

## Trifoliate Orange Seed Germination Enhancing Method in Mid Hill of Nepal

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### ABSTRACT

Trifoliate orange seedlings are used as rootstock for citrus crops due to cold hardiness and phytophthora disease tolerance. Nursery owners usually prepare raised bed in open field to sow seeds of trifoliate. The trifoliate seed production in government farms are very low compared to its demand due to limited number of fruiting trees. Further the germination percent in open nurseries are very low. Hence, production of grafted sapling is very much affected by unavailability of rootstock. Therefore, a study was carried out in National Citrus Research Program, Dhankuta to increase germination rate of trifoliate orange in the year 2016 and 2017. Trifoliate seeds were extracted from three stages of fruits (green mature, half yellow and full yellow) and sown at three dates (September 1<sup>st</sup> week, September 3<sup>rd</sup> week and October 1<sup>st</sup> week) at three raised bed (open field, 50-cm low plastic tunnel with or without sides open). The data on percentage seed germination was taken at 35, 50, 65, 90 and 210 days after seed sowing. The fruit maturity had no effect on germination percentage irrespective of sowing date and method of beds used. The date of sowing resulted significantly higher germination at earlier days but there was non-significant effect during final germination count. Plastic tunnel with open sides resulted 80% germination compared to 46% in tunnel side closed nursery. Hence, low plastic tunnel raised bed having open sides is the best option to raise trifoliate seed sown during 1<sup>st</sup> week of September to 1<sup>st</sup> week of October in agro-climatic conditions like Dhankuta.

**Keywords:** Citrus, germination percent, maturity, nursery beds, seed

### सारांश

तीनपाते सुन्तलालाई सुन्तला बर्गका फलफूल कलमी गर्न रुटस्टकको रूपमा प्रयोग गरिन्छ। नर्सरी धनीहरूले यसको विरुवा बीउबाट खुल्ला आकाशमा नर्सरी बेड बनाएर तयार गर्छन्। एक त तीनपातेको बीउ कम मात्रामा सरकारी फार्महरूबाट उपलब्ध हुन्छ भने उमार प्रतिशत कम भएकोले भने जति मात्रामा कलमी विरुवा निकाल्न नसकी रहेको अवस्था छ। तीनपातेको उमार शक्ति कसरी बढाउन सकिन्छ भनेर सन् २०१६ र २०१७ मा राष्ट्रिय सुन्तलाजात अनुसन्धान कार्यक्रम, धनकुटामा परिक्षण संचालन गरिएको थियो। यस परिक्षणमा तीनवटा अवस्थाका तीनपाते फलहरू (पूर्ण वयस्क हरियो, आधा पाकेको र पूर्ण पाकेको) बाट बीउ निकाली तीन वटा मितीमा (सेप्टेम्बर पहिलो, सेप्टेम्बर तेश्रो र अक्टोबर पहिलो हप्ता) तीन प्रकारका नर्सरी बेडहरूमा (खुला जमिन, ५० सेमी उचा प्लास्टिक टनेलको दुई मुख खुल्ला र बन्द गरि) बीउ छरिएको थिए। बीउको उमार शक्तिको रेकर्ड बीउ रोपेको ३५, ५०, ६५, ९० र २१० दिनमा लिइएको थियो। विभिन्न पाकेको अवस्थामा रहेका फलको कारण बीउ उमार शक्तिमा कुनै असर नपरेको पाइयो। त्यसै गरि बीउ लगाउने मितीको असर २१० दिन सम्ममा उस्तै पाइयो तर अघिल्ला मितीहरूमा रोपेको बीउमा अघिल्ला दिनहरूमा धेरै उमार शक्ति पाइयो। नर्सरी बेडको बीउ उमार शक्तिमा राम्रोसंग असर परेको देखियो। टनेलको मुख बन्द राख्दा (४६ प्रतिशत) भन्दा खुल्ला राख्दा (८० प्रतिशत) बढी बीउको उमार शक्ति पाइयो। धनकुटा जस्तो आवहावा भएको स्थानमा सेप्टेम्बर पहिलो साता देखि अक्टोबर पहिलो सातासम्म टनेल बनाइ मुख खुला राखी तीनपाते सुन्तलाको बीउ रोप्न सुझाव दिइन्छ।

