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Effect of sterilizing agent and seed treatment method on *In vitro* seed germination of guava cv. Arka Kiran

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ABSTRACT

The *in vitro* germination of seeds of guava cv. Arka Kiran was mainly affected by contamination and seed coat dormancy. For successful *in vitro* germination of guava seeds, contamination control and breaking of seed dormancy are the two main steps one should fallow. An experiment on "Effect of sterilizing agent and seed treatment method on *in vitro* seed germination of guava cv. Arka Kiran" was carried out at College of Horticulture, Sirsi, Uttar Kannada, Karnataka during 2021-2023. The study revealed significant influence of different sterilizing agents and seed treatment method on contamination and germination parameters. The seeds sterilized with $HgCl_2$ (0.05%) for 6 minutes showed minimum contamination (9.81%) and the seeds treated with 10% HCl for two minutes reported maximum germination (95.52%) and minimum days taken for germination (14.93).

Key words: *Guava, Arka kiran, Sterilizing agent, Contamination and Germination.*

Introduction

Guava (*Psidium guajava* L.) is one of the most common and important commercial fruit crops cultivated in both subtropical and tropical regions of the world. The major share of the fruits production in India is utilized for fresh consumption. Genus *Psidium* have about 150 species, most of which are fruit bearing trees. It is native to tropical America, stretching from Mexico to Peru (Singh, 2016). It is widely adopted and tolerate in frost, drought and saline conditions. It has good amount of ascorbic acid, dietary fibres, vitamin-A (about 250 IU/100 g), pectin, calcium, phosphorus, iron and other nutrients. The roots, bark, leaves and green fruits are used as medicine for gastrointestinal problems, diarrhoea and dysentery (Rathore, 1976). In India, it has become an important fruit crop, contributing 0.4 per cent of total fruit production with estimated production of 4.05 million tons from 0.26 million ha (NHB, 2018). Major guava producing states are Maharashtra, Bihar, Odisha, Uttar Pradesh, Gujarat, Madhya Pradesh, Karnataka, West Bengal and Tamil Nadu (Singh, 2007).

Arka Kiran variety of guava is gaining importance in nowadays mainly due to semi vigorous, prolific and precocious bearing nature, yields medium sized fruits (200-220g) with firm, deep red

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pulp and with high lycopene content (7.14 mg/100g). Fruits having TSS of 11-12^0 Brix, good vitamin C (190-200 mg/100g) with medium soft seeds. Crop will come to harvest after two years of planting. Economic yield starts after five years with average fruit yield of 20 ton per acre in a spacing of 4m × 3m.

Contamination and seed coat dormancy are the main factor that hinders the *in vitro* germination of guava seeds. For successful *in vitro* germination of guava seeds, contamination control and breaking of seed dormancy are the two main steps one should fallow. Some workers used different sterilizing agents and seed treatment methods to reduce contamination and improve seed germination in guava respectively. Not much work was recorded in cultivar Arka Kiran. Hence, the investigation was carried out College of Horticulture, Sirsi, Uttar Kannada, Karnataka to study the effect of sterilizing agent and seed treatment method on *in vitro* seed germination of guava cv. Arka Kiran.

Materials and Methods

The freshly extracted seeds (cv. Arka Kiran) were washed in running tap water for 3-4 times and seeds were treated with different seed treatment methods followed by treated seeds were sterilized by using different sterilizing agents at different exposure time under laminar hood and washed with sterile water for five times. Thoroughly washed seeds were placed in culture bottle containing basal half MS media without any growth regulators and allowed to germinate. After finalizing the best sterilizing agent and seed treatment method, further 500 mg/l activated charcoal was added to MS media for better germination and growth of seedling. The observations taken were contamination, days taken for germination and germination (%). To access the effect of sterilizing agents on contamination of seeds, the experimental design used was completely randomized design with seven treatment and three replication. The outcome was recorded at 30 days of inoculation which is presented below.

