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Effect of seed priming on germination and seedling growth in Amlook (*Diospyros lotus* L.)

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Abstract

To investigate the effect of seed priming on enhancement of germination and seedling growth in amlook (*Diospyros lotus* L.) was done at Department of Seed Science and Technology, Dr YS Parmar UHF, Nauni, Solan during the year 2019-20. Under field conditions, there were eleven different seed priming treatments i.e. GA₃ (250, 500, 750 ppm), Thiourea (2500, 5000, 7500 ppm), KNO₃ (2500, 5000, 7500 ppm), hydro-priming and control. Among all treatments GA₃ @ 500 ppm significantly enhanced the seed germination, seedling height, seedling diameter, number of leaves per seedling, leaf area, leaf chlorophyll content, fresh weight of shoots and roots, dry weight of shoots and roots, total root length, biomass of seedlings, SVI-I, SVI-II, survival rate and number of graftable seedlings. This treatment also significantly increased the days taken to germination. Therefore, seed primed with GA₃ @ 500 ppm could be recommended for improving the germination and seedling growth in amlook.

Keywords: Amlook, GA₃, KNO₃, seed priming, thiourea

Introduction

Amlook (*Diospyros lotus* L.) is commonly used as a rootstock for raising cultivated persimmon because of its high grafting affinity and strong resistance to drought and low winter temperatures. Persimmon, a member of the Ebenaceae family, is the most economic species of the genus *Diospyros*. In India, persimmon is locally called Japaniphal or the Japanese fruit and is grown on a semi-commercial scale in the states like Himachal Pradesh, Jammu and Kashmir, Uttarakhand and Tamil Nadu and as a scattered plantation in some parts of North East and Nilgiri Hills in South India. The total area under persimmon cultivation in Himachal Pradesh was 523 ha with a production of 877 MT (Anonymous 2019)^[4].

Persimmon is commercially propagated by grafting the desired variety on seedling rootstocks. Hard seed coat affects seed germination through inhibiting and mechanically restricting the embryo growth (Taha 1987)^[23]. Therefore, persimmon seeds are hard to germinate and need additional treatments to break dormancy. Such germinated seeds are used as rootstocks for grafting with suitable variety. Due to low germination of seeds, there is a need to enhance the germination through stratification and treatment of the seeds with certain chemicals i.e. seed priming so as to increase the production of quality seedlings of persimmon for rootstock purpose (Peché *et al.*, 2016)^[18].

During seed germination, embryonic gibberellic acid is released that triggers the weakness of seed cover by stimulating gene expression involved in cell expansion (Finkelstein *et al.*, 2008)^[7]. Gibberellins represent a natural regulator of the processes involved in seed germination to stimulate the production of hydrolytic enzymes i.e. α -amylase in the aleurone layer of germinating seed grains. Gibberellic acid has been reported to increase cell division (Sachs *et al.*, 1959)^[20] and cell enlargement (Haber and Leopold 1960; Haber *et al.*, 1969)^[9, 10]. Gibberellins stimulate cell elongation by altering the rheological properties of the cell wall. As a consequence, the water potential of the cell wall is lowered allowing for water uptake and therefore an increase in cell volume (Jones and Kaufman 1983)^[13].

Certain chemicals like thiourea and potassium nitrate are widely used to break seed dormancy (Agrawal and Dadlani 1995)^[3]. Thiourea overcomes certain types of dormancy, such as the

seed- coat inhibiting effect of deep embryo-dormant seeds (Hartmann *et al.*, 1997) ^[11]. Thiourea improve the germination as it has a strong neutralizing effect on inhibitors present in seed or may be due to variation in characteristic of thiourea (Hore and Sen 1994) ^[12]. Potassium nitrate has also been known as suitable chemical approach for promoting germination in various plant species and generally as a halo-priming agent for germination (Shim *et al.*, 2008) ^[22]. The present investigation aimed to study the effect of seed priming with GA₃, Thiourea and KNO₃ on seed germination, seedling growth and quality parameters in amlook.

