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Sangamesh R Sabarad
Kittur Rani Channamma College of Horticulture, University of Horticultural Sciences, Bagalkote, Karnataka, India

Manu Kumar HR
College of Horticulture, University of Horticultural Sciences, Bagalkote, Karnataka, India

Anil I Sabarad
Kittur Rani Channamma College of Horticulture, University of Horticultural Sciences, Bagalkote, Karnataka, India

Chandan K
College of Horticulture, University of Horticultural Sciences, Bagalkote, Karnataka, India

Prashantha A
Kittur Rani Channamma College of Horticulture, University of Horticultural Sciences, Bagalkote, Karnataka, India

Shivakumar KM
College of Horticulture, University of Horticultural Sciences, Bagalkote, Karnataka, India

Suhasini Jalawadi
College of Horticulture, University of Horticultural Sciences, Bagalkote, Karnataka, India

Corresponding Author:
Sangamesh R Sabarad
Kittur Rani Channamma College of Horticulture, University of Horticultural Sciences, Bagalkote, Karnataka, India

Influence of seed priming on germination and seedling growth of wood apple (*Limonia acidissima* L.)

Sangamesh R Sabarad, Manu Kumar HR, Anil I Sabarad, Chandan K, Prashantha A, Shivakumar KM and Suhasini Jalawadi

Abstract
An experiment was conducted during 2020-21 to study the different concentration of chemicals, growth regulators and soaking time on the germination of wood apple seeds at Kittur Rani Channamma College of Horticulture, Arabhavi, Karnataka. The experiment was laid out in factorial completely randomized design comprising of three soaking time (6 hours, 12 hours and 24 hours) and eight different seed treatment chemicals (GA_3 @ 50 ppm and 100 ppm, KNO_3 @ 50 ppm and 100 ppm, Thiourea @ 50 ppm and 100 ppm, Soaking in water and Control *i.e.*, without any treatment). The result of the present investigation revealed that amongst the different seed treatments, soaking the seeds in GA_3 @ 100 ppm for 24 hours was most effective to ensure maximum germination percentage (78.42, 75.04%), number of leaves per seedling (30.98, 24.78), height of the seedlings (14.23, 13.09 cm), fresh weight of the seedlings (13.61, 10.76 g), dry weight of the seedlings (5.26, 4.16 g), seedling vigour index-I (1123.63, 997.24), seedling vigour index-II (416.70, 323.63) and shoot to root ratio (1.41, 1.35).

Keywords: GA_3, KNO_3, recalcitrant, soaking time, thiourea and wood apple

Introduction
Wood apple (*Limonia acidissima* L.) is a thorny tree belongs to Rutaceae family, having a chromosome number 2n= 18. In India, it is also known by many different names like Elephant apple, Monkey fruit, Curd fruit and Katbhel in Bengali, Kaitha in Hindi, Kadu nimbe and Bellada hannu in Kannada. The name Limonia in allusion to its lemon like fruit. Its original home is India and Sri Lanka (Lande *et al*., 2010) [11] and prefers a dry climate for optimum flowering and fruiting.

Dual purpose use of wood apple i.e., raw as well as processed products makes it more economically preferred commodity. Pulp of wood apple is loaded with immense quantity of medicinally important compounds like umbelliferol, dictamnine, xanthotoxol, sco-parone etc. those could be used in the pharmaceuticals industries. From ancient time, in India the ripe fruits are used as a liver and cardiac tonic, while unripe ones as an astringent for checking diarrhoea and dysentery. In addition, the fruits serve as effectual treatment for sore throat, hiccough, and diseases of the gums. The fruit pulp is also used for the preparation of chutney, fruit bar, jam, jelly, and ready-to-serve beverages (Vidya and Narain, 2011) [25].

The fruit pulp contains 2.66 per cent pectin, moisture 64.2 per cent, protein 7.1 per cent, fat 3.7 per cent, minerals 1.9 per cent, fibre 50 per cent, carbohydrates 18.1 per cent, calcium 0.13 per cent, phosphorus 0.11 per cent, and iron 0.048 per cent of the fruit weight (Roy and Mazumdar, 1988) [19].

Seed propagation in fruit plants are well acknowledged where seed is the only means of multiplication. Though, commercial method of propagation in wood apple is chiefly through seeds, vegetative propagation by budding, cutting, softwood grafting (Raghavendra *et al*., 2011) [18], and inarching is also successful. Nevertheless, decline in seed viability is noticed within a very short period following its (seed) extraction from fresh fruits of wood apple. As a consequence, low seed viability along with poor germination is encountered as a severe menace during commercial cultivation of wood apple. Such low viability coupled with poor germination occurs presumably due to the physiological event of low metabolic activity referred as ‘dormancy’ or ‘quiescence’ that is associated with hard seed coat, immature embryo or with the result of endogenous chemical germination inhibitors (Wareing and Phillips, 1981; Sharma *et al*., 2014) [26, 22].