### INTRODUCTION

Quality planting material of citrus crop means budded and grafted plants. Grafted plants require scion of a distinct variety with a compatible and recommended rootstock variety. Citrange and trifoliate oranges are the preferred rootstock varieties in Nepal due to cold and dry hardiness as well as phytophthora disease tolerance (Pun et al 2015). A rootstock for citrus plant is usually of seedling

origin. The seeds of rootstock varieties (esp. trifoliate orange) are produced in limited quantities (approx. 200kg) by three horticulture farm/stations (Central Horticulture Centre, Kritipur; Horticultural Research Station, Dailekh; and National Citrus Research Program, Dhankuta) in Nepal (NCDP 2016, NCRP 2017). Depending upon geography, the trifoliate fruits mature from September to November in Nepal before the onset of winter season. The seeds are extracted and shade dried for few days (3-5 days) before sowing in open field by the nursery man (Kaini 2003). Usually half to fully ripe (yellow) fruits are harvested to extract seeds. The trifoliate seeds being recalcitrant need to be stored at 4°C if not immediately used. The seeds can be stored up to six month with varying degree of viability loss and further affected by fungus if not treated with proper fungicide before storage (Mobayen 1980). Depending upon soil temperature and other climatic factor the seed may require 25-35 days to initiate germination (Wiltbank et al 1995) and up to 8 months for full germination (NCRP 2016). Nursery men usually plant seed in raised bed with herb mulch. In horticulture farm/stations seeds are sown in raised bed covered with herb mulch under an arched polythene low tunnel of 50 cm height. There is an issue of poor germination (around fifty percent) under NCRP farm condition (NCRP 2015). Therefore, a study was designed to find out appropriate harvesting and sowing time of trifoliate seed which could give the best germination rate under different types of nursery beds.

## MATERIALS AND METHODS

To mitigate poor germination problem of trifoliate seed, an experiment was laid out in factorial RCB design with three replications in year 2016/17 at NCRP farm located at an altitude of 1250 m with geographical location 27°1' north latitude and 87°18' east longitude. Trifoliate seed extracted from three maturity stages of fruit (green mature, half yellow and full yellow) were sown on four dates (12<sup>th</sup> August, 20<sup>th</sup> August, 28<sup>th</sup> August and 7<sup>th</sup> September) in three types of nursery beds. In farmers' method, raised bed was prepared, seed were sown without using plastic cover; where as in other two methods: 50 cm high arched plastic tunnel (200 micron) was established using bamboo with both sides open in Dailekh method and closed in NCRP method. Based on first year's finding, the experiment was repeated in year 2017/18 with four replications comprising same three maturity stages of fruit but with three different dates of sowing (1<sup>st</sup> week of Sept, 3<sup>rd</sup> week of Sept and 1<sup>st</sup> week of Oct) in three type of nursery beds (Farmer's method in Dhankuta, Dailekh and NCRP method). Seeds were sown in 10-m x 0.75-m dimension bed at 10-cm line distance with 3-cm seed to seed distance. The seed beds were prepared one month earlier and covered with plastic mulch for soil solarization (100 micron). During seeding time 30kg vermi-compost was applied to each bed. In each sub-plot, 100 seeds (with seed coat) were sown two cm deep into soil and mulched with dried herbs (*Imerata cylindrical*, Cogon grass). The seeds used in this experiment were extracted 6 days before first sowing date, shade dried, treated with 2 g/kg Bavistin fungicide and kept refrigerated at 4°C until sown. The germination percentage was recorded after seven months in first year trial while it was recorded at 35, 50, 65, 90 and 210 days after sowing (DAS) in second year trial.

