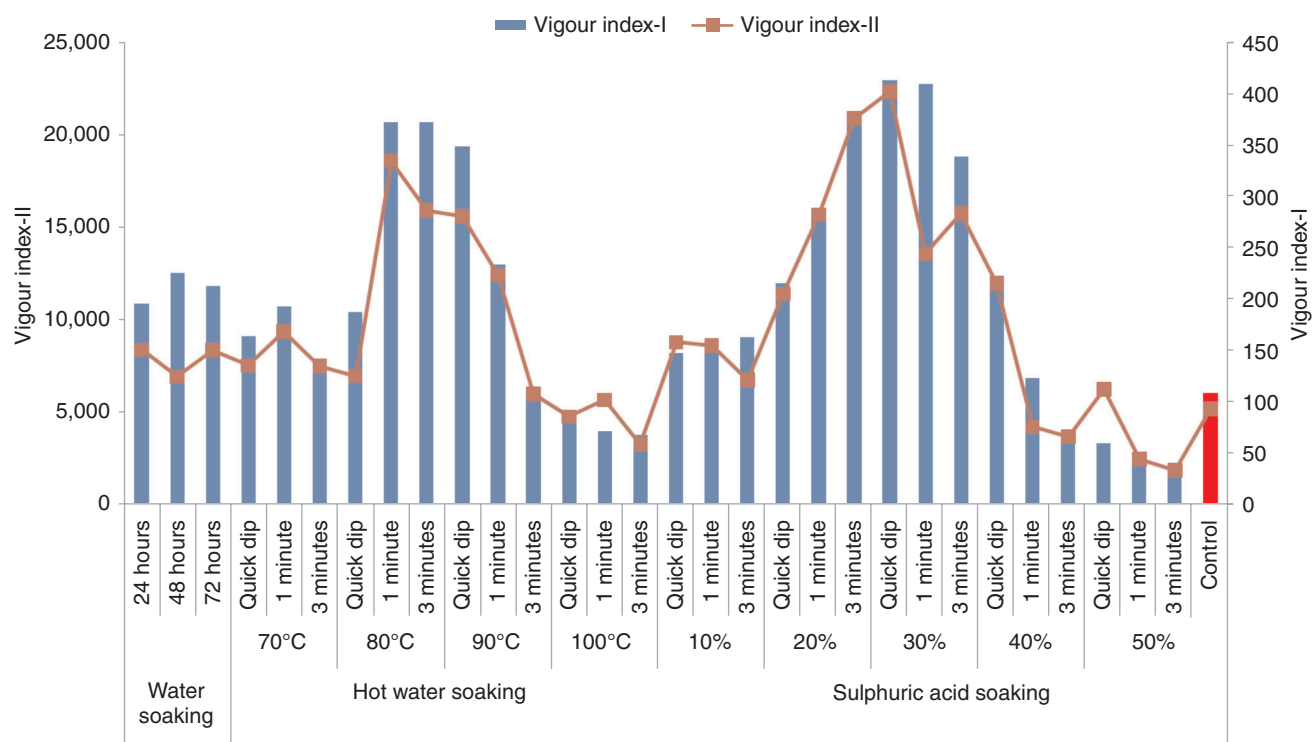


Fig 2 Effect of different scarification treatments on vigour index I and vigour index II.

treatments. Seeds treated with 30% sulphuric acid soaking as quick dip duration had maximum vigour index I (414.7) and vigour index II (22404). Minimum vigour index I (30.0) and vigour index II (1878) was recorded in seeds treated with 50% sulphuric acid soaking for 3 min. duration.

This may be due to their corresponding standard germination, seedling length and seedling dry weight. Similar result was reported in aonla by Nayaka (2006) as he reported that sulphuric acid scarified seeds gave maximum germination, took minimum number of days for

50% germination and had very high seed vigour indices as compared to control.

