Effect of Various Treatments on Seed Germination and Seedling Vigour of Aonla cv. Chakaiya

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ABSTRACT

Background: Aonla is considered as one of the important indigenous minor fruit crop. Being rich in nutrients the demand of its planting material is increasing day by day which is not fulfilled due to poor seed germination and seedling growth. Therefore there is a dire necessity to standardize the techniques for improving germination and growth of seedlings. The aim of the current study is to overcome the dormancy and to enhance the germination in aonla seeds.

Methods: The present investigation was carried out during 2019-2020 under field conditions with the pre soaking application of the aonla seeds with thiourea, GA₃, cow urine, sulphuric acid, water and seed stratification for time interval of 12 and 24 hours. In the laboratory the selected samples were analysed morphologically based on shoot, root and biomass traits.

Result: Out of the various treatments the application of GA₃ @ 500 ppm for 24 h have ensured early germination with an increase in seed germination. This treatment also improved the seedling growth with an increase in root formation and vigour index. This study will be useful in boosting the cultivation of aonla which will be beneficial for the growers on commercial basis.

Key words: Aonla, Dormancy, Germination, GA₃, Seedling vigour.

INTRODUCTION

Aonla or Indian gooseberry (Phyllanthus emblica L.) belongs to the family Euphorbiaceae and sub family Phyllanthoideae. In India, it is called by various names as Aonla, Nelli, Amla, Amlika, Dhothri, Emblica and Usuri (Barathkumar 2019). A seedling selection from Banarasi, with prolific bearing and flat fruits was named as Chakla and now it is known as ‘Chakaiya’. It is known for its medicinal and therapeutic properties from the ancient time in India and considered as a wonder fruit for health conscious population (Choudhary et al. 2016). Aonla lowers the risk of cancer, increases red blood cells and haemoglobin. Its fruit is one of the main ingredient of chyawanprash and one of the three ingredients of triphala which is useful to treat constipation, headache, enlarge liver and biliousness. In old scriptures, aonla is known as Amritphal. It has great importance in preparation of ayurvedic medicines (Verma et al. 2019). So in traditional medicine, it is known as one of “the best rejuvenating herbs”. Maximum vitamin C is found in mature fruit than immature fruit. Aonla fruit contains several chemical constituents like tannins among which ellagic acid and gallic acid possess biological activity.

Aonla can be propagated both by sexual and asexual means. However, freshly harvested seeds do not germinate even when exposed to favourable environmental conditions owing to seed dormancy. Various factors may cause seed dormancy in aonla seeds such as hard and thick testa or due to incorrect storage or handling (Mousavi et al 2011). Various chemicals like GA₃, thiourea, sulphuric acid and cow urine have been reported to influence germination and seedling growth (Rajamanickam et al. 2002). Availability of quality planting material is the prime need of the day in this cultivar. Therefore, there is a necessity to standardize the techniques for improving germination and seedling growth in aonla.

MATERIALS AND METHODS

The present investigation was carried out at the nursery of Department of Horticulture, Khalsa College, Amritsar during 2019-2020. The experiment was conducted with 14 treatments viz. (T₁ – Soaking in tap water for 12 h; T₂ – Soaking in tap water for 24 h; T₃ – Soaking in cow’s urine for 12 h; T₄ – Soaking in cow’s urine for 24 h; T₅ – Soaking in thiourea@2% for 12 h; T₆ – Soaking in thiourea@2% for 24 h; T₇ – Soaking in GA₃ @ 250 ppm for 12 h; T₈ – Soaking in GA₃ @ 500 ppm for 12 h; T₉ – Soaking in GA₃ @ 250 ppm for 24 h; T₁₀ – Soaking in GA₃ @ 500 ppm for 24 h; T₁₁ – Soaking in cold water for 3h; T₁₂ – Cold stratification at 5°C for 10 days; T₁₃ – Acid scarification –(Sulphuric acid 30 seconds) and T₁₄ – Control (without soaking). The experiment was conducted by randomised block design replicated thrice. Freshly harvested uniform and healthy seeds were selected and imposed with the above treatments. 100 seeds per treatment were sown for study. For stratification treatment, wooden boxes of suitable size were filled with sand and then seeds were placed on it and then again covered with 3-5 cm thick layer of sand. Then, these boxes were placed in refrigerator at 5°C for 10 days. In acid scarification, seeds were

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transferred to beaker containing concentrated sulphuric acid and stirred with the help of a glass rod for 30 seconds. Then, they were washed in running water for few minutes to remove traces of acids. The germinating seeds were counted daily and the day on which the first germination of seed was initiated from the date of sowing considered as days required for germination. Half number of seeds germinated from the date of sowing considered as days taken for 50 percent germination and the days after which no further germination takes place were taken for complete germination.