## RESULTS

There was no difference on seed germination percent due to maturity stage of fruit on all observation days (Table 1 and 2). However, date of sowing has shown significantly different on germination percent at 35 DAS and 65 DAS with no difference in the result at final count (Table 2). Moreover, types of nursery bed showed significantly different germination percentage on all observation dates except 90 DAS (Table 2) while there was non-significant effect of date of sowing when seeds were sown on 12<sup>th</sup> August to 7<sup>th</sup> September in first year (Table 1). Therefore, in second year seed sowing was started from first week of September. Farmers could sow seed from any maturity stage fruit from 1<sup>st</sup> September to 1<sup>st</sup> week of October provided the seeds are kept in 4-8°C condition treating with Bavistin (2g/kg) if not used immediately after extraction. In case of nursery bed, there was earlier and more germination with NCRP method (tunnel side closed) but seedlings died due to heat stress within one month of sowing and that was not the problem with Dailekh method (tunnel sides open) (Table 2). There was cold injury symptom with farmer's method three months after sowing seed and plastic tunnels were raised to protect the seedlings during the winter season. The highest percentage germination on Farmers' method is due to this reason otherwise Dailekh method is hassle free way of seed sowing. Therefore there is still hope of more than 80% germination with closing tunnel sides for

30 DAS and opening the sides till 90 DAS during day time and closing it at night until winter ceases (Table 2).

**Table 1.** Trifoliolate seed germination percentage after seven months of sowing as affected by maturity stage, sowing date and nursery bed structure at NCRP Dhankuta in year 2016/17

Fruit Maturity	Germination %
Mature green	34.69
Half yellow	41.78
Full yellow	42.61
Date of Sowing	
12 <sup>th</sup> Aug	32.81
20 <sup>th</sup> Aug	35.70
28 <sup>th</sup> Aug	28.78
7 <sup>th</sup> Sept	61.48
Nursery Method	
Dailekh method	44.98
Farmer method	34.96
NCRP method	39.83
Mean	39.69
P value (Maturity)	Ns
(Sowing date)	***
(Nursery bed)	Ns
LSD (0.05%)	10.08
CV%	44.01



**Figure 1.** Mean weekly air temperature (°C) at NCRP, Dhankuta during experimental period in year 2017/18.

**Table 2.** Trifoliolate seed germination percentage affected by maturity stage, sowing date and nursery bed structure at NCRP Dhankuta in year 2017/18

Fruit Maturity	35 DAS	50 DAS	65 DAS	90 DAS	210 DAS
Mature green	25.89	33.92	32.39	38.42	64.81
Half yellow	23.19	31.81	35.47	31.33	70.72
Full yellow	22.89	32.17	35.03	32.12	67.28
Date of Sowing					
1 <sup>st</sup> wk of Sept	19.86	29.78	32.36	33.14	67.69
3 <sup>rd</sup> wk of Sept	23.33	35.33	40.22	-	68.75
1 <sup>st</sup> wk of Oct	28.78	32.78	30.31	34.78	66.36
Nursery Method					
Dailekh	16.36	37.58	40.86	38.92	75.83
Farmer	20.61	31.75	33.00	33.33	80.00
NCRP	35.00	28.56	29.03	29.62	46.97
Mean	23.99	32.63	34.30	33.96	67.60
P value (Maturity)	Ns	Ns	Ns	Ns	Ns
(Sowing date)	*	Ns	*	Ns	Ns
(Nursery bed)	***	*	**	Ns	***
LSD (0.05%)	6.71	7.08	7.47	9.12	7.72
CV%	57.81	46.37	46.58	46.62	24.41

