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Studies on Various Pre Sowing Treatments on Seed Germination and Seedling Growth of English Walnut (*Juglans regia* L.)

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ABSTRACT

Field experiment was conducted to determine the most suitable pre sowing treatments for seed germination and seedling growth of walnut. The study shows that germination and seedling growth of walnut was improved with combine application of all pre sowing treatments. Nut cracking + gibberellic acid @ 500 ppm + stratification for 60 days showed highest germination percentage where as early germination was observed under the treatment combination of nut cracking + gibberellic acid @ 1000 ppm + stratification for 60 days. Highest shoot height, shoot diameter, number of leaves per plant, root length, root area, shoot fresh weight, root fresh weight, total fresh weight and survival percentage were recorded in treatment with hot water + gibberellic acid @ 500 ppm + stratification for 60 days. Therefore, the results of present experiment indicate that it is the combination of various pre sowing treatments which was found effective in improving the germination as well as growth of walnut seedlings as compared to the control.

Keywords: *Walnut, Seed Germination, Gibberellic acid, Stratification, Cracking, Hot water treatment*

I. Introduction

Walnut (*Juglans regia* L.) is an important temperate nut crop with delicious kernel. It belongs to family Juglandaceae. The genus *Juglans* has 21 species of which *Juglans regia* is the most important species. Walnut is believed to have originated in Iran. Walnuts are rich in proteins, fats and minerals and are a concentrated source of energy. These contain a good amount of vitamin B group and are the richest in vitamin B_6 among all the nuts (Vermudy, 2011). Seed dormancy is the major barrier to walnut seed germination. Seed dormancy in walnut has been attributed to one or more factors (Stockes, 1965) such as due to hard and impermeable seed coat, immaturity of embryo, after ripening in dry storage, presence of inhibitors and germination stimulators and may be due to light sensitivity of walnut seeds. In case of walnut the seed dormancy has been correlated with physiological dormancy that is controlled by seed coat and embryo dormancy. Seed germination is a complex process which started with the absorption of water and after a short period the enzyme is activated (Matilla and Matilla-Vazquez, 2008). Many practices which are commonly followed to break the dormancy in walnut, in order to improve or stimulate germination i.e. scarification, stratification and gibberellic acid. The objective of this experiment was to evaluate different pre sowing treatments which are helpful to improve seed germination percentage and seedling growth.

II. Material and Methods

The present experiment was conducted at the Horticulture Research Block of Shri Guru Ram Rai School of Agricultural Sciences, SGRRU, Dehradun, Uttarakhand during 2017-18. Fully mature walnut seeds were purchased from Srinagar. Selected seeds were washed, float-checked, air dried and then subjected to various pre sowing treatments viz. Nut cracking (T_1), Hot water treatment (T_2), Nut cracking+GA3@500ppm (T_3), Nut cracking+GA3@750ppm (T_4), Nut cracking+GA3@1000ppm (T_5), Hot water+GA3@500ppm (T_6), Hot water+GA3@750ppm (T_7), Hot water+GA3@1000ppm (T_8), Nut cracking + Stratification for 60 days (T_9), Hot water + Stratification for 60 days (T_{10}), Nut cracking + GA3@500ppm + Stratification for 60 days (T_{11}),
Nut cracking + GA3@750ppm + Stratification for 60 days (T_{12}), Nut cracking + GA3@1000ppm + Stratification for 60 days (T_{13}), Hot water + GA3@500ppm + Stratification for 60 days (T_{14}), Hot water + GA3@750ppm + Stratification for 60 days (T_{15}), Hot water + GA3@1000ppm + Stratification for 60 days (T_{16}) and Control (T_{17}) The above treatments were sowed in Randomized Complete Block Design with three replications at the spacing (30 x 30) cm^2 in open ventilated polyhouse.