The observations pertaining to germination percentage was calculated as germination (%) = Number of seeds germinated/Total number of seeds sown x 100. Seedling length was measured from the root tip to growing tip in centimeters with the help of a scale (120 days after sowing). It was measured for all the five seedlings which were tagged and later on mean data was arrived. Shoot girth of five tagged seedling was measured separately with the help of digital vernier caliper and expressed in millimeters. Fresh weight of shoot was taken on electronic balance and average weight was calculated. In case of dry weight, five tagged shoots were collected and placed in paper bags and were dried in oven at 85°C till constant weight was attained. After drying, the dry weight of shoot was measured on electronic balance and average weight was calculated. The survival percentage was calculated as number of survived seedlings/Total number of seedlings x 100. Vigour index (I) was calculated as mean seedling length x germination % and vigour index (II) as dry weight of seedling x germination % respectively after 120 days of sowing.

RESULTS AND DISCUSSION

Days for initiation of germination

The data in Table 1 clearly indicated that the seeds subjected to GA₃ 500 ppm soaking for 24 hrs (T₁₃) took minimum (7.12) days for initiation whereas maximum days (12.20) to commencement of seed germination was recorded in T₄ Control. The soaking of seeds in GA₃ solution for 24 hrs might have helped to increase the permeability of seed coat to GA₃ solution. Further, the soaking might have helped in leaching out the inhibitors of seed germination and increases endogenous gibberellin like substances (Mathur et al.1971). The growth regulators like GA₃ have been reported to play a great role in the process of germination. With the imbibed water, the embryo gets activated and the process of germination is initiated. GA₃ synthesized by the enlarged embryo acts on the living cells and causes de nova synthesis of hydrolyzing enzymes particularly a-amylase which converts the starch into simple sugars during the process of germination. These sugars provide energy that is required for various metabolic and physiological process associated with germination. Other enzymes activated by GA include those which weaken the seed coat and allow the axis to burst through. GA also enhances cell elongation, so the radicle can push through the endosperm and seed coat that restrict its growth (Hartman and Kester. 1979). The present results are in agreement with the findings of Gholap et al. (2000), Bagal (2004), Manekar et al. (2011), Lilabati and Sahoo (2015) in aonla, Ratan et al. (2004) in custard apple, Barche et al. (2010) and Pratibha et al. (2015) in papaya, Hota et al. (2018) in jamun, Harshvardhan and Rajashekar (2012) in jackfruit, Pampanna et al. (1995) in sapota.

Time taken for 50% germination (days)

It was observed that the seeds treated with GA₃ 500 ppm (T₁₃) for 24 hrs took minimum (8.93) for 50% germination. Maximum days (15.33) taken for 50% germination were recorded in T₄ Control as depicted in Table 1. This might be attributed to the synergistic effects of these combined inputs (GA₃ and soaking period) on the stimulation of combined growth through cell division and expansion, improved physico-chemical properties of protoplasm, respiration, nucleic acid metabolism etc. It is believed that GA₃ increases de novo synthesis of hydrolyzing enzymes particularly amylase and protease. The hydrolyzed food was subsequently utilized for growth of embryo which in turn enhanced germination (Chaudhary et al. 2016). The present findings are in agreement with the findings of Bagal (2004), Manekar et al. (2011), Lilabati and Sahoo (2015) and BarathKumar (2019) in aonla, Ratan and Reddy (2004) in custard apple, Barche et al. (2010) and Pratibha et al. (2015) in papaya, Harshvardhan and Rajashekar (2012) in jackfruit, Pampanna et al. (1995) in sapota.

