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Effects of physical treatments and gibberellic acid on germination success of Aonla seed

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Abstract
Aonla (*Emblica officinalis*) is an important minor fruit crop of India. Demand for Aonla fruit is steadily expanding owing to its use in the cosmetic, pharmaceutical and processing industries. Aonla commercially propagated through patch budding but the availability of the quality seedling rootstock for budding technique is a major constraint in expanding area under aonla cultivation. Therefore, various physical treatments (hot water treatment and mechanical scarification using sand paper) and concentrations (200 ppm, 400 ppm and 600 ppm) of GA_3 were used for treating to aonla seeds in the experiment. The result indicated that mechanical scarification using sand paper is found to be the most effective for better seed germination (days taken to first germination, germination percentage and survival percentage) and seedling growth (length of seedling, total dry matter of seedling, Vigour Index I and Vigour Index II). Among GA_3 concentration, GA_3 @400 ppm recorded higher values for seed germination and seedling growth parameters of aonla. The treatment combination of scarification with sand paper + GA_3 @400 ppm showed better germination and seedling growth. Therefore, it is concluded that the combination of scarification with sand paper + GA_3 @400ppm was found most suitable for seed germination and seedling growth of aonla.

Keywords: seedling, physical treatment, sandpaper, scarification, GA_3

Introduction

Aonla (*Emblica officinalis*), also known as Indian Gooseberry, is an important minor fruit crop of India. It is a subtropical fruit belonging to the *Euphorbiaceae* family and is native to tropical Southeast Asia. With an estimated area of 0.08 mha and annual production of 0.97 mt, India is the world leader in Aonla production (Anonymous, 2019). It has been frequently utilized in Indian medicine as a remedy for a variety of ailments (Goyal *et al.*, 2008) [6]. Although considered as a minor fruit, Aonla has commercial significance and is considered as a wonder fruit primarily due to its medicinal properties (Choudhary *et al.*, 2016) [5]

Demand for Aonla fruit is steadily expanding as a result of its use in the cosmetic, pharmaceutical and processing industries. One of the biggest obstacles to expanding the area under cultivation of Aonla is lack of quality planting material. Aonla can be propagated by both sexual and asexual methods. However, Aonla is commercially propagated through patch budding (Negi *et al.*, 2010) [19] as seeds show poor and delayed germination owing to seed dormancy. Dormancy may be due to internal (physiological) factors affecting embryo or morphological factor such as hard, thick testa, or due to incorrect storage or handling (Mousavi *et al.*, 2011) [17]. To overcome dormancy, such seeds may require special treatments such as stratification, scarification, soaking in water or seed treatments with growth regulators.

The quality of rootstocks is critical to budding success. Because aonla seedlings grow slowly and require a long time to reach the buddable stage, nursery procedures must be learned in order to improve seed germination and seedling growth. Water soaking and heat transfer through water are basic methods for overcoming seed coat impermeability. Plant growth regulators are frequently used to improve seed germination and root and shoot growth in most of the horticultural crops (Lal *et al.*, 2013) [14]. These growth regulators not only increase the seed germination percentage but also hasten seedling growth (Lal and Das, 2017) [13]. Many fruit crops have reported increased germination and vigorous seedlings, or both after soaking seeds in gibberellins.

Being a minor crop, limited work has been reported on pre-germination seed treatment with gibberellins, water soaking, heat through water and mechanical scarification in Aonla. Therefore, this experiment was undertaken to find effects of several combinations of physical treatments and seed treatment with GA_3 on germination success of Aonla seeds.

Materials and Methods

The present study entitled "Effects of Physical Treatments and Gibberellic Acid on Germination Success of Aonla Seed" was carried out at Hi-Tech Polyhouse, School of Agriculture, Lovely Professional University, Phagwara, Punjab during the year 2022. Experiment was laid out in the Factorial Completely Randomized Design (F-CRD) comprising Physical treatments and concentration of GA_3. The treatments included 3 levels of physical treatments i.e., water soaking (P1), mechanical scarification - using sand paper (P2) and scarification - hot water (70 ^{0}C) treatment for 10 minutes (P3) and 4 levels of concentration of Gibberellic acid *viz*. GA_3 @200 ppm (G1), GA_3 @400 ppm (G2), GA_3 @600 ppm (G3) and control (G_0). During the experiment, 12 treatment combinations were performed on Anola seeds with three replicatons of each treatment. 20 seeds were used for each experimental unit.