III. Results and Discussion

Seed germination and Germination percentage

The combine application of various pre sowing treatments resulted in minimum days taken for germination and maximum germination. Seeds of walnut showed early germination (12.68 days) as well as best germination (75.68%) when combination of nut cracking with GA3 @ 750 ppm stratification for 60 days and combination of nut cracking with GA3 @ 500 ppm for 60 days stratification were used respectively as compared to control and single treatments. The maximum germination might be due to the fact that GA3 involved in the activation of cytological enzymes which stimulates α – amylase enzyme that converts insoluble starch into soluble sugars (Babu *et al.*, 2012) and early germination might be due to the fact that, GA3 plays an important role in two stages of germination one at initial enzyme induction and other in activation of reserve food mobilizing system which help in enhancement of germination (Jha *et al.*, 1997). In the present experiment, it has been observed that gibberellic acid was required in relatively lower concentration with stratification and scarification for the maximum germination. The inability of walnut seeds to germinate may be due to the presence of hard seed coat. As the scarification treatment given to the seed helped in water uptake, growth hormones and air which is essential for seed germination (Çetinbaş *et al.*, 2006; Conner, 2008 and Al-Absi, 2012). Prechilling stratification had a significant effect on breaking of seed dormancy. It can be attributed that at low temperature more oxygen dissolves in water and therefore more oxygen is available for embryo (Young and Young, 1998).

Shoot growth parameters

The maximum shoot height (37.85 cm), shoot diameter (4.27 mm), number of branches per plant (8.20) and number of leaves per plant (42.00) were recorded under combination of hot water with GA3 @ 500 ppm under 60 days stratification period. It might be due to the effect of GA3 and stratification which helps to promote growth due to the solubility of fats and sugars caused by stratification and also due to the increase in gibberellin biosynthesis. In addition, the improving effect of GA3 and stratification on seed germination might have reflected on enhancement of the shoot parameters. These findings are in agreement with Dahkaei (2013) on *Danae racemosa*, Rawat *et al.*, (2010) on *Punica granatum* and Hassan and Fetouh (2014) on seeds of *Magnolia grandiflora*. The seedling which was raised from combination of hot water with GA3 @ 500 ppm under 60 days stratification period attained more shoot height which reveals that as the height of seedlings increased, there was a simultaneous increase in the

number of leaves. Similar observations were also reported by Mathur (1964) in peach and apricot seedlings. The GA3 hormone increases cell size by stimulating the cell wall to release and transmit its calcium into the cytoplasm that provides a condition for absorption of water and cell growth and moreover in stratification, endosperm is disrupted permitting the growth of embryo. On the other hand, low temperature stimulates the breakdown of proteins into soluble nitrogenous compounds and formation of the amino acids such as glycine and argentine, which are beneficial for embryo growth (Baskin and Baskin, 2011 and Razavi *et al.,* 2009).

Root growth parameters

The highest root length (28.52 cm), root diameter (5.59 cm) as well as root area (19.82 cm^2) was recorded with the combination of hot water + gibberellic acid @ 500 ppm + stratification for 60 days which might be due to the fact that the more shoot growth resulted in production of photosynthates which were translocated through phloem to the root zone and was responsible for increase in root length. Pravin *et al.,* (2016) who reported maximum root length and root area of walnut with combination of gibberellic acid and stratification. The effect of GA3 and stratification on root parameters followed the same trend as on the shoots. The positive effect of GA3 and stratification on root parameters might be explained through the role of GA3 and stratification in enhancing gibberellin biosynthesis which also leads to increase the growth, root branching and overall increase in root fresh weight (Penfield *et al.*, 2015).

Shoot and root fresh weight

The maximum shoot fresh weight (14.27 g), root fresh weight (12.16 g) and total fresh weight (24.64g) were observed with the treatment combination of hot water, gibberellic acid @ 500 ppm and stratification for 60 days. This could be explained through the role of stratification in enhancing gibberellin biosynthesis which also leads to increase in the growth, root branching and overall increased roots fresh weight. The production of maximum shoot height, root height, diameter of both and maximum number of leaves which indicates that as the all growth parameters of shoot as well root of seedlings increased, there was a simultaneous increase in the shoot and root fresh weight. Similar observations were also reported and confirmed by Pawar *et al.,* (2014) in Jatropa, Farhoudi *et al.,* (2015) in *Echinacea purpurea* and Parvin *et al.*, (2014) in black walnut.

