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Effect of Seed Priming on Germination and Growth of Rough Lemon and Rangpur Lime Seedlings

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ABSTRACT: Citrus fruit belongs to Rutaceae family and it is one of the major horticultural crops grown in more than 100 countries worldwide. Rough lemon (*Citrus jambhiri*) and Rangpur lime (*Citrus limonia Osbeck*) is the most promising rootstock used for propagating mandarin in Central India. However, seed germination of these crops is very poor as well as late germination occurs. Slow seedlings growth bound to use as rootstocks. Rapid germination, maximum germination percentage and healthy seedlings stock are very much essential to fulfill the growing demands for budded and grafted quality planting material. Several studies have been indicated that seed priming will improve the germination and subsequent growth of seedlings in many fruits species. Therefore, pre-sowing seed treatment is very important and it can be done either by physical method such as scarification or by soaking in chemicals, growth regulators, organic substance etc. Cow urine affects the inhibitory response to seed germination, shoot growth and seedling vigor. Gibberellic acid (GA_3) is an environment-friendly growth regulator which being used in couples of fruits crops to enhance phenotypic traits besides breaking the dormancy. The present experiment was carried out in Asymmetric Factorial Completely Randomized Design with four replications. Treatments include seed priming (GA_3 200 ppm, cow urine 20% & control) and rootstock (rough lemon & rangpur lime). Seed priming with GA_3 @ 200 ppm found maximum germination percent, survival percent, height of seedling, number of leaves/ seedling, root shoot ratio, seedling vigour index I & II and minimum days taken to first germination. Maximum germination percent, survival percent, seedling height, root shoot ratio and seedling vigour index I and minimum days taken to first germination at 60 days after sowing was recorded under the seed of rough lemon whereas maximum seedling vigour index II was observed in rangpur lime. The effect of seedling on number of leaves with different rootstocks was observed as non-significant.

Seed priming in GA_3 200 ppm with Rough lemon seeds significantly influenced the root shoot ratio and seedling vigour index II but not significant in germination (%), taken to first germination, survival percent of seedlings, height of seedling, number of leaves/ seedling and seedling vigour index I. Hence, combination as seed priming in GA_3 200 ppm with Rough lemon seeds may be used for preparing seedlings/ rootstocks for further vegetative multiplication to fulfil the demand of quality planting material.

Keywords: Seed priming, GA_3, cow urine and Rough lemon.

INTRODUCTION

Citrus fruit belongs to Rutaceae family and it is one of the major horticultural crops grown in more than 100 countries worldwide. Citrus is the third most important fruit crop of India after mango and banana since it is grown almost all the tropical and subtropical region of the country. Citrus is widely grown in India with the area of 10,39,000 hectare and production 1,31,83,000 MT whereas mandarin occupied 4,37,000 hectare and production 53,80,000 MT (Anonymous, 2019).

Rough lemon (*Citrus jambhiri*) and Rangpur lime (*Citrus limonia Osbeck*) is the most promising rootstock for mandarin. Rough lemon is a cold-hardy citrus and commonly known as Jamberi is widely used rootstocks for propagating mandarin in Central India and it is faster growing rootstock as compared to other rootstocks as well as having better success rate. Rough lemon is an ancient hybrid variety crossed between mandarin and citron. Rough lemon grows as evergreen tree that can reach 3 to 6 meters in height. It is a very good rootstock for producing medium size healthy trees

however it is more prone to damage from root diseases, nematodes and water logging condition.

Rangpur, *Citrus reticulata* × *medica*, sometimes called rangpur lime, mandarin lime or lemandarin. It is the most utilized rootstock for its specific qualities *i.e*. due to its heavy and early bearing, and drought resistance. Rangpur lime is slow growing rootstock however it has maximum productivity and resistance to tristeza disease (Kharpe, 2015). It is the principal rootstock in Brazil and Argentina for sweet oranges, mandarins and grapefruit. It is highly resistant to tristeza and does well in heavy soils, more tolerant to salts than others, susceptible to exocortis and trees on this stock are vigorous, precocious and prolific with quality produce.

Rootstocks modulate several characteristics of citrus trees, including vegetative growth, yield, quality and tolerance to different biotic and abiotic stresses. Rough lemon (*Citrus jambhiri*) and Rangpur lime (*Citrus limonia* Osbeck) is the most promising rootstock for mandarin.

