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Research Article

STUDIES OF PRE-SOWING SEED TREATMENTS ON GERMINATION AND SEEDLING GROWTH OF GUAVA

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Abstract: An investigation was carried out at Fruit Nursery and Analytical laboratory, Department of Fruit Science, Dr Panjabrao Deshmukh Krishi Vidyapeeth, Akola during the year 2020-21 with objective, to study the effect of physical and chemical treatments on higher seed germination and seedling growth of guava and to find out the suitable pre-sowing physical and chemical treatment for higher seed germination and seedling growth of guava. The experiment was laid out in Factorial Completely Randomized Design (FCRD) with two factors, as Factor 'A' constitutes physical treatment *i.e.*, scrapping of seed coat with sand paper and without scrapping of seed coat with sand paper and Factor 'B' constitutes chemical treatment *i.e.*, soaking seeds for 24 hrs in different chemical solutions of different concentrations *viz.*,GA_3 @ 500 ppm, GA_3 @ 1000 ppm, Thiourea @ 2000 ppm, Thiourea @ 4000 ppm and Tap water with ten treatment combinations and replicated thrice. The observations in respect of germination and growth parameters were recorded from the initial days of germination to periodically at 30 day's interval up to 90 days after sowing. The results of an investigation indicated that, germination and growth of guava significantly influenced by physical treatment *i.e.*, scrapping of seed coat with sand paper and showed early germination and better response for germination percentage, vigour index, height of seedling, number of leaves per seedling, diameter of stem, length of shoot, length of root per seedling, fresh weight of shoot, root and seedling, dry weight of shoot, root and seedling, root: shoot ratio, absolute and relative growth rate and final survival percentage. With reference to different chemical treatments, soaking seeds in GA_3 @ 1000 ppm recorded significantly best results over all the treatments for early germination, highest germination percentage, vigour index, height of seedling, number of leaves per seedling, diameter of stem, length of shoot, length of root per seedling, fresh weight of shoot, root and seedling, dry weight of shoot, root and seedling, root: shoot ratio, absolute and relative growth rate and final survival percentage.
The interaction effect of physical and chemical treatments indicated that, guava seeds treated with treatment combination scrapping of seed coat with sand paper + soaking seeds in GA_3@ 1000 ppm for 24 hrs recorded significant performance for number of days to germinate, germination percentage and final survival percentage. While, it showed non-significant effect regarding other germination and growth parameters. The highest benefit: cost ratio was recorded in treatment combination of scrapping of seed coat with sand paper + soaking seeds in GA_3 @ 1000 ppm.

Keywords: *Seed Treatment, Guava, Germination*

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Introduction

Guava is one of the most popular fruit crops grown in tropical and sub-tropical regions of India. It is considered as 5th most important fruit crop after mango, banana, papaya and citrus in India. The botanical name of Guava is *Psidium guajava*. It is a small monoecious evergreen tree growing upto a height of 2-10 m belongs to the family Myrtaceae. It has the popularity as poorman's apple. It is available in plenty to people of all standards at reasonable price in all the seasons. It is superior in all the aspects of nutrition.

It is native to tropical areas of Southern Mexico and Northern region of South America although guava trees have now grown by many other countries having tropical and sub-tropical climates, which therefore allows production around the world. Major guava producing countries are South Asian countries, Hawaiian Islands, Cuba and India. India is the leading producing country of guava with an area of 276 thousand hectares under cultivation and production of 4336 thousand MT (NHB, 2018-19). In India major guava producing states are Maharashtra, Bihar, Uttar Pradesh, Gujarat, Madhya Pradesh and Andhra Pradesh. Uttar Pradesh is by so far the most important guava producing state in the country and Allahabad has the reputation of growing the best in the country as well as in the world. These trees are quite resilient, highly productive, requires minimal care and have high economic returns. They adapted to areas with hot summers and cool winters. Guava fruit is often consumed fresh. The fruits are berries consisting of fleshy pericarps and seed cavities with fleshy pulps and numerous small seeds. The fruit contains high levels of vitamin C (up to 228.3 mg/100 g fresh weight) with the highest concentration in the unpeeled fruits.