Multiple researches have been conducted on pre-germination treatments of seeds with different seed priming chemicals (GA3, KNO3, thiourea, salicylic acid, hot water as well as cow dung slurry) in different fruit crops like custard apple, passion fruit, fig, yoshino cherry, papaya etc. (Farooq *et al*., 2005; Basra *et al*., 2007; Kim, 2019) [6, 4, 10]. These chemicals and multiple other seed priming treatments improve seed germination and seedling growth mainly by reducing internal growth inhibitor content (Agrawal and Dadlani, 1995; Hassini *et al*., 2017) [2, 8] and increasing the growth promoter concentration in seed; improving entry of water into the cell (Arteca, 1996; Sharma *et al*., 2014) [3, 22] and improving enzymatic activity to initiate several metabolic processes (Maiti *et al*., 2013) [12].

In breeding programmes, wood apple seeds are the key component for developing hybrid plants to enrich genetic diversity, and to produce quality rootstock plant (Morton, 1987) [14]. It has been proven that seed priming is one of the competent approaches to obtain uniform and healthy seedling but exhaustive information of this approach in wood apple is still scarce. Keeping these facts in concern, the present experiment was conceived to study the effect of different concentration of chemicals, growth regulators and soaking time on the germination of wood apple seeds.

Material and Methods

Details of the experimental site

The experiment was conducted at the K.R.C. college of Horticulture, Arabhavi, of the University of Horticultural Sciences, Bagalkot, Karnataka, India. Arabhavi is situated in Northern dry Zone of Karnataka state (Zone-3, Region-2) at 16°15' N latitude and 74°45' E longitude, 612 m above mean sea level.

Experimental details

The present experiment was laid in factorial completely randomized design with three replications. Observations were recorded in respect of germination percentage, which was worked out after complete germination i.e., after stoppage of germination. It was calculated by dividing total number of seeds sown with the number of seeds germinated and was multiplied by 100. Whereas other growth parameters such as seedling height and number of leaves at 30, 45 and 60 days after sowing, fresh weight of the seedlings, dry weight of the seedlings, seedling vigour index-I, seedling vigour index-II and shoot to root ratio were recorded at 60 days after sowing. Seedling vigour index was calculated using the formula listed below (Abdul-Baki and Anderson, 1972) [1].

1. Vigour index-I = Mean seedling height × germination %
2. Vigour index-II = Dry weight of seedling × germination %

Treatment details

Factor-A (Soaking time)

S1 - 6 hours
S2 - 12 hours
S3 - 24 hours

Factor-B (Seed treatments)

C1 - GA3 @ 50 ppm
C2 - GA3 @ 100 ppm
C3 - KNO3 @ 50 ppm
C4 - KNO3 @ 100 ppm
C5 - Thiourea @ 50 ppm
C6 - Thiourea @ 100 ppm
C7 - Soaking in water
C8 - Control (without any treatment)
Note: Seeds sown under shade net conditions

Result and Discussion

Germination percentage (%)

Seeds soaked for 24 hours in GA_3 100 ppm showed significantly highest germination percentage (75.05% and 78.42%, respectively). The minimum germination percentage was recorded in 6 hours soaking time and without any treatment-control (64.54% and 53.46%, respectively) and data presented in Table 1. It might be attributed to GA_3, which acts directly on embryo relieving them from dormancy through promoting protein synthesis and elongation of coleoptiles and leaves also helps in the production of ethylene. This ethylene invokes the synthesis of hydrolases, especially amylase, which favours the seed germination. Thus, the enhanced enzymatic reactions along with the suppression of inhibitors by these growth substances might have acted in faster germination (Stewart and Freebairn, 1969) [23]. The results of study are in close agreement with the findings of Muralidhara *et al*. (2015) and Venkatrao and Reddy (2005) [24] in mango.