Seed germination is a complex physiological process which is mainly depends on environmental signals such as moisture, temperature, humidity, light, nitrate etc. While, poor seed germination is the major limiting factor of some of the important fruit crops including Rough Lemon and Rangpur Lime. It has been postulated that seed coat (testa) of many fruit species contains considerable amount of germination inhibitor viz., benzoic acid, cinnamic acid, coumarin, naringenin, jasmonic and abscisic acid (ABA), which prevent their germination. However, the seed germination percentage of many fruit crops is very poor as well as late germination occurs. Slow seedlings growth bound to use as rootstocks. Rapid germination, maximum germination percentage and healthy seedlings stock are very much essential to fulfil the growing demands for budded and grafted quality planting material. Seed without use of growth regulatators showed poor response to germination and growth (Dongre, 2007).

Several studies have been indicated that seed priming will improve the germination and subsequent growth of seedlings in many fruits species. Therefore, pre-treating seeds of fruit crops is very important and can be done either by physical method such as scarification or by soaking in chemicals, growth regulators water, organic substance like cow urine, cow dung slurry etc. Cow urine may bring a breakthrough in the present context as it is free of cost and easily available. Cow urine contains nitrogen, sulphur, ammonia, copper, iron, urea, uric acid, phosphate, sodium, potassium, manganese, carbolic calcium, salt, vitamins, lactose, enzyme, water, creatinin, aurum hydroxide etc (Dongre *et al*., 2014). The cow is a mobile medical dispensary and cow urine is a panacea of all diseases (Pathak and Kumar 2003). Cow urine contains iron, urea, uric acid, estragon and progesterone which affect the inhibitory response to seed germination, shoot growth and seedling vigor (Dilrukshi, 2009).

There is a lot of information that show how plant growth regulators encourage the biochemical changes in plants, which in induce vegetative and reproductive responses (Dongre *et al*., 2021). Growth regulators play a vital role in improving the vegetative growth and yield traits (Bose *et al*., 2009). Gibberellic acid (GA_3) is an environment-friendly growth regulator which being used in multiple fruits crops to enhance phenotypic traits including dormancy breaking. Therefore, the present study was planned to compare germination performance of different seed priming application in rough lemon and rangpur lime seedlings.

MATERIAL AND METHODS

The experiment was carried out at Zonal Agricultural Research Station, Chhindwara (M.P.) India under Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur. The experimental was consisted in Asymmetric Factorial Completely Randomized Design with four replications. Treatments include seed priming (GA_3 200 ppm, cow urine 20% & control) and rootstock (rough lemon & rangpur lime) with six treatments combination i.e. R1P1 - Rough lemon seed priming with water, R1P2 -Rough lemon seed priming with GA_3 200 ppm, R1P3 -Rough lemon seed priming with cow urine 20%, R2P1 - Rangpur lime seed priming with water, R2P2 - Rangpur lime seed priming with GA_3 200 ppm and R2P3 - Rangpur lime seed priming with cow urine 20%. The normal, healthy and uniform sized 100 seeds, each of rough lemon and Rangpur lime were selected separately for six treatments. Observations were recorded on germination parameters i.e. germination percent, days taken to first germination , survival percent of seedlings, seedling height, number of leaves/ seedling , root shoot ratio, seedling vigour index I & seedling vigour index II at 60 days after sowing influenced by the seed priming, rootstocks and its interaction.

Collection of fruits and seed extraction. Fully ripened, uniform sized and true to type fruits were collected from genuine and healthy rough lemon and rangpur lime tree from mother block of ZARS, Chhindwara. Subsequently fruits are cut into two pieces and seeds were extracted carefully, washed with clean water and spread in shade for 3-4 hours.

Preparation of Seed priming solution. The priming of seeds for each treatment was carried out for 3 hours. Seeds soaked with distilled water were considered as control. For making of 200 ppm GA_3 solution, 200 mg GA_3 weighed by the help of electronic balance and dissolved ethyl alcohol 10 ml in beakers. The distilled water was added to make up the volume equal to one liter to obtain desired concentrations of solutions. For making of 20 % cow urine taken 20 ml cow urine and added it in 100 ml water and thoroughly mixed it for solution. Fresh one liter cow urine is collected from Desi (indigenous) breed cow, in the morning from the cattle shade.

Seed priming. The normal, healthy and uniform sized 100 seeds, each of rough lemon and Rangpur lime were selected separately for six treatments. The concentrations of GA_3 200 ppm, cow urine 20% and distilled water 100 ml solution of each concentration was taken in 100 ml beaker and seeds were primed thoroughly.

