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Effect of stratification and gibberellic acid on seed germination and seedling growth of walnut under Kashmir valley conditions

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ABSTRACT

Off-season seed sowing in walnut in order to reduce sprouting period and extend propagation season under field conditions of Kashmir valley was undertaken during 2011 and 2012. Common walnut seeds at cold and ambient conditions treated with GA_3 (50 and 100 ppm), ethrel (1000 and 1500 ppm), sulphuric acid (7.5 and 9.5%) and potassium nitrate (0.2 and 0.4%) were evaluated in split plot design (SPD) having 32 treatment combinations and three replications. GA_3 100 and 50 ppm produced the maximum sprouting (58.24 and 54.23%). Ethrel 1500 and 1000 ppm showed low bud sprouting (38.07 & 35.58%, respectively), but was significantly higher than control (29.99%). Cold treated nuts showed significantly higher germination (46.51%) than ambient conditions (42.21%). Correlation between cold × unsoaked × GA_3 was statistically significant. Unsoaked seeds × cold also produced sprouting rate (47.73%) higher than soaked with ambient storage (42.21%). Cold-30-day × 100 ppm GA_3 interaction showed the maximum sprouting rate (63.29%). Interaction between unsoaked × 30-day-cold × GA_3 100 ppm showed higher sprouting rate (65.33%) than soaked × 30-day-ambient storage × ethrel 1000 ppm (33.33%). Whereas, minimum sprouting (34.33%) was obtained in ambient-30-day × ethrel 1000 ppm. Vegetative growth *viz.*, seedling height, number of days from germination leaf fall and stem diameter (mm) were the similarly influenced. Research findings indicate that off-season sowing in walnut and GA_3 soaking could be economically viable method for enhancing seed germination. Refrigerated cold (30 days) and 100 ppm GA_3 is feasible procedure for forcing seed germination and to extend the period of propagation in walnut.

Key words: Cold treatment, GA_3, seedling height, walnut.

INTRODUCTION

The Persian walnut (*Juglans regia* L.) is the most valuable commercial species in its genus belonging to family Juglandaceae, it has originated in eastern Europe, Asia minor, extending from Turkey, Iran and western China to eastward to the Himalayan regions (Lesile and McGranahan, 9). In India, the state of Jammu and Kashmir occupies an important position, producing about 85 per cent of total production of the country. At present, Jammu & Kashmir has an area of about 93,641 ha with a production of 1, 65,024 MT, giving an average productivity of 1.89 metric tonnes per ha (Anon, 2). The traditional walnut plantations in J&K consist of mature seedling trees characterized by long juvenile period, poor productivity, late nut sprouting and inconsistent fruiting behavior. The germination of walnuts does not take place uniformly due to the fact that seeds are mainly obtained from seedling origin trees and from mixed nut populations. Secondly, during last few years, it has been observed that due to worldwide phenomenon of climate change, snowfall and/ or rainfall takes place during October-December, which otherwise used to be dry periods. The early precipitation does not allow nurserymen to go for seed sowing during normal sowing months, *i.e.*, November-December so that nuts get stratified under field conditions. Under these situations the nuts need artificial treatment to germinate.

Various chemicals as well as seed treatment procedures have been tried in different countries so as to ensure uniform germination of walnut seeds. Furthermore, walnut seed germination needs to be re-evaluated to shorten the time of sprouting, by way of finding alternatives to compensate chilling requirement. Eike *et al*. (3) considering the climatic changes which show rise in average air temperature all chilling models have predicated substantial decrease in winter chill at all sites. The present study was therefore undertaken to identify most reliable chemical to be used as alternative means for enhancing walnut seed germination considering imminent effects of climate change and to produce rootstocks for epicotyl grafting of walnut.