Material and Methods

The study was conducted at the Experimental Farm and laboratory of Department of Seed Science and Technology, Dr YS Parmar UHF, Nauni, Solan (H.P.) during the year 2019-20. In field trial, there were eleven different seed priming treatments viz. T₁: Priming with GA₃ @ 250 ppm, T₂: Priming with GA₃ @ 500 ppm, T₃: Priming with GA₃ @ 750 ppm, T₄: Priming with Thiourea @ 2500 ppm, T₅: Priming with Thiourea @ 5000 ppm, T₆: Priming with Thiourea @ 7500 ppm, T₇: Priming with KNO₃ @ 2500 ppm, T₈: Priming with KNO₃ @ 5000 ppm, T₉: Priming with KNO₃ @ 7500 ppm, T₁₀: Hydro-priming, T₁₁: No priming (control).

Amlook seeds were stratified for 45 days in the alternate layers of sand in a wooden box and kept at the lower chamber of refrigerator during the month of January. The seeds were soaked in given concentrations of GA₃, Thiourea and KNO₃ solutions for 24 hours and then shade dried for two hours before sowing in nursery beds. Seeds were sown in these beds in the first week of March, 2019. Observations on different parameters were performed as per the standard procedures.

The height and diameter of the seedlings was measured with a measuring tape and digital Vernier Calipers, respectively, at the end of the season. For leaf area, five randomly selected leaves from each replication were measured with the help of Leaf Area Meter (LI-COR Model-3100).

At the end of growing season, survival percentage of each treatment was recorded

$$\text{Survival percentage} = \frac{\text{Total number of survived seedlings}}{\text{Total number of germinated seedlings}} \times 100$$

During December, seedlings having diameter of 5 mm or above were considered as graftable seedlings and was calculated on percentage basis. To determine the fresh weight of shoots and roots, after uprooting the seedlings at the end of season, shoot and root portion was separated and cut into small pieces. The

fresh weight of shoot and root segments was then recorded separately on electronic balance. To record the dry weight, the root and shoot segments were dried at 65±5 °C in an oven until a constant weight was obtained. Total root length was measured with the help of root length scanner (Comair Root Length Scanner). Total biomass of seedlings was obtained by adding the dry weight of shoots and roots. For computation of germination percentage, the germination was recorded from the emergence of seedlings after sowing till the emergence has stopped.

$$\text{Germination \%} = \frac{\text{Total number of germinated seeds}}{\text{Total number of seeds sown}} \times 100$$

Seedling vigour index-I and II was calculated (Abdul-Baki and Anderson 1973) ^[11]

Seedling vigour index-I = Germination percentage (%) × Seedling length (cm)

Seedling vigour index-II = Germination percentage (%) × Seedling dry weight (mg)

Statistical analysis: Data generated from the experiment were subjected to statistical analysis with the standard procedure as suggested by Gomez and Gomez (1984) ^[18]. The level of significance was tested at 5% value of significance using computer software OP Stat.

Results and Discussion

Plant growth: The growth of amlook seedlings was significantly affected by the different seed priming treatments (Table 1 and 2). The growth of seedlings with application of GA₃ was significantly higher as compared to control. The maximum seedling height (29.10 cm), seedling diameter (6.27 mm), number of leaves per seedlings (17.07), leaf area (86.04 cm²), fresh weight of shoots and roots (16.32 g and 11.97 g), dry weight of shoots and roots (8.57 g and 6.24 g), biomass of seedlings (14.81 g) and total root length (1.45 m) was recorded in seeds primed with T₂ i.e., GA₃ @ 500 ppm.

The highest survival percentage and number of graftable seedlings was also recorded when seeds were primed with GA₃ @ 500 ppm. Whereas, the minimum seedling height (23.50 cm), seedling diameter (4.70 mm), number of leaves per seedlings (12.00), leaf area (60.61 cm²), fresh weight of shoots and roots (12.64 g and 8.94 g), dry weight of shoots and roots (6.31 g and 5.10 g), biomass of seedlings (11.41 g) and total root length (0.76 m) was observed with T₁₁. The minimum survival percentage and number of graftable seedlings was also recorded in T₁₁.