Time taken for complete germination (days)

It is evident from the data in Table 1 that the seeds treated with GA₃ 500 ppm for 24 hrs (T₁₃) took minimum (13.66) days for complete germination whereas seeds soaked in cow urine for 12 hrs (T₄) took maximum (20.33) days for complete germination. The possible reason for minimum days taken for complete germination by GA₃ treated seeds might be due to that GA₃ activates the hydrolysis of starch and their translocation facilitated the complete germination (Kumari 2006). Similar findings on germination were reported by Ratan and Reddy (2004) in the seeds of custard apple, Vasantha et al. (2014) and Rajendrakumar (2017) in tamarind, Harshvardhan and Rajashekar (2012) in jackfruit and Pampanna et al. (1995) in sapota, Gholap et al. (2000), Bagal (2004), Manekar et al. (2011), Lilabati and Sahoo (2015) in aonla.

Germination percentage

The data furnished in Table 1 regarding germination percentage as influenced by various seed treatments showed that the germination percentage was significantly increased due to GA₃ and other treatments as compared to control. Maximum germination (72.45%) was recorded in T₁₃ GA₃ 500 ppm for 24 hrs. Soaking of aonla seeds in cow urine for 24 and 12 hr gave less germination of 44.67 and 42.71 per cent followed by T₄ Control which gave minimum germination 36.21 per cent respectively. The increase in germination might be due to the reason that the exogenous application of GA antagonizes the ill effect of inhibitors and
increases endogenous gibberellins like substances. GA helps in the synthesis of enzymes and one of them is amylase which converts the starch into simple sugars during the process of germination.

**Survival percentage**

The data in Table 2 reveals that percentage survival of seedlings ranged from 52.32 to 86.39. Among the different seed treatments, the maximum survival of seedlings (86.39%) was observed in seeds treated with 500 ppm GA$_3$ for 24 hrs (T$_3$), whereas, significantly minimum per cent survival of seedlings (52.32%) was observed in T$_2$- Tap water soaking for 12 hrs and T$_1$- Control (55.21%). Maximum survivability in gibberelic acid might be due to quicker root and shoot development and making the seedling stouter and resisting root diseases (Barche et al. 2010). These findings are in the accordance with the finding of Meena et al. 2003 and Bagal 2004. The probable cause for high survival percentage of seedlings might be due to early germination of seeds which helps in successful acclimatization of seedlings in field conditions and vigour of seedlings ultimately leads to better growth, thus less mortality i.e. higher survival percentage of seedlings (Kumari 2006).

**Seedling length (cm)**

The data of 120 DAS in Table 2 clearly showed significant differences showing T$_3$ (GA$_3$ 500 ppm for 24 hrs) to be the treatment which gave maximum seedling length (110.44 cm). Minimum seedling length (82.48 cm) was recorded in seeds treated with thiourea 2% for 24 hrs (T$_2$). The maximum seedling length in GA$_3$ treated seeds might be attributed to the fact that this hormone increased osmotic uptake of nutrients, causing cell multiplication and elongation in the cambium tissue of the internodal region leading to an increase in length of the shoots because GA$_3$ apparently activates the metabolic processes or nullifies the effect of growth inhibitors (Barathkumar 2019).

**Shoot diameter (mm)**

According to data depicted in Table 2 the maximum shoot diameter (5.85 mm) was recorded in T$_3$- Tap water soaking for 24 hrs while minimum shoot diameter (4.27 mm) was recorded under controlled conditions (T$_1$) after 120 days of sowing. The increase in shoot diameter was due to greater cell division and elongation at the stem portion (Sen et al. 1990). The present findings are in line with the research findings of Lilabati and Sahoo (2015) and Kumari (2006) in amla and Bhavya et al. (2017) in karonda.

**Fresh weight of shoot (g)**

Seed treatment with GA$_3$ 500 ppm for 24 hrs gave maximum fresh shoot weight (22.75 g) followed while minimum fresh weight of shoots (13.64 g) was found in T$_2$- Cow urine soaking for 12 hrs at 120 DAS (Table 2). The increase in fresh weight of shoot with GA$_3$ treatment might be due to overall growth of the seedling and increased rate of photosynthesis that lead to the overall assimilation and redistribution of photosynthates within the seedling and hence, resulted in higher fresh weight of shoot. The results are in close conformity with findings of Rajendrakumar (2017) in tamarind, Pratibha et al. (2015), Anburani and Shakila (2010) in papaya, Parmar et al. (2016) in custard apple, Kadam et al. (2010) in kaiqi lime, Venkatrao and Reddy (2005), Muralidhara et al. (2015) in mango and Gurung et al. (2014) in passion fruit. Barche et al. (2010) in amla and Parvin et al. (2015) in walnut also reported the same.