Conclusion

On the basis of present findings, scarification of guava seeds with 30% concentrated sulphuric acid for 5 seconds, resulted in maximum germination performance and comparatively higher seed viability. Hot water soaking at 80°C for 1 min. improved germination and resulted in maximum seedling length (6.03 cm). Untreated seeds and seeds treated with higher sulphuric acid concentrations or soaked in hot water at higher temperature showed poor germination as well as poor growth.

REFERENCES

- Abdul-Baki A A and Anderson J D. 1973. Vigour determination in soybean seed by multiple criteria. *Crop Science* **13**: 630–632.
- Ali N, Mulwa R M S, Morton M A and Skirvin R M. 2007. Radical disinfestation protocol eliminates *in vitro* contamination in guava (*Psidium guajava* L.) seeds. *Plant Cell Tissue Organ Culture* **91**: 295–298.
- Baskin C C. 2003. Breaking physical dormancy in seeds – focusing on the lens. *New Phytologist* **158**: 227–238.
- Brijwal M, Kumar R and Mishra D S. 2013. Effect of pre-sowing treatments on seed germination of guava (*Psidium guajava* L.) under Tarai region of Uttarakhand. *Progressive Horticulture* **45**(1): 154–159.
- David F H and Midcap J T. 2007. *Seed Propagation of Woody Ornamentals*. University Press, University of Florida, Florida, USA.
- Gomez K A and Gomez A A. 1984. *Statistical Procedures for Agricultural Research*, 2nd edn, p 680. John Wiley and Sons, New York.
- Harb A M. 2013. Reserve mobilization, total sugars and proteins in germinating seeds of durum wheat (*Triticum durum* Desf.) under water deficit after short period of imbibition. *Jordan Journal of Biological Sciences* **6**: 67–72.
- ISTA. 2001. Rules amendments 2001. *Seed Science and Technology* **29**(supplement): 13–33.
- Maslin B R and McDonald M W. 2004. *Acacia search: evaluation of Acacia as a woody crop option for southern Australia*. Rural Industries Research and Development Corporation, Barton, ACT.
- Moradi Dezfouli P, Sharif-zadeh F and Janmohammadi M. 2008. Influence of priming techniques on seed germination behavior of maize inbred lines (*Zea mays* L.). *ARP Journal of Agricultural and Biological Science* **3**(3): 22–25.
- Musara, C, Chitamba J and Nhuvira C. 2015. Evaluation of different seed dormancy breaking techniques on okra (*Abelmoschus esculentus* L.) seed germination. *African Journal of Agricultural Research* **10**(17): 1952–1956.
- Nayak G and Sen S K. 1999. Effect of growth regulators, acid and mechanical scarification on germination of bael (*Aegle marmelos* C.). *Environment and Ecology* **17**(3): 768–769.
- Nayaka G. 2006. 'Propagation studies in aonla (*Phyllanthus emblica* L.)'. MSc thesis, University of Agricultural Science, Dharwad, Karnataka, India.
- Ostle B and Mensing R W. 1975. *Statistics in Research. Basic Concepts and Techniques for Research Workers*, 3rd edition. Ames: IOWA State University Press, USA.
- Pandey D and Singh G. 2000. Effect of seed pretreatment on promotion of germination in guava. *Annals of Agricultural Research* **21**: 279–281.
- Saxena M and Gandhi C P. 2017. Indian Horticulture Database, National Horticulture Board, Ministry of Agriculture, Government of India, India.
- Sedbrook J. 2006. *Gardening and Horticulture*. Denver County Cooperative Extension, Colorado, USA.
- Srinivasan K and Saxena S. 2007. Removal of tenacious glumes enhances the seed germination and seedling vigour in eight *Aegilops* species for efficient conservation in genebank. *Indian Journal of Agricultural Sciences* **83**(4): 437–40.
- Teketay D. 1998. Germination of *Acacia origina*, *A. pilispina* and *Pterolobium stellatum* in response to different pre-sowing seed treatments, temperature and light. *Journal of Arid Environment* **38**: 551–560.