Table 1: Effect of seed priming treatment on growth characters of amlook seedlings

Treatments	Seedling height (cm)	Seedling diameter (mm)	Number of leaves per seedling	Leaf area (cm ²)	Survival* (%)	Number of graftable seedlings* (%)
T ₁	28.25	5.60	14.97	73.02	80.71 (63.92)	87.38 (69.17)
T ₂	29.10	6.27	17.07	86.04	93.84 (75.64)	96.99 (80.17)
T ₃	28.83	6.00	16.43	85.34	90.68 (72.20)	93.23 (74.91)
T ₄	27.47	5.53	14.80	72.04	78.00 (62.01)	82.11 (64.95)
T ₅	26.38	5.43	14.47	70.19	75.65 (60.41)	79.51 (63.07)
T ₆	25.82	5.30	13.90	70.40	72.86 (58.59)	77.04 (61.35)
T ₇	26.17	5.37	13.27	66.71	74.76 (59.82)	78.26 (62.19)
T ₈	27.70	5.73	13.77	78.21	83.06 (65.68)	88.33 (70.04)
T ₉	28.00	5.87	14.60	79.15	86.06 (68.07)	91.02 (72.56)
T ₁₀	24.75	4.97	12.53	63.88	69.32 (56.35)	71.79 (57.89)
T ₁₁	23.50	4.70	12.00	60.61	68.18 (55.65)	69.73 (56.60)
CD _(0.05)	0.51	0.31	1.24	5.29	1.61	1.91

*Values in parenthesis are angular transformed values

Table 2: Effect of seed priming treatment on growth characters of amlook seedlings

Treatment	Fresh weight of shoots (g)	Fresh weight of roots (g)	Dry weight of shoots (g)	Dry weight of roots (g)	Biomass of seedlings (g)	Total root length (m)
T ₁	15.66	10.71	7.80	6.02	13.82	1.33
T ₂	16.32	11.97	8.57	6.24	14.81	1.45
T ₃	16.00	11.41	8.30	6.10	14.40	1.40
T ₄	14.73	10.64	7.73	5.86	13.60	1.32
T ₅	14.39	10.04	7.23	5.64	12.87	1.27
T ₆	13.87	9.94	6.90	5.57	12.47	1.23
T ₇	13.53	9.69	6.81	5.30	12.11	1.17
T ₈	13.67	9.93	6.85	5.40	12.25	1.24
T ₉	14.64	10.52	7.41	5.74	13.16	1.34
T ₁₀	13.25	9.37	6.63	5.18	11.81	0.84
T ₁₁	12.64	8.94	6.31	5.10	11.41	0.76
CD _(0.05)	0.58	0.58	0.33	0.20	0.38	0.08

The increase in height of seedling with application of GA₃ treatment might be due to early germination, cell division and quick cell elongation (Shanmugavalli *et al.* 2007) ^[21]. Our findings are in consonance with the investigations of Kumar and Shahnaz (2013) ^[14], who also recorded the maximum seedling length with seed treatment of 500 ppm GA₃, in an experiment on the effect of growth regulators in wild apricot. More number of leaves may also be due to more height of seedling and faster rate of cell multiplication and cell elongation as suggested by Agha *et al.* (1990) ^[2] in sour orange and citrange. The higher leaf area with the GA₃ treatments may be due to its contribution in cell division, elongation and multiplication (Mane *et al.* 2018) ^[15]. The increase in the seedling diameter by the treatment of gibberellins may be due to the fact that it caused the stimulation of cambium cells and immediate cell progeny by cell multiplication process (Dhankhar and Singh 1996) ^[6].

The increase in seedling height and diameter along with well developed root system in the seed treatment of GA₃ may be the possible reason for higher number of graftable seedlings. The highest survival rate of seedlings resulted from GA₃ treated seeds may be due to the earlier and faster root initiation, which resulted into vigorous and sturdy seedlings (Palepad *et al.* 2017) ^[17].