Number of leaves per seedling

The data in respect to number of leaves per seedling at 30, 45 and 60 days after sowing were recorded and presented in Table 1. Among the soaking time and different seed treatment, 24 hours soaking time (11.84, 17.54 and 24.78, respectively) and GA_3 100 ppm (14.42, 21.92 and 30.98, respectively) recorded the maximum number of leaves per seedling. Whereas, minimum number of leaves was recorded in 6 hours soaking time (10.18, 15.38 and 21.73, respectively) and without any treatment-control (8.02, 11.74 and 16.55 respectively). The increase in number of leaves per seedlings might be due to activity of GA_3 at the apical meristem resulting in more synthesis of nucleoprotein responsible for increasing leaf initiation (Sen and Ghunti, 1976) [20]. The observation analogues to these findings were reported by Jadhav *et al*. (2015) [9] in custard apple.

Seedling height (cm)

The data in respect to seedling height at 30, 45 and 60 days after sowing were recorded and presented in Table 2. The maximum seedling height was observed with 24 hours soaking time (6.54, 9.82 and 13.09 cm, respectively) and GA_3 100 ppm (7.12, 10.62 and 14.23 cm, respectively). While minimum seedling was recorded in 6 hours soaking time (5.49, 8.24 and 10.97, respectively) and without any treatment-control (4.92, 7.61 and 9.84, respectively). It might be due to soaking wood apple seeds for 24 hours may have accelerated the hydrolysis of complex sugar into simple sugars which are then utilized in the synthesis of auxins and proteins. It is a well-known fact that proteins are utilized in the production of new tissues and that auxins promote growth. This probably explains the higher values recorded for various growth parameters under 24 hours soaking time. The increase in seedling height with GA_3 treatment was due to the fact that this hormone increased osmotic uptake of nutrients, causing cell elongation and thus increased height of the seedlings (Shanmugavelu, 1966) [21]. Such type of findings was also reported by Nimbalkar, *et al*. 2012 [16] in karonda.

Effect on biomass

The data in respect to fresh and dry weight of the seedlings were recorded and presented in Table 3. Significantly the maximum fresh and dry weight of the seedlings were recorded with 24 hours soaking time (10.76 and 4.16 g) and GA_3 100 ppm (13.61 and 5.26 g) at 60 days after sowing and minimum fresh and dry weight of the seedlings were recorded in 6 hours soaking time (7.43 and 2.87 g) and without any treatment-control (5.71 and 2.20 g). It might be due to overall growth of the seedling and increased rate of photosynthesis that led to the overall assimilation and redistribution of photosynthates within the seedling and hence, resulted in higher fresh and dry weight of the seedlings. Thus increased growth is a consequence of increased dry matter accumulation. This type of result was also observed by Parmar *et al*. (2016) [17] in custard apple.

Seedling vigour

The data in respect to seedling vigour was recorded and presented in Table 3. Among the soaking time and different seed treatments, 24 hours soaking time (997.24 and 323.63) and GA_3 100 ppm (1123.63 and 416.70) were recorded significantly highest seedling vigour index-I and vigour index-II at 60 days after sowing. While the lowest seedling vigour index-I and vigour index-II were observed in 6 hours soaking time (716.92 and 188.82) and without any treatment-control (527.53 and 118.29). The increase in vigour index-I and vigour index-II might be attributed to enlarged embryos, higher rate of metabolic activity and respiration, better utilization and mobilization of metabolites to growth points and higher activity of enzymes as influenced by GA_3. This altered enzymatic and hormonal mechanism stimulate metabolic process such as sugar mobilization, protein hydrolysis, oxidation *etc*. (Earlplus and Lambeth, 1974) [5], which leads to increase in root length, shoot length and seedling dry weight, in turn increase in seedling vigour. The results are in close conformity with findings of Gurung *et al*. (2014) [7] in passion fruit

Shoot to root ratio

The data in respect to shoot to root ratio was recorded and presented in Table 4. Among the soaking time and different seed treatments, 24 hours soaking time (1.35) and GA_3 100 ppm (1.41) recorded significantly maximum shoot to root ratio at 60 days after sowing. Whereas, minimum shoot to root ratio was observed in 6 hours soaking time (1.23) and without any treatment-control (1.14). The results of study are in close agreement with the findings of Meshram *et al*. (2015) [13] in acid lime.

