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Improving seed germination and seedling traits by presowing treatments in khirni (*Manilkara hexandra*)

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ABSTRACT
Khirni or Rayan is an important underutilized fruit crop of tropical deciduous forests of Indian subcontinent. Khirni is also known to be the most suitable and widely used rootstock for sapota. The edible fruits are small, oval, sweet and nutritionally rich. Poor germination due to hard seed coat and continuously increasing pressure on the natural wild population is gradually eroding its genetic variability. Thus, the present study was undertaken to develop an optimal treatment for improving the germination and seedling characteristics of khirni. The treatments applied were soaking in distilled water, GA_3 9100 and 200 ppm) , Thiourea (1 and 2%) and KNO_3 (1 and 2%). Growth features of khirni seedlings were recorded at 75 and 150 days after sowing (DAS). Significant variations in growth attributes of khirni seedlings were observed. The highest germination percentage, shoot length, number of leaves, shoot fresh weight, shoot dry weight, seedling vigour index - I and seedling vigour index II were obtained in the seeds treated with 100 ppm GA_3 while root length, stem diameter, tap root diameter, root fresh weight, root dry weight and root: shoot ratio 75DAS were higher in the seeds treated with 200 ppm GA_3. Seeds treated with 1% thiourea had higher root: shoot ratio 150DAS. The findings suggest that treatment of seeds with 200 ppm GA_3 showed efficacy in improving seed germination, growth characteristics and vigour of khirni seedlings.
Keywords: *GA_3, khirni, KNO_3, rayan, seed treatment, Thiourea*

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INTRODUCTION

Khirni (*Manilkara hexandra*) playing an important role in the socio-economic upliftment of tribal population in south and central India [6] is valued for its fruits, wood, leaves and latex. The evergreen tree is slow in growth and is very commonly found growing wild in forests and backyards [12]. The bark, fruits and seeds are valued for their nutritional as well as medicinal significance. Fruits are rich in minerals, sugars, protein, carbohydrates and vitamin A [9]. The strong and dense hardwood timber has multipurpose uses. Leaves are used as fodder for cattle. The seeds contain approximately 25 per cent oil which can be used for cooking purpose. The different parts of this plant find their use in treatment of ulcers, dyspepsia, opacity of the cornea, bronchitis, urethrorrhea, leprosy, etc. It is also the most popular and extensively used rootstock for Sapota because of its vigorous root system which helps in proper anchorage and efficient absorption of nutrients and water. The Sapota plants grafted on Khirni rootstocks are more healthy and strong resulting in higher production and better quality fruits.

Although the fruits and plants have immense importance and potential, the commercial cultivation of khirni is restricted due to lack of healthy planting material. The work on vegetative methods of propagating khirni is very meagre, so new plants are prepared by raising seedlings only. The hard seed coat imposed dormancy or 'hardseededness' and recalcitrant nature of khirni seeds results in low germination. [12]. The tough seed coat hinders the imbibition of water and limits the gaseous exchange. The short viability of seeds prevents long term storage which further reduces bulk availability of planting material. Besides, the slow growth rate of khirni seedlings is also a drawback in its rapid and mass multiplication.

The importance of khirni either for its nutritive fruits or for providing bulk rootstocks for sapota or for conserving its genetic diversity, there is an urgent need to produce large stock of healthy and vigorous

seedlings in short duration. Presowing treatments have been reported to enhance seed germination, seedling vigour and seedling growth in many tree species. Several efforts like treatment with chemicals, growth regulators hot water, cattle urine and cow dung slurry have been used to overcome hard seed coat dormancy. Therefore, in the present investigation, response of various chemicals for rapid and synchronized seed germination for improvement in seedling characteristics of khirni has been studied.

MATERIALS AND METHODS

Experimental site

This research was conducted in the fruit nursery of Horticultural Research Centre, Patherchatta, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand under protected conditions. The experimental site is located at 29º N latitude, 79.3º E longitude and at an altitude of 243.8 m above mean sea level.