Sowing of treated seeds. The treated seeds of both

rootstocks (rough lemon and rangpur lime) were sown separately on raised bed by keeping the distance of 10 cm row to row and 5 cm plant to plant in plastic tray. Randomization was followed for treatment distribution within replication. Each treatment contained 150 seeds and 25 seeds were placed replication wise. After sowing the intercultural operations like regular watering, weeding and plant protection measures were followed properly. Drenching of copper fungicide (1.0%) was taken twice for control of damping off disease during the early period of investigation.

Germination (%). The germination count was taken 10 days after sowing at an interval of 5 days. The seeds germinated were counted till completion of germination and the rate and percentage of seed germination was calculated by the following formula.

$$\text{Germination (\%)} = \frac{\text{Total no. of seeds germinated}}{\text{Total no. of seeds sown}} \times 100$$

Days taken to first germination. The days taken to seed germination were recorded in days from date of seed sowing to first emergence of seedling.

Survival of seedling (%) at 60 DAS. The survival percentage of each treatment was recorded at 180 days after seed sowing. The survival percentage was calculated by using formula as given below:

$$\text{Survival (\%)} = \frac{\text{No. of survived seedlings}}{\text{Total no. of seedlings}} \times 100$$

Height of seedling (cm) at 60 DAS. The seedling height was recorded at 60 days after sowing. The length from the collar region to the tip of the shoot apex was measured. The height of ten plants in each treatment was measured in centimeters with the help of meter scale and the average value was reported.

Number of leaves at 60 DAS. The Number of leaves of ten plants in each treatment was counted at 60 DAS and the average value was reported.

Root shoot ratio (%) at 60 DAS. Separated the root part from the top portion (cut at soil line base), separately weighed and recorded the root and shoot for each plant.

$$\text{Root/ Shoot ratio (\%)} = \frac{\text{Dry weight for roots (g)}}{\text{Dry weight for shoots (g)}} \times 100$$

Seedling vigor index I (cm). It was calculated by adding the values of root length and shoot length at 30 days after sowing. These were randomly selected and multiplied with their corresponding germination percentage. Thus, the values were recorded.

Seedling vigour index **I** = dry weight of seedlings (g) × [root length (cm) + shoot length (cm)]

Seedling vigor index II (g). It was calculated by multiplying dry weight of seedlings with their corresponding germination percentage.

Seedling vigour index II = dry weight of seedlings (g) × germination percentage

RESULT AND DISCUSSION

The results of the present investigation show that the germination parameter i.e. germination percent, days taken to first germination, survival percent of seedlings, seedling height, number of leaves/ seedling, root shoot ratio, seedling vigour index I and seedling vigour index II significantly influenced by the seed priming, rootstocks and its interaction.

Seed priming Effect. The maximum germination percent (85.09%), survival percent of seedlings (78.96 %), height of seedling (7.97 cm), maximum number of leaves/seedling (7.33), root shoot ratio (0.54), seedling vigour index I (685.23 cm), seedling vigour index II (23.01 g) and minimum days taken to first germination (15.13) was recorded under seed priming with GA_3 @ 200 ppm.

The promoting of germination may be due to the antagonistic effect of GA_3 against influence of inhibitors (Brain and Hemming, 1958 and Wareing *et al*., 1968) and endogenous gibberellin increased by soaking (Mathur *et al*., 1971). Additionally, GA_3 participates in stimulation of protein synthesis which causes production of mRNA thereby the DNA replication is increased and analysis of seed endospermic materials are induced (Lahuti *et al*., 2003). The hormone of GA_3 induces different emergence processes in the seed such as absorption of growth inhibitors and initiation of enzymes which is essential for seed germination.

Seed priming with gibberellic acid exhibited significant effect on seedling survival percent and found maximum survival percent of seedling at 60 days after sowing. GA_3 might have enhanced the early germination and fast growth of seedling by cell multiplication and cell elongation subject to the enhanced seedling growth resulting maximum percent of seedling survival. The results found in present investigation are in conformity with the results of Parmer *et al*, (2019) in acid lime and Yadav *et al*. (2018) in custard apple.

The enhancement in seedling height under GA_3 treatment may have occurred due to increased osmotic uptake of nutrients by this hormone which caused cell elongation and enhancement of seedling height (Shanmugavelu, 1966).