Table 1: Effect of seed treatment and soaking time on germination percentage and number of leaves per seedlings of wood apple seeds

Seed treatments	Germination percentage				Number of leaves per seedlings											
					30 DAS				45 DAS				60 DAS			
	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean
C_1	69.54	77.92	83.66	77.04	11.35	12.47	13.28	12.37	17.66	18.89	19.93	18.83	24.93	26.67	28.20	26.60
C_2	68.37	79.81	87.09	78.42	12.12	14.92	16.23	14.42	18.89	22.56	24.30	21.92	26.67	31.87	34.40	30.98
C_3	71.34	75.33	81.11	75.93	12.03	10.68	11.24	11.32	15.93	16.06	16.94	16.31	22.53	22.73	23.93	23.06
C_4	71.26	78.13	82.17	77.19	11.44	10.71	12.45	11.53	17.35	17.73	18.89	17.99	24.47	25.07	26.67	25.40
C_5	59.30	69.83	73.51	67.55	9.90	10.41	10.84	10.38	15.00	15.63	16.19	15.61	21.20	22.00	22.93	22.04
C_6	65.43	70.52	75.25	70.40	8.97	11.78	11.78	10.84	15.28	15.76	16.42	15.82	21.60	22.40	23.13	22.38
C_7	59.32	58.05	61.14	59.50	8.31	10.15	10.27	9.58	12.38	14.68	14.82	13.96	17.53	20.67	21.00	19.73
C_8	51.74	52.26	56.39	53.46	7.33	8.13	8.60	8.02	10.57	11.86	12.79	11.74	14.93	16.73	18.00	16.55
Mean	64.54	70.23	75.04	-	10.18	11.16	11.84	-	15.38	16.65	17.54	-	21.73	23.52	24.78	-
	SEm (±)		CD @ 5%		SEm (±)		CD @ 5%		SEm (±)		CD @ 5%		SEm (±)		CD @ 5%	
Soaking time (S)	0.38		1.08		0.43		1.22		0.43		1.22		0.40		1.14	
Seed treatments (C)	0.62		1.76		0.69		1.96		0.69		1.96		0.66		1.88	
Interaction (SxC)	1.08		3.07		1.20		NS		1.20		NS		1.14		NS	

Treatment details

DAS: Days after sowing

Factor-A (Soaking time)

S_1 - 06 hours
S_2 - 12 hours
S_3 - 24 hours

Factor-B (Seed treatments)

C_1 - GA_3 @ 50 ppm
C_2 - GA_3 @ 100 ppm
C_3 - KNO_3 @ 50 ppm
C_4 - KNO_3 @ 100 ppm
C_5 - Thiourea @ 50 ppm
C_6 - Thiourea @ 100 ppm
C_7 - Soaking in water
C_8 - Control (without any treatment)

Table 2: Effect of seed treatment and soaking time on seedling height (cm) of wood apple

Seed treatments	Seedling height (cm)											
	30 DAS				45 DAS				60 DAS			
	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean
C_1	6.33	6.37	7.07	6.59	9.17	9.55	10.6	9.77	12.67	12.73	14.13	13.18
C_2	6.50	7.17	7.68	7.12	9.58	10.75	11.53	10.62	13.00	14.33	15.37	14.23
C_3	5.72	6.03	6.93	6.23	8.61	9.05	10.4	9.35	11.43	12.07	13.87	12.46
C_4	5.90	6.18	7.05	6.38	8.88	9.61	10.58	9.69	11.79	12.37	14.10	12.75
C_5	5.30	5.65	6.04	5.66	8.05	8.48	9.06	8.53	10.60	11.30	12.08	11.33
C_6	5.32	6.02	6.67	6.00	7.98	9.03	10.01	9.01	10.63	12.03	13.35	12.00
C_7	4.53	5.22	5.58	5.11	6.89	8.16	8.37	7.81	9.05	10.43	11.15	10.21
C_8	4.31	5.13	5.32	4.92	6.79	8.04	8.01	7.61	8.61	10.27	10.63	9.84
Mean	5.49	5.97	6.54	-	8.24	9.08	9.82	-	10.97	11.94	13.09	-
	SEm (±)		CD @ 5%		SEm (±)		CD @ 5%		SEm (±)		CD @ 5%	

Soaking time (S)	0.11	0.31	0.14	0.40	0.21	0.60
Seed treatments (C)	0.17	0.48	0.23	0.65	0.34	0.97
Interaction (SxC)	0.30	NS	0.40	NS	0.60	NS

Treatment details **DAS:** Days after sowing

Factor-A (Soaking time) **Factor-B (Seed treatments)**

S_1 - 06 hours
S_2 - 12 hours
S_3 - 24 hours

C_1 - GA_3 @ 50 ppm C_3 - KNO_3 @ 50 ppm C_5 - Thiourea @ 50 ppm C_7 - Soaking in water
C_2 - GA_3 @ 100 ppm C_4 - KNO_3 @ 100 ppm C_6 - Thiourea @ 100 ppm C_8 - Control (without any treatment)