Fruit collection and seed extraction

The fresh fruits of Khirni were collected from local market of Lucknow district of Uttar Pradesh. The fully ripened fruits were manually kneaded in water to remove the pulp. The seeds were extracted and thoroughly washed with water to remove any remains of flesh. Finally the seeds were rinsed with distilled water and dried in shade. The seeds were then stored at room temperature (25-26^{0}C) for 1 day until the experiments started. All the misshapen and aborted seeds were discarded.

Seed treatments

The seeds were subjected to following treatments : 1) T_1 – Untreated taken as control 2) T_2 – soaking of seeds in distilled water 3) T_3 - soaking of seeds in 100 ppm GA_3 4) T_4 - soaking of seeds in 200 ppm GA_3 5) T_5 - soaking of seeds in 1% thiourea 6) T_6 - soaking of seeds in 2% thiourea 7) T_7 - soaking of seeds in 1% KNO_3 and 8) T_8 - soaking of seeds in 2% KNO_3 solution. All the solutions were prepared in distilled water. The soaking of seeds was done for 36 hours in an incubator (24^{o}C) to ensure uniform imbibition. After incubation, the seeds which sank at the bottom of the beaker were selected for sowing while the seeds floating on the surface were separated out. The seeds were then shade dried for 10 minutes at room temperature (25-26^{o}C).

Germination experiment

Seeds were germinated in root trainers (8 cm in diameter, 15 cm depth) filled with a mixture of soil: sand: FYM (1:1:1). In each treatment, three replications with 100 seeds per replication were used. The seeds were sown vertically at 1 inch depth in the root trainers. The moisture content of the soil was uniformly maintained for all the treatments by watering at 24 hours interval until the end of experiment. Weeding was carried out uniformly in all the treatments. The germination started 2 weeks after sowing and continued sporadically upto 6 weeks. The germination count was daily recorded to work out the germination percentage.

All the observations of seedling growth and characteristics were recorded at 75 and 150 days after sowing (DAS). Twenty seedlings per replication in each treatment were randomly selected for measuring the growth parameters. The observations on shoot and tap root length were recorded with a measuring scale. The shoot length measurement was taken from the collar to the apical portion of the plant. Counting of leaves per plant was done visually. Stem and tap root diameter were measured with digital vernier callipers calibrated in millimetre. The seedlings were gently uprooted and the root and shoot of the seedlings were separated to determine their fresh and dry weight which was recorded with the help of an electronic balance. For the estimation of dry weight, the shoots and roots were dried in an oven for 24 hours at 60^{o}C until constant weight was attained. Seedling vigour index - I was calculated by multiplying germination percentage and total seedling length (cm) while seedling vigour index II was determined by multiplying germination percentage and seedling dry weight (gm), respectively [1].

Statistical analysis

The experiment was laid out in completely randomized block design (CRD) with eight treatments replicated thrice. All data were subjected to analysis of variance (ANOVA) and significant differences were determined at 5 % level of significance[4].

RESULTS AND DISCUSSION

Presowing treatment of khirni seeds with different growth regulators and chemicals greatly affected the various parameters (Table 1 and 2). The pre-sowing treatments had profound influence on the vegetative characters of Khirni seedlings (Table 1 and 2). Among the different treatments, the seeds treated with 100 ppm GA_3 exhibited superior results followed by those treated with 200 ppm GA_3 in terms of germination percentage (85.80 %), shoot length (6.68 and 11.13 cm), number of leaves per seedling (4.46 and 7.01), fresh weight of shoots (540 and 1046 mg) and dry weight of shoots (186 and 351 mg) both at 75 and 150 DAS. In passion fruit also, GA_3 significantly increased the number of leaves, fresh and dry

weight of shoots as reported by Gurung *et al*. [5]. The stimulating effects of GA_3 on seedling growth are also in conformity with the findings of Bhanuprakash *et al*. [2] in khirni and Vasantha *et al*. [15] in tamarind. Similarly, the fresh and dry weights of shoots were also higher in the seeds treated with gibberellic acid. The obtained results strongly support the view that GA_3 increases the mobilization of water and nutrients at higher rate leading to enhanced production of photosynthates and their translocation to various plant parts resulting in better growth.