**Dry weight of shoot (g)**

According to the data in Table 2 maximum dry shoot weight (16.39 g) was recorded in T$_1$- GA$_3$ 500 ppm for 24 hrs while

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**Table 1: Effect of various treatments on seed germination and seedling vigour of aonla.**

| Treatments | Days taken for commencement of germination (days) | Days taken for 50% germination | Days taken for complete Germination | germination (%) | Seeding vigour index-I | Seeding vigour index-II |
|------------|-------------------------------------------------|--------------------------------|-----------------------------------|-----------------|-----------------------|-----------------------|
| T$_1$- Control | 12.20                                           | 15.33                          | 19.62                             | 36.21           | 5869.20               | 521.31                |
| T$_2$- Tap water soaking for 12 hr | 8.93                                           | 11.33                          | 16.33                             | 44.32           | 7617.40               | 682.92                |
| T$_3$- Tap water soaking for 24 hr | 8.33                                           | 12.66                          | 16.66                             | 58.17           | 10348.10              | 1089.63               |
| T$_4$- Cow urine soaking for 12 hr | 9.33                                           | 13.66                          | 20.33                             | 42.71           | 8289.26               | 702.36                |
| T$_5$- Cow urine soaking for 24 hr | 9                                               | 14.66                          | 17.33                             | 44.67           | 8525.15               | 1071.23               |
| T$_6$- Thiourea 2% soaking for 12 hrs | 9.66                                           | 15.20                          | 19.66                             | 52.23           | 9279.04               | 1109.08               |
| T$_7$- Thiourea 2% soaking for 24 hrs | 9.69                                           | 15                               | 20.16                             | 60.59           | 9212.60               |                      |
| T$_8$- GA$_3$ 250 ppm soaking for 12 hrs | 8.12                                           | 10.66                          | 15.66                             | 56.36           | 10486.20              | 1432.75               |
| T$_9$- GA$_3$ 500 ppm soaking for 12 hrs | 7.93                                           | 9.66                            | 15.33                             | 68.21           | 13312.63              | 1884.16               |
| T$_{10}$- GA$_3$ 250 ppm soaking for 24 hrs | 7.42                                           | 9.33                            | 13.86                             | 68.72           | 13548.97              | 2052.38               |
| T$_{11}$- GA$_3$ 500 ppm soaking for 24 hrs | 7.12                                           | 8.93                            | 13.66                             | 72.45           | 14612.05              | 2433.07               |
| T$_{12}$- Cold water soaking for 3 hrs | 8.92                                           | 13                               | 18.33                             | 55.77           | 9787.60               | 1538.14               |
| T$_{13}$- Stratification at 5°C for 10 days | 9.96                                           | 12                               | 17.66                             | 50.01           | 9450.17               | 924.27                |
| T$_{14}$- Acid scarification for 30 seconds | 10.80                                          | 14                               | 18.33                             | 47.07           | 8606.57               | 953.92                |
| Mean | 9.10                                           | 12.53                           | 17.35                             | 54.11           | 9924.63               | 1231.25               |
| CD at 5% level | 0.910                                          | 2.185                           | 1.878                             | 3.60            | 642.202               | 197.69                |
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Table 2: Effect of various treatments on shoot parameters of aonla