Survival percentage

The maximum survival percentage (94.64%) was reported with treatment combination of hot water + gibberellic acid @ 500 ppm + stratification for 60 days which might be due to stratification helps to increase the early germination which resulted into longest radical growth, which helps in early establishment of new germinating seedling to produce maximum food material which helped in photosynthesis that resulted into the maximum survival of seedlings. The results are in conformity with the observations of Wani (2014) who reported increase in the survival percentage with the application of gibberellic acid @ 500 ppm for 40 hours. It might be as GA3 favors the increased enzymatic activity that leads to the favorable environment for the seed germination as well as the growth of the radicle and plumule leading to better growth and survival of seedlings.

From the present study it can be concluded that among various pre sowing treatments, the best results in terms of shoot height, shoot diameter, number of leaves per plant, root length, root diameter, root area, shoot fresh weight, root fresh weight, total fresh weight and survival percentage were recorded with treatment combination of hot water + gibberellic acid @ 500 ppm + stratification for 60 days (Table 1 & 2). The combination of various pre sowing treatments was found effective in improving the germination and growth of walnut seedlings and the best treatment was T_{14} (hot water + gibberellic acid @ 500 ppm

+ stratification for 60 days) which was highly effective in enhancement of the overall growth of walnut seedlings.

References

[1] Al-Absi, K.M. 2012. The effects of different pre-sowing seed treatments on breaking the dormancy of Mahaleb cherries, (*Prunus mahaleb* L.) seeds. *Seed Sci. Technol.,* 38: 332-340.
[2] Anburani, A., and Shakila, A. 2010. Influence of seed treatment on the enhancement of germination and seedling vigor of papaya. *Acta Horticulturae,* 851: 295-98.
[3] Babu, K.D., Patel, R.K., Singh, A., Yadav, D.S., De, L.C., and Deka, B.C. 2012. Seed germination, seedling growth and vigour of papaya under North east Indian condition. *Acta Horticulturae,* 851: 299-306.
[4] Barche, S. Kirad, K.S. and Singh, D.B. 2010. Response of seed treatment on germination, growth, survivability and economics of different cultivars of papaya. *Acta Horticulturae,* 851: 279-284.
[5] Baskin, C.C. and Baskin, J. 2011. Seeds: Ecology, Biogeography and Evolution of Dormancy and Germination. San Diego, Academic Press.
[6] Çetinbaş, M. and Koyuncu, F. 2006. Improving germination of *Prunus avium* L. seeds by gibberellic acid, potassium nitrate and thiourea. *Hortsci.,* (Prague) 33(3): 119–123.
[7] Conner, P.J. 2008. Effects of stratification, germination, temperature and pretreatment with gibberellic acid and hydrogen peroxide on germination of 'Fry' muscadine *(Vitis rotundifolia)* seed. *Horticulture Sci.,* 43: 853–856.
[8] Dahkaei, M.N.P. 2013. Effect of Gibberellic acid, temperature and cold moist stratification on seed germination of Danae racemosa. *Acta Horticulturae*, 813: 445-452.
[9] Farhoudi, R., Modhej, A. and Jamshidi, A.R. 2015. *Echinacea purpurea* seed pretreatment to improve germination. *Res. J. Fishries and Hydrobiol.,* 10(9): 58-61.
[10] Hassan, M.I. and Fetouh, F.A. 2014. Seed germination criteria and seedling characteristics of *Magnolia grandiflora* L. trees after cold stratification treatments. *Int. J. Curr. Microbiol. Appl. Sci.,* 3(3): 235-241.
[11] Jha, B.N., Kumar, V., Singh, R.P. Kumari, R. and Sinha, M. 1997. Dormancy in groundnut Standardization of procedure of breaking. *J. Appl. Biol.,* 7: 23-25.
[12] Mathur, D.D., Couvilon, G.A., Vines, H.M. and Hendershott, C.H. 1971. Stratification effects on endogenous gibberellic acid in peach seeds. *Hortsci.,* 6: 538.
[13] Matilla, A.J. and Matilla-Vazquez, M.A. 2008. Involvement of ethylene in seed physiology. *Plant Sci.,* 175: 87-97.
[14] Parvin, P. Khezri, M. Tavasolian, I. and Hosseini, H. 2014. The Effect of Gibberellic Acid and Chilling Stratification on Seed Germination of Eastern Black Walnut (*Juglans nigra* L.) *J. Nuts,* 6(1): 67-76.
[15] Pawar, V.B., Gore, R.V., Patil, V.K. and Narsude, P.B. 2014. Effect of gibberellic acid on seed germination and growth of *Jatropha curcas*L. *The Asian J. Horticulture,* 5(2): 311-313.
[16] Penfield, S. Josse, E.M. Kannangara, R. Gilday, A., D. Halliday,K., J. and Graham, I.A. 2015. Cold and light control seed germination through the bHLH transcription factor spatula. *Curr. Biol.,* 15: 1998-2006.
[17] Powell, L.E. 1987. Hormonal aspects of bud and seed dormancy in temperate-zone woody plants. *Hortsci.,* 22: 845–850.
[18] Pratibha, C. Teja, T. and Krishna, P.M. 2015. Effect of Chemical Treatments on the Germination and Subsequent Seedlings Growth of Papaya *(Carica papaya* L.*)* Seeds cv. Pusa Nanha. *J. Agri. Engi. Food Technol.,* 2(3): 189-191.