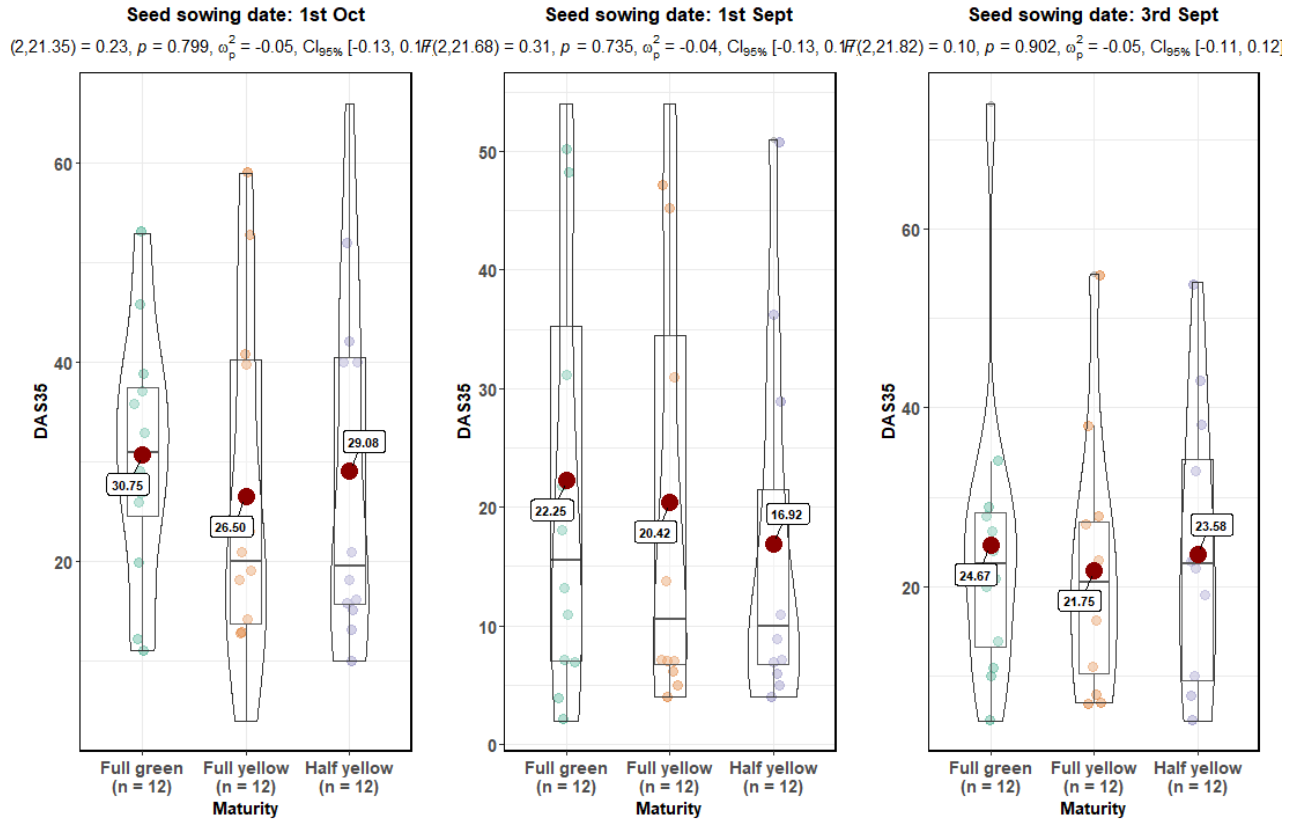


Figure 2. Effect of trifoliolate seed sowing date on seed germination at 35 days after seed sowing (the circle value in the middle of boxplot represent mean % germination)