Our results showed that treatment with 200 ppm GA_3 was the most effective treatment for increasing root length at 75 and 150 DAS (9.91 and 13.60 cm), stem diameter (1.54 and 1.88 mm), tap root diameter (1.24 and 1.43 mm), fresh weight of roots (238 and 396 mg) and dry weight of roots (121 and 212 mg). The results suggest that gibberellins play a significant role in the developmental and physiological processes in plants**.** The positive effect of gibberellins on increasing root length with GA_3 treatment has also been reported by Vachhani *et al*. [14] in khirni and Pampanna and Sulikeri [7] in sapota. The maximum root length in seedlings obtained from GA_3 presoaked seeds might be due more elongation of the cells in the sub-apical region of roots as reported by Salisbury and Ross [11]. Vigorous shoot growth due to GA_3 treatment might have led to increased production of photosynthates and their translocation through phloem to the root zone resulting in increasing the length and diameter of radical. The GA_3 also accelerates the assimilation and translocation of auxins which imparts better root growth and vegetative characters of the plant as reported by Pandiyan *et al*. [8]. The root: shoot ratio was however higher in the seedlings treated with 1% thiourea and was closely followed by treatment with 200 ppm GA_3.

The effect of GA_3 pre-sowing treatment on increment of stem girth has also been reported by Rashmi *et al*. [10] in Aonla. The maximum stem girth of seedlings obtained from seeds pre-soaked in GA_3 might be attributed to the fact that GA_3 also enhanced the rate of cell division and elongation of stem portion. The increase in fresh weight of roots can be correlated with the stimulatory effect of GA_3 in stimulating cell division, cell elongation, auxin metabolism, cell wall plasticity and permeability of cell membrane leading to enhanced growth.

Table 1. Effect of presowing seed treatments on germination, shoot length, root length, root: shoot ratio, number of leaves, stem diameter and tap root diameter of khirni seedlings.

Treatments	Germi-nation (%)	Shoot length (cm)		Root length (cm)		Root: shoot ratio		Number of leaves		Stem diameter (mm)		Tap root diameter (mm)	
		75 DAS	150 DAS	75 DAS	150 DAS	75 DAS	150 DAS	75 DAS	150 DAS	75 DAS	150 DAS	75 DAS	150 DAS
T_1- Control	41.70	5.30	8.91	7.71	11.35	1.45	1.27	3.86	5.68	1.31	1.70	1.03	1.21
T_2-Distilled water	50.80	5.38	9.05	7.78	11.56	1.45	1.28	3.90	5.92	1.32	1.75	0.99	1.23
T_3-GA_3 (100ppm)	**85.80**	**6.68**	**11.13**	9.36	12.90	1.40	1.16	**4.46**	**7.01**	1.46	1.82	1.16	1.36
T_4- GA_3 (200ppm)	80.00	6.29	10.80	**9.91**	**13.60**	**1.58**	1.26	4.15	6.80	**1.54**	**1.88**	**1.24**	**1.43**
T_5-Thiourea (1%)	75.00	5.52	9.30	8.36	12.11	1.51	**1.30**	4.04	6.63	1.40	1.80	1.11	1.29
T_6-Thiourea (2%)	58.30	5.65	9.96	8.50	12.42	1.50	1.25	3.92	6.19	1.32	1.72	1.07	1.20
T_7-KNO_3 (1%)	61.70	5.96	10.60	9.00	12.63	1.51	1.19	3.96	6.28	1.39	1.79	1.12	1.30
T_8-KNO_3 (2%)	51.70	5.69	10.36	8.63	12.50	1.52	1.21	4.00	6.40	1.36	1.75	1.14	1.35
C.D. (5%)	3.36	**0.21**	**0.47**	**0.33**	**0.47**	**0.05**	**0.04**	**0.15**	**0.31**	**0.04**	**0.05**	**0.04**	**0.04**
S. Em. ±	1.01	**0.07**	**0.16**	**0.11**	**0.16**	**0.02**	**0.01**	**0.06**	**0.10**	**0.01**	**0.02**	**0.01**	**0.02**

