Trifoliate Orange Seed Germination Enhancing Method in Mid Hill of Nepal

Umesh K Acharya and Roshan Pakka

National Citrus Research Program, Dhankuta, Nepal; umeshach@gmail.com, roshanpakkarp@gmail.com

Received 5 Jan 2019, Revised 25 Jan 2019, Accepted 25 Feb 2019, Published 28 April 2019

ABSTRACT

Trifoliate orange seedlings are used as rootstock for citrus crops due to cold hardiness and phytopthora 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 1st week, September 3rd week and October 1st 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 1st week of September to 1st week of October in agro-climatic conditions like Dhankuta.

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

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 phytopthora disease tolerance (Pun et al 2015). A rootstock for citrus plant is usually of seedling
Trifoliate orange seed germination by UK Acharya and R Pakka

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 (12th August, 20th August, 28th August and 7th September) in three types of nursery beds. In farmers’ method, raised bed was prepared, seed were sown without using plastic cover; whereas 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 (1st week of Sept, 3rd week of Sept and 1st 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 12th August to 7th 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 1st September to 1st 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.** Trifoliate 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  | Germination % |
|-----------------|----------------|
| 12th Aug        | 32.81          |
| 20th Aug        | 35.70          |
| 28th Aug        | 28.78          |
| 7th Sept        | 61.48          |

| Nursery Method  | Germination % |
|-----------------|----------------|
| 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.** Trifoliate 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  | Germination % |
|-----------------|----------------|
| 1st wk of Sept  | 19.86          |
| 2nd wk of Sept  | 35.33          |
| 3rd wk of Sept  | 35.33          |

| Nursery Method  | Germination % |
|-----------------|----------------|
| Dailekh method  | 16.36          |
| Farmer method   | 20.61          |
| NCRP method     | 35.00          |

| Mean            | 23.99          |
| P value (Maturity) | Ns             |
| (Sowing date)    | *              |
| (Nursery bed)    | **             |
| LSD (0.05%)      | 6.71           |
| CV%              | 57.81          |

64
**Figure 2.** Effect of trifoliate 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 trifoliate 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 trifoliate 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 trifoliate 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 trifoliate 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 trifoliate 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 trifoliate seed irrespective of days of data recording (Figure 2-4). The slower germination rate of trifoliate 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 trifoliate 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 trifoliate 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 trifoliate fruit sown during September under plastic tunnels having sides open is recommended to enhance the trifoliate germination under mid hills condition of Nepal.

ACKNOWLEDGMENTS
The authors would like to thank Nepal Agricultural Research Council for funding support (Project No. 609) to conduct this study.

REFERENCES
Arin L and S Ankara. 2001. Effect of low-tunnel, mulch and pruning on the yield and earliness of tomato in unheated glasshouse. J. Appl. Hort. 3(1):23–27.
Diaz-Perez JC. 2009. Root zone temperature, plant growth and yield of broccoli [Brassica oleracea (Plenck) var. Italica] as affected by plastic film mulches. Scientia Horticulturae. 123:156–163. DOI: 10.1016/j.scienta.2009.08.014
Kaini BR. 2003. Package of Practices for Junar production and post-harvest management. JICA- Nepal and JCCU, Kathmandu; First Edition.
Mobayen R. 1980. Germination of trifoliate orange seed in relation to fruit development, storage and drying. Journal of Horticultural Science. 55(3):285–289. DOI: 10.1080/00221589.1980.11514936
NCRP. 2015. Annual report 2071-72 (2014-15). Dhankuta: National Citrus Research Program.
NCRP. 2016. Annual report 2072-73 (2015-16). Dhankuta: National Citrus Research Program.
NCDP. 2017. Annual report 2073-74 (2016-17). Kritipur, Kathmandu: National Citrus Dev. Program.
NCRP. 2017. Annual report 2073-74 (2016-17). Dhankuta: National Citrus Research Program.
Pun AB, AR Ansari, MK Thakur and KK Bhandari. 2015. Nepala ma suntalajat falful kheti prabidhi. Nepal Agricultural Research Council, National Citrus Research Program, Dharan; First Edition (In Nepali language).
Saipari E, AM Goswami and M Dadlani. 1998. Effect of seed drying on germination behaviour in citrus. Scientia Horticuluture. 73:185–190. DOI: 10.1016/S0304-4238(97)00143-X
Salman SR, AF Abou-Hadid and MS El-Baltagy. 1992. Plastic house microclimate condition as affected by low tunnel and plastic mulch. Egypt J. Hort. 19(2):111–119.
Wiltbank W, RE Rouse and LN Khoi. 1995. Influence of temperature on citrus rootstock seed emergence. Proc. Fla. State Hort. Soc. 108:137–139.