The increase in the number of leaves per seedling in GA_3 possibly due to the induced cell division and cell growth by the movement of GA_3 to the shoot apex which causes in increase of the young leaves (Salisbury and Ross 1988). This also helps in invigoration of physiological process of plant and stimulatory effect of chemicals to form new leaves at faster rate as suggested by Shaban (2010).

The roots can be developed and maintained by the sufficient supply of carbohydrates from shoots. The promising effect of GA_3 on fresh weight of roots may be due to the acceleration in the translocation and assimilation of auxins, the assimilation and redistribution of materials in plants which causes better growth and vegetative characters which enhance the growth attributes (Pandiyan *et al*., 2011).

It is cleared from the data showed that seed priming with GA_3 was found significant with respect to Seedling Vigour Index I and II. The reason may be due to the increased germination percent and dry matter production of the rough lemon seedlings. The hike in vigour may be due to the direct influence on the

extensive growth of seedlings probably by increasing mobilization of reserve foods to growing tops. The findings are supported by Parmar (2019) in acid lime.

Table 1: Effect of seed priming and rootstock on germination (%), days taken to first germination, survival (%), and seedling height (cm) at 60 days after sowing.

Treatments	Seed germination			Days taken to first germination			Survival of seedling (%) at 60 DAS			Seedling height (cm) at 60 DAS		
	Rough lemon	Rangpur Lime	Mean	Rough lemon	Rangpur Lime	Mean	Rough lemon	Rangpur Lime	Mean	Rough lemon	Rangpur Lime	Mean
Distilled Water	73.82	68.14	70.98	21.20	23.03	22.11	58.76	54.17	56.46	5.42	5.08	5.25
GA_3 200 ppm	87.05	83.14	85.09	14.75	15.50	15.13	81.31	76.61	78.96	8.14	7.80	7.97
Cow urine 20%	81.22	76.28	78.75	17.80	18.80	18.30	74.48	69.56	72.02	7.70	7.59	7.65
Mean	80.70	75.85		17.92	19.11		71.51	66.78		7.08	6.82	
	R	**P**	**R × P**	**R**	**P**	**R × P**	**R**	**P**	**R × P**	**R**	**P**	**R × P**
SE(m)±	0.300	0.368	0.520	0.134	0.165	0.233	0.278	0.340	0.481	0.055	0.068	0.096
CD 5%	0.892	1.092	NS	0.399	0.489	NS	0.826	1.011	NS	0.165	0.202	NS

Table 2: Effect of seed priming and rootstock on number of leaves, root shoot ratio, seedling vigour index I (cm) and seedling vigour index II (g) at 60 days after sowing.

Treatments	Number of leaves at 60 DAS			Root shoot ratio (%) at 60 DAS			Seedling vigour index I (cm)			Seedling vigour index II (g)		
	Rough lemon	Rangpur Lime	Mean	Rough lemon	Rangpur Lime	Mean	Rough lemon	Rangpur Lime	Mean	Rough lemon	Rangpur Lime	Mean
Distilled Water	5.28	5.03	5.15	0.38	0.36	0.37	404.65	350.69	377.67	15.13	17.35	16.24
GA_3 200 ppm	7.35	7.30	7.33	0.57	0.51	0.54	715.17	655.30	685.23	23.94	22.07	23.01
Cow urine 20%	6.35	6.25	6.30	0.48	0.51	0.49	632.53	585.45	608.99	16.51	19.45	17.98
Mean	6.33	6.19		0.48	0.46		584.12	530.48		18.53	19.62	
	R	**P**	**R × P**	**R**	**P**	**R × P**	**R**	**P**	**R × P**	**R**	**P**	**R × P**
SE(m)±	0.045	0.056	0.079	0.003	0.004	0.006	4.785	5.860	8.288	0.154	0.189	0.267
CD 5%	NS	0.165	NS	0.010	0.012	0.017	14.217	17.412	NS	0.459	0.562	0.794

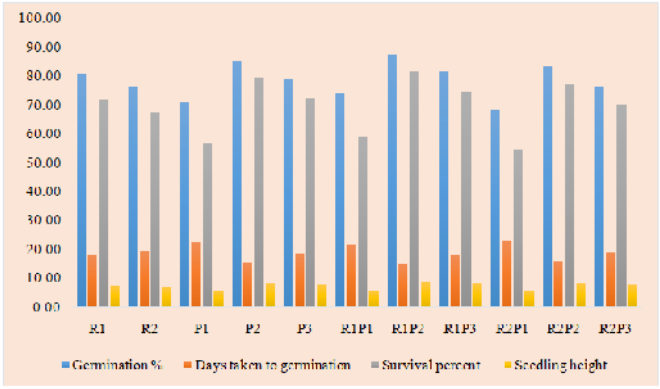

Fig. 1. Effect of different seed priming (P), rootstock (R) and its interaction (RP) effects on different parameter.