[19] Rawat, J.M.S., Tomar, Y.K. and Rawat, V. 2010. Effect of stratification on seed germination and seedling performance of wild pomegranate. *J. American Sci.,* 6(5): 97-99.
[20] Razavi, S.M. and Hajiboland, R. 2009. Dormancy breaking and germination of *Prangos ferulaceae* seeds. *Eur. Asian J. BioSci.,* 3(11): 78-83.
[21] Stokes, P. 1965. Temperature and seed dormancy. In: Ruhland W. (Eds.), Encyclopedia of Plan Physiology, 15/2. Springer-Verlag, Berlin, Heidelberg and New York, Pp. 746–803.
[22] Vermudy, V. 2011. Walnuts: Need to expand cultivation. Facts for you, Pp.13- 15.
[23] Wani, R.A., Malik, T.H., Malik, A.R., Baba, J.A. and Dar, N.A. 2014. Studies on apple seed germination and survival of seedlings as affected by gibberellic acid under cold arid conditions. *Int. J. Scientific & Technol. Res.,* 3(3): 210- 216.
[24] Young, R.A. and Young, N.A. 1998. Studies on peach seed germination and survival of seedlings under cold arid conditions. *Int. J. Scientific & Technol. Res.,* 3(6): 213- 218.

Table.1 Effect of various pre sowing treatments on seed germination and seedling growth of walnut