High density planting requires huge quality planting material and non-availability of budded and grafted plants have adversely affected the production and productivity level in guava. The good planting material *i.e.*, rootstock is very vital component in a grafted plant and it influences the vigour, longevity, tree size, yield and quality [1]. Non-availability of superior rootstock due to poor seed germination and seedling growth is attributed to inconsistent production of quality planting material. Traditionally, it is mostly propagated from seed [2]. In order of guava seeds to germinate, their dormancy should be broken. Water absorption, enzymatic activity, embryo growth, seed coat rupture and plant growth promotion are important steps of germination. Seeds with hard seed coat have delayed and non-uniform germination causing 40% loss of genetic resources if non-employed. Physical, thermal and chemical treatments are the main techniques used to make seed coat smooth and absorbent to water or oxygen. So, now a day attention has been mainly directed to use such techniques like physical or mechanical treatment with scrapping, nicking or rubbing the seeds on rough surface and chemical treatment by using different chemicals and growth regulators for better seed germination and seedling growth. Keeping these points in view an experiment was conducted to study the germination and growth behavior by using different physical and chemical treatments.

Germination of guava seed takes long time due to hard seed coat [3], impermeability of the hard seed coat results in poor seedling emergence [4].

Table-1 Effect of pre-sowing physical and chemical treatments on germination percentage (%), Vigour Index, Height of seedling, Number of leaves per seedling, Diameter of stem, Length of shoot and Length of root at 90 days.

Treatments	Germination percentage (%)	Vigour Index I	Vigour Index II	Height of seedling (cm)	Number of leaves per seedling	Diameter of stem (cm)	Length of shoot (cm)	Length of root (cm)
	90 DAS	90 DAS		90 DAS	90 DAS	90 DAS	90 DAS	90 DAS
Factor A (Physical Treatment)								
P_1	77.81 (61.90)*	687.82	23.89	8.78	13.52	0.225	10.88	7.65
P_2	74.71 (59.81)*	642.26	22.26	8.52	13.22	0.217	10.61	7.40
'F' Test	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig
SE(m)±	0.33	9.33	0.226	0.08	0.1	0.02	0.09	0.08
CD at 5%	0.98	27.73	0.67	0.25	0.3	0.07	0.27	0.24
Factor B (Chemical Treatment)								
C_1	81.15 (64.27)*	745.37	26.67	9.18	14.05	0.241	11.23	8.00
C_2	83.83 (66.29)*	806.34	29.62	9.61	14.52	0.25	11.75	8.39
C_3	73.06 (58.73)*	606.58	20.01	8.29	12.72	0.213	10.28	7.19
C_4	77.62 (61.77)*	667.61	22.68	8.59	13.31	0.221	10.76	7.48
C_5	65.65 (54.12)*	499.30	16.39	7.59	12.26	0.183	9.7	6.56
'F' Test	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig
SE(m)±	0.52	14.76	0.35	0.13	0.16	0.004	0.14	0.13
CD at 5%	1.56	43.85	1.06	0.39	0.48	0.012	0.42	0.39
	Interaction (A × B)							
'F' Test	Sig	NS	NS	NS	NS	NS	NS	NS
SE(m)±	0.74	20.87	0.50	0.18	0.22	0.006	-	0.18
CD at 5%	2.20	-	-	-	-			

Hence, guava seed becomes permeable to water and gases through different seed treatments like physical or mechanical, thermal and chemical treatments. Therefore, in the present study an effort has been made to improve the germination of seeds by different pre-sowing physical and chemical treatments. Effect of pre-sowing treatments on germination and seedling growth has been studied.

Material and Methods

Many other species possess some degree of seed coat dormancy, where seed coat is hard some form of pre-treatments are essential in artificial regeneration, in order a reasonably high germination rate in a short time.

Pre-treatments germinate, break seed coat dormancy and speed up germination is thus one important type of pre-treatment. Keeping in view the same, investigation on "Studies of pre-sowing seed treatments on germination and seedling growth of guava." was carried out at Fruit Nursery, Department of Fruit Science, Dr Panjabrao Deshmukh Krishi Vidyapeeth, Akola during the year 2020-21.

The experiment was initiated by sowing seeds in polythene bags of size (25 x15 cm). Before filling the polythene bags all bags were punctured with the help of punching machine to improve the drainage and filled with garden mixture which prepared by mixing of two parts of soil, 1 part of fine sand and 1 part of well-rotted FYM. Then polythene bags were placed in flat beds with appropriate space.