The data on seedling vigour index presented in Table 2 shows significant variations due to various presowing treatments. The results indicated highest seedling vigour index – I and II in seeds treated with GA_3-100 ppm (1376.23 and 2061.77) followed by 200 ppm GA_3 (1296 and 1944) at 75 and 150 DAS, respectively. The enhancement in vigour index of the seedlings obtained in seeds soaked in GA_3 has also been reported by Dhankhar and Singh [3] in *Phyllanthus emblica*. This might be due to increased germination and seedling length which have contributed to higher vigour index-I. Similarly treatment with 100 ppm GA_3 also resulted in higher seedling vigour index - II (25.40 and 47.16) followed by GA_3 - 200 ppm (23.92 and 44.56) at 75 and 150 DAS, respectively. The positive response of GA_3 for enhanced vigour index as presented in Table 2 might be attributed to higher germination percentage as well as

vigorous growth of shoot and root leading to higher dry matter production in the GA_3 treated seedlings. The control (untreated seeds) produced significantly inferior seedling characters as compared to rest of the treatments.

Significant increase in growth attributes of seedlings was observed in the treated seeds (especially GA_3) when compared with control. Among other treatments, soaking of seeds in KNO_3 and thiourea also improved the vegetative characters of khirni seedlings but the effect varied for different characters. Treatment with GA_3, however, showed significantly better results for all the characters undertaken in the study. The use of gibberellic acid (GA_3) for boosting the growth and vigour of various tree species is very old and well documented. Therefore, it can be concluded that treatment of khirni seeds with GA_3 before sowing enhances germination, synchronized emergence and helped in proper establishment of seedlings. Besides GA_3 also increased the seedling vigour owing to its stimulatory effect on physiology of embryos and activation of enzymes (particularly α and β-amylase) which help in digestion of the available carbohydrate into simple sugars so that optimum nutrition is easily available for the faster growth of the khirni seedlings. The results of this research can be of use for nursery owners to enhance the growth rate of khirni seedlings for achieving high economic profits.

Table 2. Effect of presowing seed treatments on fresh weight of shoot and root, dry weight of shoot and root and seedling vigour index - I and II of khirni seedlings.

Treatments	Shoot fresh weight (mg)		Root fresh weight (mg)		Shoot dry weight (mg)		Root dry weight (mg)		Seedling vigour Index - I (cm)		Seedling vigour Index - II (gm)	
	75 DAS	150 DAS	75 DAS	150 DAS	75 DAS	150 DAS	75 DAS	150 DAS	75 DAS	150 DAS	75 DAS	150 DAS
T_1- Control	348	761	160	282	139	320	83	164	542.52	844.84	9.25	20.18
T_2-Distilled water	354	844	172	294	143	328	88	170	668.53	1046.98	11.73	25.30
T_3-GA_3 (100ppm)	**540**	**1046**	225	375	**186**	**351**	110	198	**1376.23**	**2061.77**	**25.40**	**47.10**
T_4- GA_3 (200ppm)	490	960	**238**	**396**	178	345	**121**	**212**	1296	1952	23.92	44.56
T_5-Thiourea (1%)	432	943	218	352	166	340	106	191	1041	1605.75	20.4	39.83
T_6-Thiourea (2%)	380	844	186	333	148	326	92	182	824.95	1304.75	13.99	29.61
T_7-KNO_3 (1%)	402	856	195	326	160	332	100	185	923.03	1433.30	16.04	31.90
T_8-KNO_3 (2%)	366	810	182	301	155	323	91	170	740.34	1181.86	12.72	25.49
C.D. (5%)	**19.78**	**36.60**	**7.98**	**17.41**	**7.51**	**16.07**	**3.10**	**6.56**	**36.34**	**69.23**	**0.94**	**1.61**
S. Em. ±	**6.60**	**12.21**	**2.67**	**5.81**	**2.51**	**5.36**	**1.03**	**2.19**	**12.12**	**23.10**	**0.31**	**0.54**

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