| Treatments                          | Mean seedling length (cm) | Shoot diameter (mm) | Fresh wt. of Shoot (g) | Dry wt. of shoot (g) | Survival (%) |
|-------------------------------------|--------------------------|---------------------|------------------------|----------------------|--------------|
| T<sub>1</sub> Control               | 95.82                    | 4.27                | 19.15                  | 7.37                 | 36.21        |
| T<sub>2</sub> Tap water soaking for 12 hr | 94.47                    | 5.78                | 14.93                  | 4.74                 | 44.32        |
| T<sub>3</sub> Tap water soaking for 24 hr | 96                      | 5.85                | 18.36                  | 9.60                 | 58.17        |
| T<sub>4</sub> Cow urine soaking for 12 hr | 106.50                   | 5.36                | 13.64                  | 7.79                 | 42.71        |
| T<sub>5</sub> Cow urine soaking for 24 hr | 102.33                   | 5.22                | 15.52                  | 9.65                 | 44.67        |
| T<sub>6</sub> Thiourea 2% soaking for 12 hrs | 97.23                    | 4.72                | 14.23                  | 8.22                 | 52.23        |
| T<sub>7</sub> Thiourea 2% soaking for 24 hrs | 82.48                    | 4.32                | 14.02                  | 8.07                 | 60.59        |
| T<sub>8</sub> GA<sub>3</sub> 250 ppm soaking for 12 hrs | 100.73                    | 5.62                | 18.15                  | 12.18                | 56.36        |
| T<sub>9</sub> GA<sub>3</sub> 500 ppm soaking for 12 hrs | 107.67                    | 5.76                | 19.51                  | 13.26                | 68.21        |
| T<sub>10</sub> GA<sub>3</sub> 250 ppm soaking for 24 hrs | 107.89                    | 5.78                | 20.37                  | 14.43                | 68.72        |
| T<sub>11</sub> GA<sub>3</sub> 500 ppm soaking for 24 hrs | 110.44                    | 5.82                | 22.75                  | 16.39                | 72.45        |
| T<sub>12</sub> Cold water soaking for 3 hrs | 98.28                    | 4.28                | 15.46                  | 10.53                | 55.77        |
| T<sub>13</sub> Stratification at 5°C for 10 days | 105.38                    | 4.32                | 18.64                  | 11.29                | 50.01        |
| T<sub>14</sub> Acid scarification for 30 seconds | 100.26                    | 5.28                | 16.68                  | 10.22                | 47.07        |
| Mean                               | 102.40                    | 5.17                | 17.24                  | 10.46                | 54.11        |
| CD at 5% level                     | 1.712                     | 0.796               | 1.168                  | 1.137                | 3.607        |

minimum dry shoot weight (7.37 g) was recorded in T<sub>1</sub> Control. This seems to be due to the effect of mobilization of water and nutrients transported at higher rate which might have promoted more production of photosynthetic products and translocated them to various plant parts which might have resulted in better growth of seedlings and hence, more fresh and dry weight. The research findings of Rajendra kumar (2017) in tamarind, Pratibha et al. (2015), Anburani and Shakila (2010) in papaya, Parmar et al. (2016) in custard apple, Kadam et al. (2010) in kagzi lime, Venkatrao and Reddy (2005), Muralidhara et al. (2015) in mango and Gurung et al. (2014) in passion fruit are in agreement with the present results.

Seedling vigour index – I(cm)

Significantly maximum seedling vigour index-I (14612.05 cm) was found in T<sub>11</sub>–GA<sub>3</sub> 500 ppm for 24 hrs followed by T<sub>10</sub>–GA<sub>3</sub> 250 ppm soaking for 24 hrs (13548.97 cm) whereas minimum seedling vigour index- I (5869.20 cm) was recorded in T<sub>1</sub>–Control (Table1). The increase in vigour index-I 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. Enzymatic and hormonal mechanism stimulate metabolic process such as sugar mobilization, protein hydrolysis, oxidation etc. (Earlplus and Lambeth 1974), 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 Barathkumar (2019), Ponni et al. (2011), Lilabati and Sahoo (2015) in aonla, Yadav et al. (2018) in custard apple, Rajendra kumar (2017) in tamarind and Hota et al. (2018) in jamun.

Seedling vigour index-II (g)

According to data presented in Table 1, maximum seedling vigour index – II (2433.07 g) was recorded in T<sub>11</sub>–A<sub>3</sub> 500 ppm soaking for 24 hrs whereas minimum seedling vigour index– II (521.31 g) was found in T<sub>1</sub>–Control. The highest seedling vigour index in GA<sub>3</sub> was 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. Enzymatic and hormonal mechanism stimulates metabolic process such as sugar mobilization, protein hydrolysis, oxidation etc. (Verma et al. 2019). The present results are in line with the findings of Barathkumar (2019), Shakila and Ponni (2008), Manekar et al. (2011), Lilabati and Sahoo (2015) in aonla, Yadav et al. (2018) in custard apple, Rajendra kumar (2017) in tamarind and Hota et al. (2018) in jamun are in collaboration with the present results. Parvin et al. (2015) in walnut also reported the same.

CONCLUSION

Based on the results of the experiment it can be concluded that among various seed treatment chemicals the application of GA<sub>3</sub> was found to be effective with respect to germination, growth parameters, production of vigorous seedlings and higher survival percentage as compared to other seed treatment chemicals. Therefore, soaking of seed in GA<sub>3</sub> 500 ppm as a seed treatment chemical can be used for better seed germination and growth of aonla seedling.

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