Guava seeds, sand papers or scrapping papers and different chemicals such as gibberellic acid (GA_3), thiourea, acetone and tap water. The treated seeds of guava were sown on 1st Feb, 2021 in properly filled polythene bags which were labelled with tags and placed as per layout properly. Regular watering was done to seeds which were sown in polythene bags by rose cane to maintain the moisture level in it and bags were watered on alternate days.

To protect the seedlings from infection of insect, pest and disease, the plant protection schedule followed during the course of investigation. Just after sowing of seeds in polythene bags and 60 days interval copper oxychloride was drenched @ 3 g/liter. The seedlings were watered on alternated days and timely weeds were removed from polythene bags. The experiment was conducted in Factorial Completely Randomized Design with ten treatments which were replicated thrice. The guava fruits of variety L-49 were obtained from Fruit Nursery, Department of Fruit Science, Dr Panjabrao Deshmukh Krishi Vidyapeeth, Akola (M.S.). Seeds from fruits were extracted and allowed to dry in shade for few hours. Then these seeds were selected for treatments.

Result and Discussion

The result of investigation based on the various observations *viz*., number of days to germinate, germination percentage (%), vigour Index, height of seedling (cm), number of leaves per seedling, diameter of stem (cm), length of shoot (cm), length of root per seedling (cm), fresh weight of shoot, root and seedling (g), dry weight of shoot, root and seedling (g), root: shoot ratio, absolute and relative growth rate (g/day) and final survival percentage (%).The above observations recorded during the course of investigation, statistically analysed and the results obtained are presented under appropriate headings and sub-headings.

The data presented in [Table-1] revealed that, treatment P1 *i.e*., scrapping of seed coat with sand paper showed higher germination. The data presented in [Table-1] in respect of germination percentage was significantly influenced by chemical treatment. The germination percentage was found to be maximum in treatment C2 (83.83%) which was significantly superior than rest of all the treatments. It was followed by treatments C1 (81.15%) and C4 (77.62%). However, minimum germination percentage was recorded in treatment C5 (65.65%).

Vigour index II recorded significantly maximum in treatment C2 (29.62). This was followed by treatments C1 (26.67) and C4 (22.68). However, minimum vigour index II was observed in treatment C5 (16.39).

The data presented in [Table-1] illustrated that, maximum height of seedling was obtained in treatment P1 *i.e*., scrapping of seed coat with sand paper. This might be due to as earlier germination occurs, that results into earlier growth of plant with favourable environment. The data presented in [Table-1] indicated that, height of seedling at different growth stages was significantly influenced by chemical treatment. At 90 days after sowing, treatment C2 (9.61 cm) recorded maximum height of seedling which was significantly superior to rest of all the treatments. It was followed by treatments C1 (9.18 cm) and C4 (8.59cm). However, minimum height of seedling was observed in treatment C5 (7.59 cm).

Number of leaves per seedling were significantly influenced by physical treatment at 90 days after sowing, significantly maximum number of leaves were observed in treatment P1 (13.52) and minimum number of leaves per seedling were observed in treatment P2 (13.22). Number of leaves per seedling also influenced by chemical treatment at 90 days after sowing, significantly maximum number of leaves per seedling were showed by treatment C2 (14.52). This was followed by treatments C1 (14.05) and C4 (13.31). However, minimum number of leaves per seedling was observed in treatment C5 (12.26).

The diameter of stem was significantly influenced by physical treatment at 90 days after sowing maximum diameter of stem was recorded in treatment P1 (0.225 cm) and minimum diameter (0.217 cm) of stem was recorded in treatment P2.

The data presented in [Table-1] clearly indicated that, maximum length of shoot in guava seedling were obtained in treatment P1 *i.e*., scrapping of seed coat with sand paper. The data in respect of length of shoot as influenced by different chemical treatments at 90 days after sowing maximum length of shoot was recorded in treatment C2 (11.75 cm) which was significantly superior to rest of all the treatments. This was followed by treatments C1 (11.23 cm) and C4 (10.76 cm). However, minimum length of shoot was recorded with treatment C5 (9.70 cm).