Treatments	Days taken for germination	Germination percentage	Shoot height	Shoot diameter	Number of branches/ plant	Number of leaves/plant
T_1 (Nut Cracking)	59.33± 0.67	49.89 (7.13±0.05)	20.07± 0.88	2.06±0.30	5.17 ± 0.60	20.00 ± 0.00
T_2 (Hot Water)	56.33± 0.88	47 (6.93 ± 0.13)	22.21± 0.67	2.52±0.05	5.83 ± 0.60	25.61 ± 1.75
T_3(Nut Cracking+GA3@500ppm)	25.67± 1.20	53.54(8.07 ± 0.06)	26.75± 0.99	3.08±0.32	6.67 ± 0.44	31.40 ± 4.20
T_4(Nut Cracking+GA3@750ppm)	25.33± 1.33	64.07(7.87 ± 0.05)	28.92± 0.71	3.11±0.17	7.50 ± 0.58	34.01 ± 1.03
T_5(Nut Cracking+GA3@1000ppm)	26.00± 1.15	60.99(7.38 ± 0.11)	25.00± 0.86	2.43±0.05	6.67 ± 0.67	31.48 ± 1.48
T_6(HotWater+GA3@500ppm)	19.67± 0.67	60.71(7.85± 0.07)	27.18± 1.73	3.30±0.15	6.83 ± 0.73	30.97 ± 0.73
T_7(HotWater+GA3@750ppm)	19.00 ±1.15	55.63(7.52 ± 0.16)	30.38± 1.18	3.45+0.00	7.33 ± 0.60	35.24 ± 1.95
T_8(HotWater+GA3@1000ppm)	19.33± 0.33	53.72(7.39 ± 0.29)	25.12± 0.39	3.19+0.14	6.17 ± 1.30	30.87 ± 3.66
T_9(Nut Cracking + Stratification for 60 days)	19.00± 0.58	52.05(7.28 ± 0.09)	26.35± 1.57	3.12+0.23	6.00 ± 0.76	30.07 ± 2.37
T_{10} (Hot Water + Stratification for 60 days)	18.00± 1.53	50.15(7.15 ± 0.04)	29.03± 0.86	3.05+0.35	6.23 ± 0.43	25.99 ± 2.06
T_{11}(Nut Cracking+GA3@500ppm+ Stratification for 60 days)	14.33± 0.33	75.68(8.77 ± 0.11)	34.79± 0.35	3.48+0.19	7.57 ± 0.07	36.33 ± 0.90
T_{12}(Nut Cracking+GA3@750ppm+ Stratification for 60 days)	12.68± 0.33	73.05(8.60 ± 0.04)	34.73± 0.52	3.58+0.16	7.33 ± 0.60	35.47 ± 2.24
T_{13}(Nut Cracking+GA3@1000ppm +Stratification for 60 days)	14.33± 0.33	71.63(8.52 ± 0.04)	30.50± 0.91	3.09+0.07	6.50 ± 0.58	30.74 ± 0.74
T_{14} (HotWater+GA3@500ppm+ Stratification for 60 days)	13.33 ± 0.33	74.46(8.67 ± 0.07)	37.85± 0.34	4.27+0.31	8.20 ± 0.00	42.00 ± 0.00
T_{15} (HotWater+GA3@750ppm+ Stratification for 60 days)	14.00 ± 0.58	72.34(8.56 ± 0.07)	35.28± 0.22	3.30+0.07	7.17 ± 0.00	35.67 ± 0.67
T_{16}(HotWater+GA3@1000ppm+ Stratification for 60 days)	14.67 ± 0.33	71.63(8.52 ± 0.04)	31.08± 0.85	3.24+0.16	7.00 ± 0.88	32.71 ± 1.40
T_{17} (Control)	76.67± 9.26	45.66(6.90 ± 0.29)	18.58± 0.88	2.17±0.34	5.00 ± 0.58	23.33 ± 4.41
± SE (m)	3.38	1.89 (0.18)	1.28	0.31	0.98	3.10
$CD_{0.05}$	6.88	3.79 (0.37)	2.61	0.63	NS	6.31

Table.2 Effect of varoius pre sowing treatments on seed germination and seedling growth of walnut