Table 3: Effect of seed treatment and soaking time on fresh weight of the seedlings, dry weight of the seedlings, seedling vigour index-I and seedling vigour index-II of wood apple

Seed treatments	Fresh weight of the seedlings				Dry weight of the seedlings				Seedling vigour index-I				Seedling vigour index-II			
	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean
C_1	8.24	10.52	13.42	10.73	3.19	4.07	5.19	4.15	881.72	993.48	1181.98	1019.06	221.70	317.02	434.40	324.37
C_2	12.21	13.32	15.31	13.61	4.72	5.15	5.92	5.26	888.81	1144.67	1337.41	1123.63	323.09	411.20	515.81	416.70
C_3	7.24	7.72	11.50	8.82	2.80	2.99	4.45	3.41	813.87	908.65	1125.51	949.34	199.85	225.09	360.84	261.93
C_4	8.21	8.33	12.32	9.62	3.17	3.22	4.76	3.72	841.47	967.22	1158.23	988.97	226.24	251.96	391.61	289.94
C_5	5.88	6.13	8.64	6.88	2.27	2.37	3.34	2.66	629.18	788.16	888.28	768.54	134.88	165.80	245.64	182.11
C_6	6.45	6.61	11.50	8.19	2.50	2.56	4.45	3.17	696.26	849.86	1006.3	850.81	163.44	180.38	334.78	226.20
C_7	5.69	5.74	7.35	6.26	2.20	2.22	2.84	2.42	537.68	606.12	681.16	608.32	130.56	128.93	173.72	144.40
C_8	5.54	5.54	6.05	5.71	2.11	2.14	2.34	2.20	446.37	537.16	599.05	527.53	110.78	111.89	132.20	118.29
Mean	7.43	7.99	10.76	-	2.87	3.09	4.16	-	716.92	849.42	997.24	-	188.82	224.03	323.63	-
	SEm (±)		CD @ 5%		SEm (±)		CD @ 5%		SEm (±)		CD @ 5%		SEm (±)		CD @ 5%	
Soaking time (S)	0.05		0.14		0.02		0.06		16.60		47.20		3.01		8.56	
Seed treatments (C)	0.08		0.23		0.03		0.09		27.10		77.06		4.92		13.99	
Interaction (SxC)	0.14		0.40		0.06		0.17		46.94		NS		8.52		24.23	

Treatment details

Factor-A (Soaking time) **Factor-B (Seed treatments)**

S_1 - 06 hours
S_2 - 12 hours
S_3 - 24 hours

C_1 - GA_3 @ 50 ppm C_3 - KNO_3 @ 50 ppm C_5 - Thiourea @ 50 ppm C_7 - Soaking in water
C_2 - GA_3 @ 100 ppm C_4 - KNO_3 @ 100 ppm C_6 - Thiourea @ 100 ppm C_8 - Control (without any treatment)

Table 4: Effect of seed treatment and soaking time on shoot to root ratio of wood apple Seedlings

Seed treatments	Shoot to root ratio			
	S_1	S_2	S_3	Mean
C_1	1.25	1.36	1.35	1.32
C_2	1.40	1.37	1.46	1.41
C_3	1.17	1.30	1.37	1.28
C_4	1.36	1.39	1.40	1.38
C_5	1.15	1.29	1.44	1.29
C_6	1.29	1.36	1.32	1.32
C_7	1.11	1.11	1.27	1.16
C_8	1.09	1.17	1.16	1.14
Mean (Soaking time)	1.23	1.29	1.35	-
	SEm (±)			CD @ 5%
Soaking time (S)	0.02			0.06
Seed treatments (C)	0.04			0.11
Interaction (SxC)	0.06			NS

Treatment details

Factor-A (Soaking time) **Factor-B (Seed treatments)**

S_1 - 06 hours
S_2 - 12 hours
S_3 - 24 hours

C_1 - GA_3 @ 50 ppm C_3 - KNO_3 @ 50 ppm C_5 - Thiourea @ 50 ppm C_7 - Soaking in water
C_2 - GA_3 @ 100 ppm C_4 - KNO_3 @ 100 ppm C_6 - Thiourea @ 100 ppm C_8 – Control (without any treatment)

Conclusion

Germination of wood apple seeds can be enhanced when seeds were treated with GA_3 @ 100 ppm for 24 hours. Further, this treatment resulted in maximum number of leaves per seedlings, height of seedlings, fresh weight of the seedlings, dry weight of the seedlings, seedling vigour index-I, seedling vigour index-II and shoot to root ratio. The lowest results were obtained in 6 hours soaked and without any treatment (Control).

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