Length of root was significantly influenced by physical treatment. Maximum length of root in guava seedling was recorded in treatment P1 (7.65 cm) and treatment P2 (7.40 cm) recorded minimum length of root. Length of root of guava seedling was significantly influenced by chemical treatment. At 90 days after sowing, maximum length of root was recorded with treatment C2 (8.39 cm) which was found to be at par with treatment C1 (8.00 cm). However, minimum length of root was recorded with the treatment C5 (6.56 cm).

The data presented in [Table-2] with respect to fresh weight of shoot, root and seedling was significantly influenced by physical treatment. Significantly maximum fresh weight of shoot recorded in treatment P1 (0.43 g) and treatment P2 (0.42 g) recorded minimum fresh weight of shoot.

Table-2 Effect of pre-sowing physical and chemical treatments on fresh weight of shoot, root and seedling (g) at 90 DAS

Treatments	Shoot (g)	Root (g)	Seedling (g)
	90 DAS		
Factor A (Physical Treatment)			
P_1	0.43	0.24	0.67
P_2	0.42	0.23	0.65
'F' Test	Sig	Sig	Sig
SE(m)±	0.001	0.001	0.002
CD at 5%	0.003	0.003	0.005
Factor B (Chemical Treatment)			
C_1	0.46	0.26	0.72
C_2	0.47	0.27	0.75
C_3	0.41	0.22	0.63
C_4	0.43	0.23	0.66
C_5	0.36	0.21	0.58
'F' Test	Sig	Sig	Sig
SE(m)±	0.001	0.001	0.003
CD at 5%	0.004	0.004	0.008
Interaction (A × B)			
'F' Test	NS	NS	NS
SE(m)±	0.002	0.002	0.004
CD at 5%	-	-	-

Treatment P1 (0.24 g) recorded significantly maximum fresh weight of root and minimum fresh weight of root was recorded in treatment P2 (0.23 g).

Similarly, fresh weight of seedling was recorded significantly maximum in treatment P1 (0.67 g) and treatment P2 (0.66 g) recorded minimum fresh weight of seedling.

Fresh weight of shoot, root and seedling was significantly influenced by different chemical treatments. Maximum fresh weight of shoot was recorded in treatment C2 (0.47 g) which was found to be at par with treatment C1 (0.46 g). However, minimum fresh weight of shoot was recorded in treatment C5 (0.36 g).

Fresh weight of root was recorded maximum in treatment C2 (0.27 g) which was found to be at par with treatment C1 (0.26 g). However, minimum fresh weight of root was recorded in treatment C5 (0.21 g).

Similarly, Fresh weight of seedling recorded significantly maximum in treatment C2 (0.75 g). This was followed by treatments C1 (0.72 g) and C4 (0.66 g). However, treatment C5 (0.58 g) recorded minimum fresh weight of seedling.

The data presented in [Table-3] indicated that, dry weight of shoot, root and seedling was significantly influenced by physical treatments. Maximum dry weight of shoot was recorded in treatment P1 (0.20 g) and treatment P2 (0.19 g) recorded minimum dry weight of shoot.

Significantly maximum dry weight of root was recorded in treatment P1 (0.10 g) and minimum dry weight of root was recorded in treatment P2 (0.09 g).

Similarly, dry weight of seedling was recorded significantly maximum in treatment P1 (0.30 g) and treatment P2 (0.29 g) recorded minimum dry weight of seedling.

Dry weight of shoot, root and seedling was significantly influenced by different chemical treatments. Significantly maximum dry weight of shoot was recorded in treatment C2 (0.23 g). This was followed by treatments C1 (0.21 g) and C4 (0.19 g) and were found to be at par with each other. However, minimum dry weight of shoot was recorded in treatment C5 (0.17 g).

Significantly maximum dry weight of root recorded in treatment C2 (0.12 g) which was found to be at par with treatment C1 (0.11 g). However, minimum dry weight of root was recorded in treatment C5 (0.07 g). Similarly, dry weight of seedling recorded significantly maximum in treatment C2 (0.35 g). This was followed by treatment C1 (0.32 g) and C4 (0.29 g). However, minimum dry weight of seedling was recorded in treatment C5 (0.25 g).

The data presented in [Table-3] with respect to root: shoot ratio was significantly influenced by physical treatment. Significantly maximum root: shoot ratio was recorded in treatment P1 (0.49). Whereas, minimum root: shoot ratio was recorded in treatment P2 (0.47).