For experimental purpose seeds were collected from the market of Jalandhar, Punjab. The extracted seeds were air dried for 24 hours before being placed in a jar of water and subjected to a float test to determine seed viability. The seeds that sank to the bottom were deemed healthy and viable, making them appropriate for the experiment. For mechanical scarification seeds were rubbed against the sand paper to break down the hard seed coat until evident scratches were observed on the seed surface. Hot water treatment was given by soaking the seeds in hot water for 10 minutes while maintaining the temperature of water at 70 ^{0}C. Likewise seeds were soaked in 200 ppm, 400 ppm, and 600 ppm stock solutions of GA_3 for 24 hours as per treatment combinations. The treated seeds were sown at a depth of 2-3 cm in root trainers filled with a 2:1:1 mix of soil, cocopeat, and vermicompost growth media. Watering was done on a regular basis with a rose can, and various plant protection measures were implemented at all growth stages.

The germinating seeds were observed daily and the day on which the first germination of seed was initiated from the date of sowing was considered as days taken to first germination. Observation pertaining to germination percentage was calculated using formula;

$$\text{Germination Percentage (\%)} = \frac{\text{Total number of germinated seeds}}{\text{Total number of seeds sown}} \text{X } 100$$

For seedling growth parameters five seedlings were randomly selected and tagged for recording observations at 60 DAS. Length of seedling was measured from the root tip to growing tip of shoot with the help of a scale. For total dry matter of seedling, seedlings were chopped and oven dried at 60 ± 2 ^{0}C temperature till a constant weight. The weight was taken with the help of electronic balance and average value was computed.

Seedling vigour was calculated using the following formula given by Bewley and Black, 1982. Vigour Index I = Germination percentage (%) × Length of seedling and

Vigour Index II = Germination percentage (%) × Total dry matter of seedling. Survival

Percentage of seedlings was computed by the formula

$$\text{Survival Percentage (\%)} = \frac{\text{Total number of survived seedlings}}{\text{Total number of seeds sown}} \text{X } 100$$

The data collected from this investigation were recorded and analysed using the statistical software OPSTAT (Panse and Sukhatme, 1985) [20].

Result and Discussion

Days taken to first germination

The data depicted in Table 1 clearly indicates that among various physical treatments, P2 (Mechanical scarification – using sand paper) recorded significantly the lowest days taken to first germination (8.33 days) followed by 9.58 days in P1 (Water soaking), whereas maximum days taken to first germination (9.83 days) was observed in P3 (hot water 70 ^{0} C treatment for 10 minutes). Among the different concentrations of GA_3, minimum days taken to first germination (7.11 days) was noticed in G2 (GA3 @400 ppm) followed by 8.56 days in GA_3 (GA3 @600 ppm). However maximum days taken to first germination (12.11 days) was recorded in G0 (control). Interaction effect between physical treatment and GA3 varied significantly with respect to days taken to first germination. It was noticed that P2G2 (Mechanical scarification - sand paper + GA_3 @400 ppm) recorded the least number of days taken to first germination (6.33 days) which was the best result among all treatment combinations. Significantly highest days taken to first germination (13.33 days) was recorded in P1G0 (Water soaking + control). This might be because scarification with sand paper help in weakening of seed coat and overcoming the physical barriers thereby increasing the absorption of water and GA_3 by the seeds (Anshu *et al.*, 2021). GA_3 takes part in an important role in the germination process by promoting the synthesis of hydrolyzing enzymes, particularly α – amylase. This aids in the conversion of starch into simple sugars during the germination process. These sugars provide energy for multiple metabolic and physiological processes associated with germination. GA also promotes cell elongation, which allows the radicle to penetrate the endosperm and seed coat, which otherwise limit its growth (Hartman and Kester, 1979) [10], resulting in faster germination. These findings corroborate those of Lilabati and Sahoo (2015) [15] in aonla and Hota *et al.*, (2018) [11] in jamun.