Rootstock Effect. With regards to rootstocks maximum germination percent (80.70 %), minimum days taken to first (17.92) and maximum survival of seedlings (71.51 %), seedling height (7.08 cm), root shoot ratio (0.48) and seedling vigour index I (584.12 cm) at 60 days after sowing were recorded under seed of rough lemon rootstock whereas maximum seedling vigour index II (19.62) was observed in rangpur lime rootstock seedlings. The effect of rootstock on number of leaves with different rootstock was observed to be non-significant.

Rough lemon is a faster growing rootstock as compared to other rootstocks as well as having better success rate. Rough lemon is the quickest growing and high vigour of all common rootstocks. Due to fast and early germination character of rough lemon gives maximum germination percent and take minimum days for germination of all seeds. After germination seedling growth quickly and attain maximum height. Plant height is assumed to be a controlled character genetically. Maximum germination percent and length of shoot and root directly co related to root shoot ratio and seedling vigour index I. It is also the easiest rootstock to propagate and has the longest propagation season. The Rough Lemon is cold hardy and well established in dry environments also. It grows easily in old citrus soil. The result was closely related with findings of Qadri *et al.* (2021) who reported that the rough lemon seed has shown a significant effect on seed growth and germination.

Whereas maximum seedling vigour index II (19.62 g) at 60 days after sowing was recorded in Rangpur lime rootstock seeds. Rangpur lime seedlings found small spines along with more leaves hence fresh weight found maximum. If fresh weight is maximum then ultimately dry weight of seedling would be higher additionally spines have woody nature and less moisture content comparison to leaves. Seedling vigour index II is dry weight of seedlings with their corresponding germination percentage. Dry weight was found more in rangpur lime seedling because of more fresh weight.

Interaction Effects. Combined effect of seed priming

and rootstocks significantly influenced the root shoot ratio (0.57), seedling vigour index II (23.94 g) was recorded under rough lemon priming of seed with GA_3. The effect of seed priming with different rootstock on germination (%), taken to first germination, survival percent of seedlings, height of seedling (cm), number of leaves/seedling, and seedling vigour index I (cm) was observed to be non-significant. Rough lemon is a faster growing rootstock as compared to other rootstocks as well as having better success rate. Gibberellins are well known for inter nodal cell elongation and higher accumulation of photosynthesis through the improved rate of photosynthesis by GA_3 thereby leading the increase in most of the germination and growth parameter such as root shoot ratio, seedling vigour index.

CONCLUSIONS

On the basis of results found in the present investigation, it is, concluded that seed priming with GA_3 @ 200 ppm found maximum germination percent , survival percent, height of seedling, number of leaves/seedling, root shoot ratio, seedling vigour index I & II (23.01 g) and minimum days taken to first germination.

With regard to rootstock in rough lemon rootstock found maximum germination percent, survival percent, seedling height, root shoot ratio and seedling vigour index I and minimum days taken to first germination at 60 days after sowing was recorded under seed of rough lemon rootstock whereas maximum seedling vigour index II was observed in rangpur lime rootstock seedlings. The effect of rootstock on number of leaves with different rootstock was observed to be non-significant.

Seed priming in GA_3 200 ppm with Rough lemon seeds significantly influenced the root shoot ratio and seedling vigour index II but not significant in germination (%), taken to first germination, survival percent of seedlings, height of seedling (cm), number of leaves/seedling, and seedling vigour index I (cm).

FUTURE SCOPE

Seed priming with GA_3 200 ppm along with Rough lemon seeds effectively useful for maximum germination percent, seedling height and overall growth of seedlings. The combination of treatment may be used for preparing seedlings/ rootstcoks for further vegetative multiplication i.e. micro budding. Extensive research is therefore required towards Seed priming in GA_3 200 ppm with Rough lemon seeds for producing early and healthy seedlings.

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Conflict of Interest. None.

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