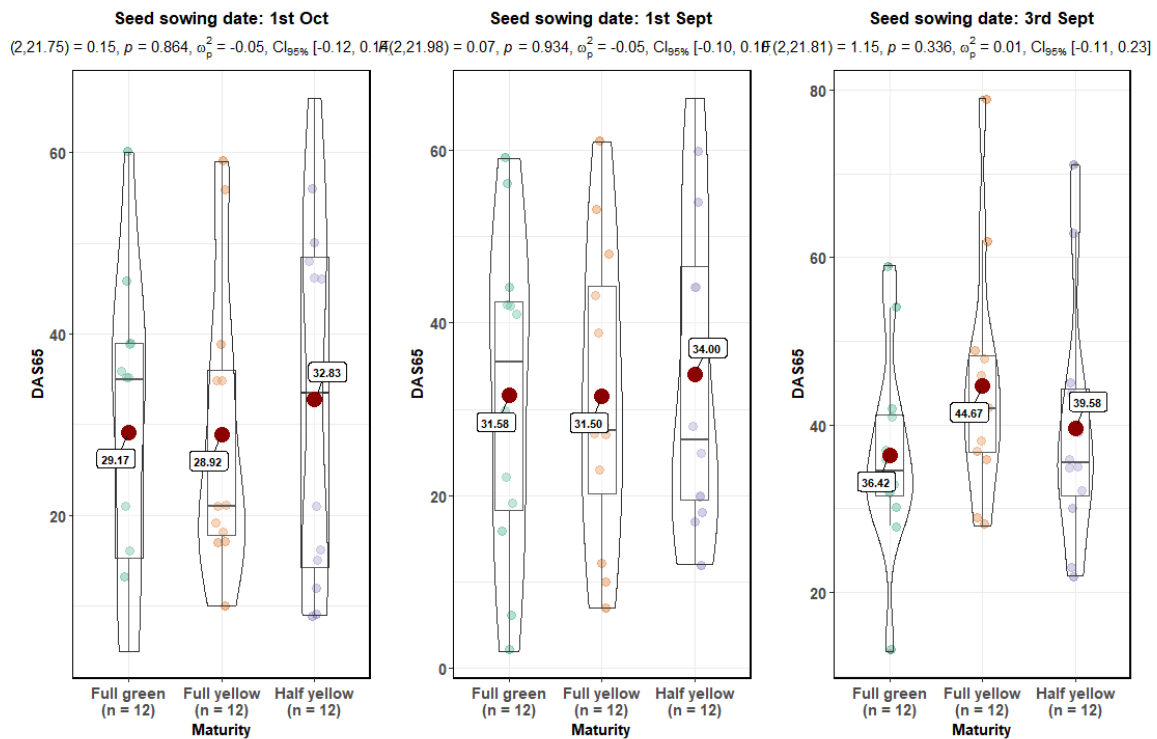
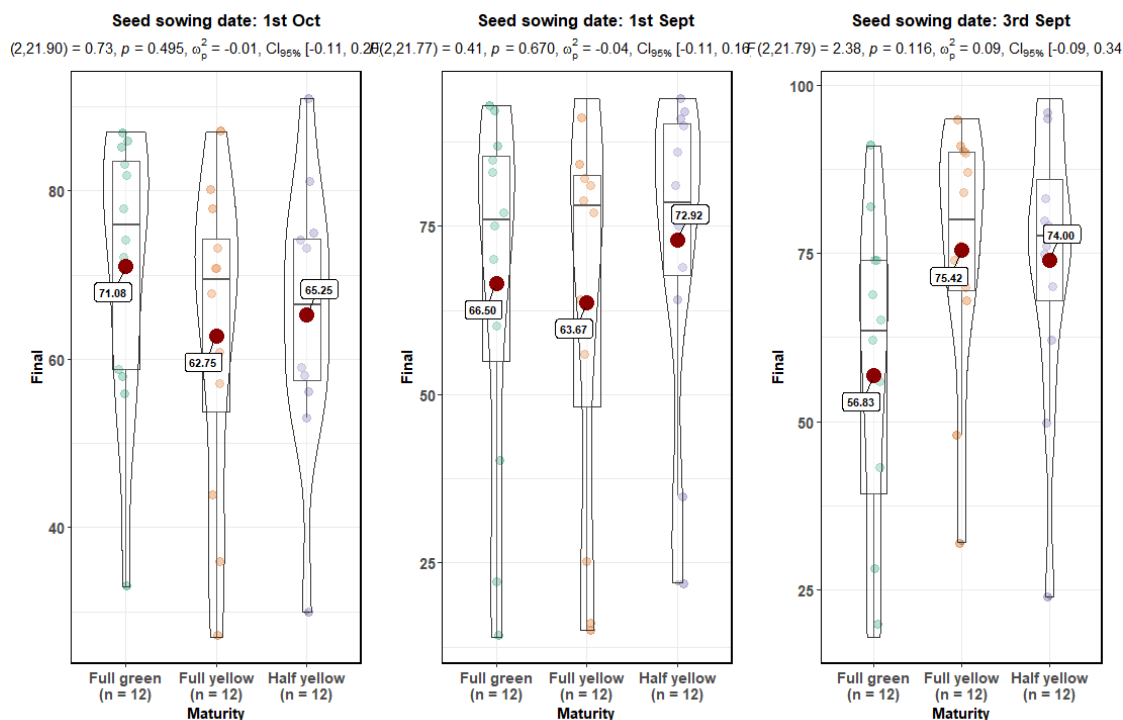