Treatments	Root length	Root diameter	Root area	Shoot fresh weight	Root fresh weight	Total fresh weight	Survival percentage
T_1 (Nut Cracking)	15.02± 0.73	3.14 ± 0.42	12.40 ± 0.29	5.40 ± 0.40	5.33 ± 0.83	10.73 ± 0.72	69.87 (8.42 ± 0.04)
T_2 (Hot Water)	13.56± 0.95	3.36 ± 0.18	11.74 ± 0.37	5.71 ± 0.11	4.22 ± 0.38	9.93 ± 0.49	72.56 (8.58 ± 0.08)
T_3(Nut Cracking+GA3@500ppm)	19.71± 0.41	5.15 ± 0.21	14.71 ± 0.14	7.17 ± 1.33	6.67 ± 0.78	13.84 ± 1.41	86.91 (9.38 ± 0.01)
T_4(Nut Cracking+GA3@750ppm)	21.26± 0.51	5.23 ± 0.12	15.41 ± 0.47	8.58 ± 0.17	7.40 ± 0.70	15.98 ± 0.81	78.62 (8.92 ± 0.12)
T_5(Nut Cracking+GA3@1000ppm)	17.70± 0.45	4.24 ± 0.10	14.19 ± 0.82	6.50 ± 0.80	6.72 ± 0.75	13.22 ± 0.35	73.85 (8.65 ± 0.05)
T_6(HotWater+GA3@500ppm)	21.63± 1.07	4.82 ± 0.11	16.78 ± 0.82	7.77 ± 0.47	7.39 ± 0.77	16.59 ± 1.22	87.42 (9.40 ± 0.11)
T_7(HotWater+GA3@750ppm)	23.65± 0.75	5.25 ± 0.83	16.03 ± 0.20	9.19 ± 0.30	7.18 ± 0.09	14.95 ± 0.21	85.26 (9.29 ± 0.04)
T_8(HotWater+GA3@1000ppm)	18.48± 1.52	5.05 ± 0.42	14.48 ± 0.76	6.74 ± 0.25	6.50 ± 0.49	13.23 ± 0.28	83.18 (9.17 ± 0.10)
T_9(Nut Cracking + Stratification for 60 days)	17.61± 0.46	3.64 ± 0.25	14.39 ± 0.14	6.93 ± 0.53	6.09 ± 0.49	13.02 ± 0.75	82.78 (9.15 ± 0.08)
T_{10} (Hot Water + Stratification for 60 days)	20.23± 0.42	3.43 ± 0.49	13.70 ± 0.44	7.13 ± 0.32	6.33 ± 0.65	13.47 ± 0.80	85.62 (9.31 ± 0.04)
T_{11}(Nut Cracking+GA3@500ppm+ Stratification for 60 days)	25.25± 0.37	3.42 ± 0.65	18.65 ± 0.12	11.89± 0.29	10.75 ± 0.56	22.00 ± 0.70	91.60 (9.62 ± 0.14)
T_{12}(Nut Cracking+GA3@750ppm+ Stratification for 60 days)	25.04± 0.45	3.27 ± 0.21	17.94 ± 0.53	10.51± 0.60	7.28 ± 0.29	17.79 ± 0.81	90.33 (9.55 ± 0.13)
T_{13}(Nut Cracking+GA3@1000ppm +Stratification for 60 days)	23.35± 1.52	3.37 ± 0.55	17.57 ± 0.54	9.34 ± 0.41	6.11 ± 0.24	15.45 ± 0.19	87.30 (9.40 ± 0.10)
T_{14} (HotWater+GA3@500ppm+ Stratification for 60 days)	28.52± 0.60	5.59 ± 0.10	19.82 ± 0.32	14.27± 0.04	12.16 ± 0.19	24.64 ± 0.23	94.64 (9.71 ± 0.05)
T_{15} (HotWater+GA3@750ppm+ Stratification for 60 days)	24.53± 1.15	4.16 ± 0.53	18.47 ± 0.40	11.25± 0.28	7.87 ± 0.86	19.76 ± 1.09	91.19 (9.60 ± 0.09)
T_{16}(HotWater+GA3@1000ppm+ Stratification for 60 days)	23.33± 0.63	4.56 ± 0.54	17.67 ± 0.67	9.25 ± 0.25	7.12 ± 0.11	16.37 ± 0.33	88.11 (9.43 ± 0.09)
T_{17} (Control)	14.32± 0.84	2.53± 0.30	12.04 ± 0.42	5.33 ± 0.55	4.54 ± 0.13	9.87 ± 0.46	67.33 (8.26 ± 0.23)
± SE (m)	1.09	0.57	0.58	0.73	0.78	1.00	2.43 (0.13)
$CD_{0.05}$	1.09 2.23	1.16	1.18	1.48	1.59	2.04	4.96 (0.28)