Treatment details

T_1: Control
T_2: 0.05% $Hgcl_2$ for 2 minutes
T_3: 0.05% $Hgcl_2$ for 4 minutes
T_4: 0.05% $Hgcl_2$ for 6 minutes
T_5: 70% Ethyl alcohol for 1 minutes
T_6: 70% Ethyl alcohol for 2 minutes
T_7: 70% Ethyl alcohol for 3 minutes

To access the effect of seed treatment on germination per cent of seeds, the experimental design used was completely randomized design with three treatment and seven replication and the seeds were observed up to 90 days for germination study.

Treatment details

T_1: Control (12 hours soak in distilled water)
T_2: 10% HCl for 2 minutes
T_3: 500ppm GA_3 (12 hours soak)

Contamination and germination (%) was calculated based on the following formula.

$$\text{Contamination (\%)} = \frac{\text{Total number of explants contaminated}}{\text{Total number of explants inoculated}} \times 100$$

$$\text{Germination (\%)} = \frac{\text{Total number of seeds inoculated}}{\text{Total number of seeds germinated}} \times 100$$

Statistical analysis

The data recorded for all the parameters was statistically analysed (ANOVA) by following the completely randomized design (CRD) at 1% level of significance. The analysis has been done in Web Agri-Stat Package (WASP 2.0) developed by ICAR Research Complex, Goa.

Results and Discussion

Effect of sterilizing agents on contamination of seed

The different sterilizing agents showed significant influence on contamination per cent in seeds (Table 1) and (Plate 1). The contamination per cent at 30 days of inoculation was minimum (9.81%) in seeds sterilized with $HgCl_2$ (0.05%) for 6 minutes (T_4) followed by $HgCl_2$ (0.05%) for four minutes (T_3) and the maximum contamination (100%) was recorded in control and ethyl alcohol at one minute (T_5). The sterilization with ethyl alcohol was not showed superior results than $HgCl_2$. Similarly, Khattak *et al*. (1990) reported minimum contamination in guava seed when they are sterilized with $HgCl_2$ (0.05%) for five minutes. Yadav and Singh (2011) reported minimum contamination (30%) when seeds were treated with $HgCl_2$ (0.1%) for five minutes in *Albizia lebbeck*. Ali and Mirza (2006) reported minimum contamination in seeds when they were sterilized with $HgCl_2$ (0.1%) for 5 minutes in rough lemon. Similar sterilization procedure was also followed by Shah *et al*. (2008) and Zamir *et al*. (2004) in guava.

The different sterilizing agents showed signifi-

Table 1. Effect of sterilizing agents on contamination and germination per cent of seeds of guava cv. Arka Kiran

Sl. No.	Treatments		Contamination (%)	Germination (%)
1.	T_1 -	Control	100 (90.00)[a]	0 (0.28)[f]
2.	T_2 -	0.05% $Hgcl_2$ for 2 minutes	68.33 (55.94)[d]	13.85 (21.84)[b]
3.	T_3 -	0.05% $Hgcl_2$ for 4 minutes	15.14 (22.89)[e]	43.15 (41.06)[a]
4.	T_4 -	0.05% $Hgcl_2$ for 6 minutes	9.81 (18.24)[f]	6.37 (14.58)[d]
5.	T_5 -	70% Ethyl alcohol for 1 minutes	100 (90.00)[a]	0 (0.28)[f]
6.	T_6 -	70% Ethyl alcohol for 2 minutes	94.35 (76.28)[b]	4.79 (12.63)[e]
7.	T_7 -	70% Ethyl alcohol for 3 minutes	86.21 (68.58)[c]	11.80 (20.09)[c]
		SEm ±	0.47	0.37
		LSD at 0.01%	2.01	1.57
		CV (%)	**1.37**	**1.09**