Germination percentage

Germination percentage of aonla seeds as influenced by physical treatments and concentration of GA_3 are presented in Table 1. Among the physical treatments, P2 (Mechanical scarification – using sand paper) had the highest germination percentage (69.58%) followed by 60.83% in P3 (hot water 70 ^{0} C treatment for 10 minutes), while P1 (water soaking) had the lowest germination percentage (55.00%). Among the various GA_3 concentrations, G2 (GA_3 @400 ppm) had the highest germination percentage (82.22%), followed by 71.11% in G3 (GA_3 @600 ppm). However, the least percentage of germination (32.22%) was recorded in G0 (control). Among the interaction effect, P2G2 (Mechanical scarification - sand paper + GA_3 @400 ppm) had the highest germination percentage (86.67%), which was the best result of all treatment combinations. In P1G0 (Water soaking + control), the germination percentage was significantly lower (23.33%). This is due to the weakening of the seed coat and the removal of physical barriers to embryo growth, water uptake, and gaseous exchange (Anshu *et al.*, 2021). GA_3 is thought to boost the synthesis of hydrolysing enzymes from scratch, particularly amylase and protease. The hydrolysed food was then used to promote embryo growth and germination. (Chaudhary *et al.*, 2016). These findings are in compliance with those of Manekar *et al.*, (2011) [16], Lilabati and Sahoo (2015) [15] and Barathkumar (2019) [2] in aonla, Barche *et al.*, (2010) [3] and Pratibha *et al.*, (2015) [21] in papaya and Harshvardhan and Rajashekhar (2012) [9] in jackfruit.

Survival percentage

Table 1 shows that among the physical treatments, P2 (Mechanical scarification – using sandpaper) had the highest survival percentage (64.58%) followed by 55.83% in P3 (Hot water), while P1 (water soaking) had the lowest survival percentage (50.00%). Among the various GA_3 concentrations, G2 (GA_3 @400 ppm) had the highest survival percentage (77.22%), followed by 66.11% in G3 (GA_3 @600 ppm). However, the lowest survival percentage (27.22%) was recorded in G0 (control). The interaction effect of physical treatment and GA_3 on survival percentage varied significantly. P2G2 (Mechanical scarification - sandpaper + GA_3 @400 ppm) had the highest survival percentage (81.67%), which was the best result of all treatment combinations. In P1G0 (Water soaking + control), the survival percentage was significantly lower (18.33%).

Seeds scarified with sandpaper and treated with GA_3 @400 ppm showed a significantly better survival percentage than the control. The likely cause of the high survival percentage of seedlings is faster germination of seeds, which aids in acclimatization of seedlings in field conditions, and vigour of seedlings, which leads to better growth and thus less mortality, resulting in a higher survival percentage of seedlings (Kumari 2006) [12]. It may also be as a consequence of faster root and shoot development, which makes the seedling sturdier and more resistant to root diseases (Barche *et al.*, 2010) [3]. These findings are consistent with those of Singh and Kaur (2021) [24].

Table 1: Effect of physical treatments and gibberellic acid on seed germination of Aonla

Treatment		Days taken to first germination	Germination Percentage (%)	Survival Percentage (%)
Physical Treatments	Water Soaking (P1)	9.58	55.00	50.00
	Sand Paper (P2)	8.33	69.58	64.58
	Hot Water (P3)	9.83	60.83	55.83
	C.D.	0.47	3.00	3.00
	SE (m) ±	0.16	1.02	1.02
Gibberellic Acid GA_3	Control (G0)	12.11	32.22	27.22
	GA_3 @200 ppm (G1)	9.22	61.67	56.67
	GA_3 @400 ppm (G2)	7.11	82.22	77.22
	GA_3 @600 ppm (G3)	8.56	71.11	66.11
	C.D.	0.54	3.46	3.46
	SE (m) ±	0.18	1.18	1.18
Interaction of Physical Treatments and Gibberellic Acid	P1G0	13.33	23.33	18.33
	P1G1	9.33	56.67	51.67
	P1G2	7.33	78.33	73.33
	P1G3	8.33	61.67	56.67
	P2G0	11.33	41.67	36.67
	P2G1	8.00	71.67	66.67
	P2G2	6.33	86.67	81.67
	P2G3	7.67	78.33	73.33
	P3G0	11.67	31.67	26.67
	P3G1	10.33	56.67	51.67
	P3G2	7.67	81.67	76.67
	P3G3	9.67	73.33	68.33
	C.D.	0.94	5.99	5.99
	SE (m) ±	0.32	2.04	2.04