Root: shoot ratio was significantly influenced by different chemical treatments. Maximum root: shoot ratio was recorded in treatment C2 (0.52) which was found to be at par with treatment C1 (0.49). However, minimum root: shoot ratio was recorded in treatment C5 (0.46).

Absolute and relative growth rate was significantly influenced by physical treatment. Absolute growth rate was recorded significantly maximum in treatment P1 (0.005 g/day) and treatment P2 (0.004 g/day) recorded minimum absolute growth rate.

Significantly maximum relative growth rate was recorded in treatment P1 (0.009 g/day) and treatment P2 (0.008 g/day) recorded minimum relative growth rate.

Absolute and relative growth rate was significantly influenced by different chemical treatments. Maximum absolute growth rate was recorded in treatment C2 (0.005 g/day) which was found to be at par with treatment C1 (0.005). However, treatment C5 (0.004 g/day) recorded minimum absolute growth rate.

Treatment C2 (0.010 g/day) recorded significantly maximum relative growth rate. This was followed by treatments C1 (0.009 g/day). However, minimum relative growth rate was recorded in treatment C5 (0.008).

Final survival percentage was significantly influenced by physical treatment. Significantly maximum final survival percentage was recorded in treatment P1 (74.82 %) and treatment P2 (71.39 %) recorded minimum final survival percentage. The data in respect of final survival percentage of guava seedling as influenced by different chemical treatments was recorded and presented in [Table-3]. Maximum final survival percentage was recorded in treatment C2 (81.14 %) which was significantly superior to rest of all treatments. This was followed by treatments C1 (77.54 %) and C4 (73.44 %). However, minimum final survival percentage was recorded with treatment C5(62.49 %).

Summary

The experiment was laid out in Factorial Completely Randomized Design (FCRD) with 10 treatment combinations comprising two physical treatments (Factor A) *viz*., P1 scrapping of seed coat with sand paper, P2 without scrapping of seed coat with sand paper and five chemical treatments (Factor B) *viz*., C1 soaking seeds in gibberellic acid (GA_3) @ 500 ppm for 24 hrs, C2 soaking seeds in gibberellic acid (GA_3) @ 1000 ppm for 24 hrs, C3 soaking seeds in thiourea @ 2000 ppm, C4 soaking seeds in thiourea @ 4000 ppm for 24 hrs and C5 soaking seeds in tap water for 24 hrs which were replicated three times. The results obtained during course of investigation are summarized in following paragraphs.

Effect of pre-sowing physical treatment on seed germination and seedling growth of guava

Germination parameters

Guava seeds were treated with physical treatment, where the physical treatment P1 *i.e*., scrapping of seed coat with sand paper was found significantly superior in respect of germination parameters were noticed as number of days to germinate (20.88 days), germination percentage (77.81 %), vigour index I (687.82) and vigour index II (23.89).

Growth parameters

Regarding growth parameters, physical treatment P1 *i.e*., scrapping of seed coat with sand paper gave best results were noted as height of seedling (8.78 cm), number of leaves per seedling (13.52 cm), diameter of stem (0.225 cm), length of shoot (10.88 cm), length of root per seedling (7.65 cm), fresh weight of shoot (0.43 g), root (0.24 g) and seedling (0.67 g), dry weight of shoot (0.20 g), root (0.10 g) and seedling (0.30 g), root: shoot ratio (0.49), absolute growth rate (0.005 g/day), relative growth rate (0.009 g/day) and final survival percentage (74.82 %).

Effect of pre-sowing chemical treatment on seed germination and seedling growth of guava

Table-3 Effect of pre-sowing physical and chemical treatments on dry weight of shoot, root and seedling (g), on absolute and relative growth rate (g/day) and on final survival percentage