cance influence on seed germination per cent of seeds (Table 1). The germination per cent at 30 days of inoculation was maximum (78.06%) in seeds sterilized with 0.05% $HgCl_2$ for 4 minutes (T_3) and minimum (0%) in control (T_1). The results are similar with the findings of Khattak *et al*. (1990). They reported maximum *in vitro* germination (80%) in $HgCl_2$ (0.05%) for 5 minutes in guava. Yadav and Singh (2011) reported maximum germination (80%) when seeds were treated with $HgCl_2$ (0.1%) for five minutes in *Albizia lebbeck*. Contamination was significantly reduced with increasing concentration of 0.05% $HgCl_2$ but at the same time, it affects the germination rate of the seeds. Very less germination at high exposure time (6 minutes) of $HgCl_2$ (0.05%) was may be due to toxic effect of mercuric chloride on embryo (Narayanaswamy, 1999).

Effect of seed treatments on germination (%)

The different seed treatments showed significant influence on germination per cent (Table 2) and (Plate 1). The germination (%) at 30 days of inoculation was maximum (95.52%) in seeds treated with 10% HCl for two minutes treatment (T_2) and minimum (48.75%) was recorded in control (T_1). Similar to this, Ali *et al*. (2007) reported maximum germination (88%) in seeds that were treated with HCl (10%) for 24 hours in guava. In an another study, Butt *et al*. (2013) reported maximum germination (98.9 and 97.6% in round and pyriformed cultivar, respectively) in seeds that were treated with HCl (15%) for 12 hours in guava and stated that further increase in concentration of HCl was reduced the germination per cent in guava. The seeds of *Withania somnifera* and *Withania coagulans* showed maximum germination (80.2 and 72.6 per cent, respectively) when they were treated with HCl (20%) for 20 minutes (Sharma *et al*., 2015). In contrast to this, Bhattacharya and Khuspe (2001) reported maximum germination (79%) when the seeds were treated with 200 ppm GA_3 for 24 hours in Washington cultivar of papaya.

The different seed treatments showed significant influence on days taken for germination (Table 2). The days for germination in this study was minimum (14.93) in HCl (10%) for two minutes (T_2) treatment and maximum (26.48) was recorded in control (T_1) (12 hours soak in distilled water) treatment. Similarly, Ali *et al*. (2007) reported early germination (15days) in seeds that were treated with HCl (10%) for 24 hours in guava. Sharma *et al*. (2015) reported early germination (28 days) in *Withania* seeds when they were treated with HCl (20%) for 20 minutes.

Table 2. Effect of seed treatments on germination per cent and days taken for germination guava cv. Arka Kiran

Sl. No.	Treatments		Germination (%)	Days taken for germination
1.	T_1 -	Control (12 hours soak in distilled water)	48.75[c]	26.48[c]
2.	T_2 -	10% HCl for 2 minutes	95.52[a]	14.93[a]
3.	T_3 -	500 ppm GA_3 (12 hours soak)	68.75[b]	18.81[b]
		SEm ±	0.40	0.30
		LSD at 0.01%	1.58	1.10
		CV (%)	**1.45**	**3.56**

Values with the same letter are stastically non-significant at LSD (*p d" 0.01*).

Plate 1. Effect of seed treatment and sterilizing agents on germination (%) and contamination (%) of seeds of guava *cv.* Arka Kiran. A- 10% HCl for 2 minutes showing maximum germination, B- Contamination of seeds and C- *In vitro* germinated seedlings showing better growth with incorporation of 500 mg/l activated charcoal to half MS media.

The values given in parenthesis are arc sine transformed in contamination (%) and germination (%). Values with the same letter are statistically non-significant at LSD ($p \geq 0.01$).

Conclusion

The $HgCl_2$ (0.05%) for four minutes performed better in controlling contamination of seeds when compared to all other sterilizing agents and *in vitro* germination of guava seeds can be enhanced when seeds were treated with HCl (10%) for 2 minutes and also recorded minimum days taken for germination.

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