GA also promotes cell elongation, allowing the radicle to push through the endosperm and seed coat, which limit its growth (Hartman and Kester, 1979) [10], resulting in faster germination. These results are in agreement with findings of Lilabati and Sahoo (2015) [15] in aonla, Pratibha *et al.*, (2015) [21] in papaya and Hota *et al.*, (2018) [11] in jamun. The higher survival percentage is due to gibberellic acid, which promotes faster root and shoot development and makes seedlings stronger and disease resistant (Barche *et al.*, 2010) [3]. These findings are in accordance with those of Meena *et al.*, 2003.

Length of seedling: Length of seedling as influenced by physical treatments and concentration of GA_3 are presented in Table 2. Among the physical treatments, P2 (Mechanical scarification – using sand paper) had the highest length of seedling (21.93 cm) followed by 20.65 cm in P3 (Hot water), while P1 (water soaking) had the lowest length of seedling (19.73 cm). Among the various GA_3 concentrations, G2 (GA_3 @400 ppm) had the highest length of seedling (24.56 cm), followed by 20.90 cm in G3 (GA_3 @600 ppm). However, the minimum length of seedling (17.56 cm) was observed in G0 (control). The interaction effect of physical treatment and GA_3 on length of seedling varied significantly. P2G2 (Mechanical scarification - sand paper + GA_3 @400 ppm) had the highest length of seedling (26.23 cm), which was the best result of all treatment combinations. In P1G0 (Water soaking + control), the length of seedling (16.40 cm) was significantly lower. The maximum seedling length in P2G2 (Mechanical scarification - sand paper + GA_3 @400 ppm) may be linked to the fact that GA3 enhanced nutrient uptake by osmosys, inducing cell multiplication and elongation in the internodal cambium tissue, resulting in an increase in shoot length because GA3 stimulates metabolic processes or negates the effect of growth inhibitors (Barathkumar, 2019) [2].

Table 2: Effect of physical treatments and gibberellic acid on seedling growth of Aonla

Treatment		Length of seedling (cm)	Total dry matter of seedling (g)	Vigour Index I	Vigour Index II
Physical Treatments	Water Soaking (P1)	19.73	0.289	1129.84	17.13
	Sand Paper (P2)	21.98	0.330	1568.95	24.02
	Hot Water (P3)	20.65	0.315	1303.43	19.74
	C.D.	0.410	0.009	27.46	1.65
	SE (m) ±	0.140	0.003	9.35	0.56
Gibberellic Acid GA_3	Control (G0)	17.56	0.239	573.26	7.80
	GA_3 @200 ppm (G1)	20.14	0.304	1245.73	18.87
	GA_3 @400 ppm (G2)	24.56	0.379	2024.40	31.39
	GA_3 @600 ppm (G3)	20.90	0.323	1492.91	23.13
	C.D.	0.473	0.010	31.70	1.90
	SE (m) ±	0.161	0.003	10.80	0.65
Interaction of Physical Treatments and Gibberellic Acid	P1G0	16.40	0.217	382.67	5.07
	P1G1	20.07	0.299	1137.11	16.94
	P1G2	22.73	0.352	1782.48	28.78
	P1G3	19.73	0.288	1217.10	17.73
	P2G0	18.87	0.247	786.11	10.32
	P2G1	20.90	0.333	1496.96	23.88
	P2G2	26.23	0.396	2273.56	34.99
	P2G3	21.93	0.343	1719.17	26.90
	P3G0	17.40	0.253	551.00	8.02
	P3G1	19.47	0.279	1103.11	15.79
	P3G2	24.70	0.388	2017.17	30.41
	P3G3	21.03	0.337	1542.45	24.76
	C.D.	0.820	0.017	54.91	3.30
	SE (m) ±	0.279	0.006	18.70	1.12