Figure 3. Effect of trifoliolate seed sowing date on seed germination at 65 days after seed sowing (the circle value in the middle of boxplot represent mean % germination).



**Figure 4.** Effect of trifoliolate seed sowing date on seed germination at 210 days after seed sowing (the circle value in the middle of boxplot represent mean % germination).

## DISCUSSION

All citrus rootstock species germinate within soil temperature range of 15-40°C with varying percentage of germination (Camp et al 1933). However, the congenial soil temperature for trifoliolate seed germination is between 31-35°C (Wiltbank et al 1995) which will likely reach during April to September months in mid hills of Nepal (Figure 1). Further, the trifoliolate seed enters into dormancy stage when temperature is 13.9°-15.6°C, which could be the reason for low germination during 65-120 DAS (Table 2). Nevertheless, storing a September harvested trifoliolate seed till March-April under ambient condition has no help as it loses viability very quickly and all citrus nursery owner could not store it between 4-6°C during that time (Mobayen 1980). Several researcher reported that 4°C higher soil temperature than air temperature inside the low tunnel during mid-day and nearly equal during night (Salman et al 1992, Arin and Ankara 2001, Diaz-Perez 2009). This high temperature could be the reason for newly emerged seedling mortality in sides closed tunnels (NCRP method at 90 DAS) (Table 2). In contrary, it was necessary to raise a tunnel in open field nursery (farmer's method) during December to February (Fig 1) due to temperature below 10°C accompanied with frost. This will protect young seedlings in one hand and prevent non germinated seed entering into dormancy, thus increasing seed germination as shown in Table 2.

The seed germination percentage was not significantly affected by the maturity of trifoliolate seed irrespective of days of data recording (Figure 2-4). The slower germination rate of trifoliolate irrespective of maturity stage during earlier days (12-18 weeks) and final germination percentage (near 80%) during 24-46 weeks was also reported by (Mobayen 1980). The below 90% germination percentage of trifoliolate seed was also reported by (Saipari et al 1998) and suggested that it is due to hindrance in water imbibition and gas exchange by the seed coat. Removal of seed coat is one way of increasing the seed germination but in commercial scale this is not feasible.

## CONCLUSION

The germination percent of trifoliolate seed could be increased with better nursery management practice. Open bed and low tunnel with closed sides are not good practice to follow. A seed from

mature trifoliolate fruit sown during September under plastic tunnels having sides open is recommended to enhance the trifoliolate germination under mid hills condition of Nepal.

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