The increased fresh weight of shoots with GA₃ application may be due to the early and increased vegetative growth. The higher

shoot dry weight with GA₃ priming treatment may be attributed to the overall seedling growth and an increased photosynthetic rate, ultimately lead to the overall assimilation and distribution of photosynthates in the seedlings. These findings are in agreement with the studies of Kumar and Shahnaz (2013) ^[14], who observed the maximum dry weight of shoots with the seed treatment of GA₃ (500 ppm) in wild apricot.

The more fresh weight of roots by GA₃ treatment in our investigations may be attributed to the fact that gibberellins plays an important role in cell division, cell elongation, auxin metabolism, cell wall plasticity and cell membrane permeability ultimately leading to increased vegetative growth (Rai *et al.* 2018) ^[19]. The increase dry weight of roots with GA₃ treatment in our studies may be due to the fact that higher seedling growth synthesized more photosynthetic assimilates and translocated them to the roots. The increased vegetative growth due to the faster rate of cell division, cell elongation and more synthesis and accumulation of photosynthates could be the possible reason for the improved seedling biomass and increase in total root length with the seed priming treatment of GA₃. The higher total root length in our findings through seed priming with GA₃ may be due to the fact that gibberellins increased the physiological processes in the plants, which is essential for cell division and cell enlargement (Vishwakarma 2013) ^[24].

Table 3: Effect of seed priming treatment on germination and vigour of amlook seedlings

Treatment	Days taken to germination	Seed germination (%)	Seedling vigour index-I	Seedling vigour index-II
T ₁	40.03	59.70	2,295.15	825.07
T ₂	38.40	64.26	2,547.88	951.91
T ₃	39.30	63.57	2,494.14	915.11
T ₄	42.17	57.13	2,123.85	776.86
T ₅	43.57	56.50	2,028.46	726.52
T ₆	45.05	52.13	1,826.19	650.44
T ₇	44.37	53.70	1,910.05	650.94
T ₈	43.87	55.63	2,089.00	681.56
T ₉	39.77	61.20	2,314.13	801.52
T ₁₀	52.25	40.03	1,343.08	472.87
T ₁₁	55.17	38.62	1,243.43	440.48
CD _(0.05)	7.13	9.59	215.34	77.71

Germination and vigour: The data presented in Table 3 indicate that germination and vigour of amlook seedlings was significantly affected by the different seed priming treatments. The highest seed germination (64.26%) and SVI-I (2547.88), SVI-II (951.91) was recorded in seeds primed with T₂ treatment. Significantly less days taken to seed germination (38.40 days) were recorded with treatment T₂ i.e., GA₃ @ 500 ppm as compared to T₁₀ (Hydro-priming) and T₁₁ (Control-no priming)

taking 52.25 days and 55.17 days, respectively.

The early germination with chemical priming may be attributed to their effect on enzymatic activities and various physiological processes inside the seeds. Lesser days taken to seed germination by GA₃ treatments might be due to the fact that gibberellins have antagonistic effect on germination inhibitors (Brian and Hemming 1958, Wareing and Foda 2006) ^[5, 26] and endogenous gibberellins were reported to increase due to

soaking (Mathur *et al.* 1971)^[16].

The higher germination in amlook seed by different chemical priming treatments might be due to their effect to bring about a favourable internal condition (breaking of seed dormancy) for maximum germination. According to Wagh *et al.* (1998)^[25], the application of GA₃, might have triggered the activity of specific enzymes that promoted early germination, such as α -amylase, which have brought an increase in availability of starch assimilation. The more vigorous seedlings with GA₃ treatment in our studies may be due to the earlier seed germination, more number of leaves, higher shoot and root length, which lead to more assimilation and distribution of food material to the whole plant (Brian and Hemming 1958)^[5].

Conclusion

Seed priming with GA₃ @ 500 ppm for 24 hours was found superior among all the treatments in terms of germination and growth parameters. Therefore, could be recommended for improving the germination and seedling growth in amlook.

Authors Contribution

CLS designed the study and supervised the experiment; RJ collected the data, performed the analysis and developed a draft of the manuscript; DPS contributed in supervising and drafting; DT and AC assisted in data collection and drafting; and further all the authors improved and approved the manuscript.

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