Total dry matter of seedling

Among different physical treatments on aonla seeds, highest dry matter of seedling (0.330 g) was recorded in seeds treated with P2 (Mechanical scarification – using sand paper) and least dry matter of seedling (0.289 g) was observed in P1 (water soaking) as depicted in Table 2. Among the various concentrations of GA_3, G2 (GA_3 @400 ppm) recorded maximum total dry matter of seedling (0.379 g) followed by 0.323 g in G3 (GA_3 @600 ppm), whereas the minimum dry matter of seedling (0.239 g) was observed in G0 (control). The interaction of physical treatment and GA_3 concentration showed that P2G2 (Mechanical scarification - sand paper + GA_3 @400 ppm) treatment combination produced the highest total dry matter of seedling (0.396 g) and outperformed all other treatment combinations. P1G0 (Water soaking + control) had the lowest total dry matter of seedling (0.217 g). This is because GA_3 had a significant impact on accumulation of dry matter in various parts of plant, that might be attributed to its impact on stimulating cell division, elongation of cells, metabolism of auxins, plasticity of cell wall, and cell membrane permeability, all of which led to increased growth of seedling. The findings are in accordance with Ratan and Reddy (2004) and Gurung *et al.*, (2014).

Vigour Index: Seedling Vigour Index is directly proportional to the germination percentage and overall growth of a seedling. Seedling Vigour Index I and Vigour Index II recorded from this investigation are shown in table 2. Seed treatment with sandpaper scarification (P2) recorded the highest Vigour Index I (1568.95) and Vigour Index II (24.02) as in comparison to other physical treatments. Whereas the lowest Vigour Index I (1129.84) and Vigour Index II (17.13) were observed in water soaking (P1). Among the concentration of GA_3, G2 (GA_3 @400 ppm) showed the highest values for Vigour Index I (2024.40) and Vigour Index II (31.39), followed by G3 (GA_3 @600 ppm) showing Vigour Index I (1492.91) and Vigour Index II (23.13). Minimum Vigour Index I (573.26) and Vigour Index II (7.80) were observed in control (G0). The interaction of sand paper scarification + GA_3 @400 ppm (P2G2) showed the maximum Vigour Index I (2273.56) and Vigour Index II (34.99), which was superior to any other treatment combination. Whereas the least amount of Vigour Index I (382.67) and Vigour Index II (5.07) was recorded in P1G0 (Water soaking + control). The results clearly indicates that Vigour Index I is proportional to the length of seedlings and Vigour Index II is proportional to the total dry matter of seedling. The increased vigour index could be attributed to enlargement of embryos, increased metabolic activity and respiration, improved metabolite utilization and mobilization to different growth points of seedling, and increased enzyme activity. Enzymatic and hormonal mechanisms stimulate metabolic processes such as mobilization of sugars, hydrolysis of proteins and increased oxidation (Earlplus and Lambeth 1974), resulting in an increase in root length of Aonla seedlings, length of shoot, and dry weight of seedling, and thus in higher seedling vigour. The findings are similar to those of Barathkumar (2019) [2], Manekar *et al.*, (2011) [16] and Lilabati and Sahoo (2015) [15] in aonla, Yadav *et al.*, (2018) [25] in custard apple and Rajendrakumar (2017) in tamarind [22].

Conclusion

From the findings of the present study, it may be concluded that among different physical treatments, scarification of aonla seeds with sand paper (P2) recorded better seed germination and seedling growth parameters. In different concentrations of GA_3, G2 (GA_3 @400 ppm) recorded better seed germination and seedling growth parameters in Aonla. Among the interaction, the treatment P2G2 (scarification with sand paper + GA_3 @400ppm) recorded better seed germination and seedling growth as compared to other treatment combinations of physical treatment and GA_3.

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