Treatments	Shoot (g)	Root (g)	Seedling (g)	Root: Shoot Ratio	Absolute Growth rate (g/Days)	Relative Growth rate (g/Days)	Survival percentage
	90 DAS		90 DAS	90 DAS	90 DAS	90 DAS	90 DAS
Factor A (Physical Treatment)							
P_1	0.2	0.1	0.3	0.49	0.005	0.009	74.82 (59.88)*
P_2	0.19	0.09	0.29	0.47	0.004	0.008	71.39 (57.66)*
'F' Test	Sig	Sig	Sig	Sig	Sig	Sig	Sig
SE(m)±	0.001	0.001	0.001	0.003	0	0	0.29
CD at 5%	0.002	0.002	0.004	0.009	0	0	0.87
Factor B (Chemical Treatment)							
C_1	0.21	0.11	0.32	0.49	0.005	0.009	77.54 (61.71)*
C_2	0.23	0.12	0.35	0.52	0.005	0.01	81.14 (64.26)*
C_3	0.18	0.08	0.27	0.47	0.004	0.008	70.89 (57.35)*
C_4	0.19	0.09	0.29	0.48	0.004	0.008	73.44 (58.98)*
C_5	0.17	0.07	0.25	0.46	0.004	0.008	62.49 (52.23)*
'F' Test	Sig	Sig	Sig	Sig	Sig	Sig	Sig
SE(m)±	0.001	0.001	0.002	0.005	0	0	0.46
CD at 5%	0.002	0.003	0.006	0.014	0	0	1.38
Interaction (A × B)							
'F' Test	NS	NS	NS	NS	NS	NS	Sig
SE(m)±	0.002	0.001	0.003	0.007	0	0	0.65
CD at 5%	-	-	-	-	-	-	1.95

Germination parameters

In respect of germination parameters, chemical treatment recorded significantly best results in treatment C2 *i.e.*, soaking seeds in gibberellic acid (GA_3) @1000 ppm and were recorded as number of days to germinate (18.60 days), germination percentage (83.83 %), vigour index I (806.34) and vigour index II (29.62).

Growth parameters

Guava seeds soaked in different chemical solutions, where the chemical treatment C2 *i.e.*, soaking of seeds in gibberellic acid (GA_3) @ 1000 ppm was found to be significant in relation to growth parameters were recorded as height of seedling (9.61 cm), number of leaves per seedling (14.52), diameter of stem (0.250 cm), length of shoot (11.75 cm), length of root per seedling (8.39 cm), fresh weight of shoot (0.47 g), root (0.27 g) and seedling (0.75 g), dry weight of shoot (0.23 g), root (0.12 g) and seedling (0.35 g), root: shoot ratio (0.52), absolute growth rate (0.005 g/day), relative growth rate (0.010 g/day) and final survival percentage (81.14 %).

Interaction effect of pre-sowing physical and chemical treatments on seed germination and seedling growth of guava

Germination parameters

Guava seeds treated with the combination of physical treatment P1 *i.e.*, scrapping of seed coat with sand paper + chemical treatment C1 *i.e.*, soaking seeds in gibberellic acid (GA_3) @ 1000 ppm were found to be significantly superior regarding two germination parameters and were noticed as number of days to germinate (17.68 days) and germination percentage (85.17 %). Whereas, vigour index I and vigour index II were found to be non-significant for this interaction effect.

Growth parameters

The interaction effect of physical treatment P1 *i.e.*, scrapping of seed coat with sand paper + chemical treatment C2 *i.e.*, soaking seeds in gibberellic acid (GA_3) @ 1000 ppm showed significantly superior results on survival percentage (82.95%). Whereas, it showed non-significant effect on other growth parameters.

On the basis of findings reported in present investigation, the effect of physical and chemical treatments on germination and growth parameters of guava was influenced by different treatments.

Conclusion

The response of physical treatment on germination and growth parameters of guava was found to be significant in treatment P1 *i.e.*, scrapping of seed coat with sand paper. Among different chemical treatments, the treatment C2 *i.e.*, soaking seeds in gibberellic acid @ 1000 ppm showed significantly better performance in relation to germination and growth parameters.

With respect to interaction effect of physical and chemical treatment, guava seeds treated with treatment combination P1C2 *i.e.*, scrapping of seed coat with sand paper + soaking seeds in gibberellic acid (GA_3) @ 1000 ppm gave significantly superior results regarding number of days to germinate, germination percentage, final survival percentage and benefit: cost ratio. Whereas, it showed non-significant effect on remaining other parameters.

Application of research: Study an effort has been made to improve the germination of seeds by different pre-sowing physical and chemical treatments. Effect of pre-sowing treatments on germination and seedling growth has been studied.

Research Category: Seed germination

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Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Ethical Committee Approval Number: Nil

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