MATERIALS AND METHODS

The experiment was conducted on the feasibility of sowing walnut seeds in off-season to assess uniform germination percentage under open conditions at the Fruit Plant Nursery of Division of Fruit Science, SKUAST-Kashmir, Shalimar during

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2011 and 2012. Selected walnut seeds were divided into two lots, one lot of seeds which were stored in cold temperature (3-4°C) in a refrigerator for 30-day and second lot was kept under ambient conditions (10-12°C). Each lot was divided into two sub-lots. Half of seeds were soaked in water for 12 h and half of seeds were used without soaking for treatment with chemicals, *viz*., GA_3 at 50 and 100 ppm, ethrel 1000 and 1500 ppm, sulphuric acid 7.5 and 9.5 per cent, potassium nitrate 0.2 and 0.4 per cent. GA_3 and ethrel treatments were given for 12 h, sulphuric acid and potassium nitrate for 10 min. The seeds were sown in polythene bags containing uniform rooting medium of sand, soil and organic manure in the ratio of 1:1:1, respectively. Seeds were placed approximately 7.5 cm deep in punched polythene bags and kept in the open field. The germination of seeds was recorded periodically. The seeds were considered germinated when plumules just began to be visible on the surface. Height and stem diameter of seedling was recorded at the end of growing season. The height of seedling was measured from collar region to the apex. The height was measured with digital Vernier calipers. A random sample of 10 leaflets was taken from each treatment. The leaf area was measured with the help of leaf area meter. The germination of walnut was observed and the dates were recorded accordingly till germination was almost over to count the days of seedling from germination to leaf fall. The statistical analysis was performed in split plot design consisting of 32 treatment combinations with plot size 20 and three replications.

RESULTS AND DISCUSSION

Gibberellic acid and cold temperature significantly influenced sprouting percentage in walnut seeds. Data (Table 1) revealed that 100 and 50 ppm GA_3 showed maximum germination (58.24 and 54.23%, respectively). This increase in seeds germination percentage might be related to the initial enzyme induction and to the activation of reserve food-mobilizing systems by gibberellins, which have also been used to enhance germination and stimulate early seedling emergence and growth (Hopkins and Hüner, 6). Diffusion of endogenous auxin and gibberellin-like substances might have antagonized the effects of inhibitors present in the seed (Mathur *et al*., 10). Results are in accordance with Nabil and Al-Imam (11) who also observed that pistachio seeds soaked for 12 h in 200 mg/l GA_3 showed sprouting rate ranging from 81.6 to 85.00% and for 12 h in 100 ppm GA_3 (77.83). Data indicates that minimum germination (38.07 and 35.58%) was produced at 1500 and 1000 ppm ethrel, respectively (Table 1), which was significantly higher to control. Results are in accordance with Kumar *et al.* (8) who reported higher concentrations of ethanol (5%) to be more effective in promoting maximum seed germination in pear. Data indicate that cold treated nuts showed significantly higher germination (46.51%) than ambient conditions. These results are in line with those of Aslamarz *et al*. (1) and Ji and Wang (7).

Data (Table 1) also indicate that unsoaked seeds showed germination (47.73%) significantly higher than soaked seeds. (Frutos, 4) who reported that gradient of humidity between applied chemical and tissue of seeds ought to be higher in dry nuts than soaked ones. Interaction (Table 1) between 30-day-cold × GA_3 100 and 50 ppm also indicate the higher sprouting rate (63.29%) and (57.29%), respectively. Results are in accordance with Ruduicki (15) who reported that activity of protease and lipase enzymes increases mobilization of stored food material in the

Table 1. Germination percentage in walnut seeds post chemical treatments.

Chemical treatment	Conc.	Cold seed treatment		Mean	Ambient seed treatment		Mean	Overall mean
		Unsoaked	Soaked		Unsoaked	Soaked		
GA_3	50 ppm	61.66	53.53	57.59	51.66	50.33	50.99	54.23
	100 ppm	65.33	61.26	63.29	53.33	52.66	52.99	58.24
Ethrel	1000 ppm	38.33	35.33	36.83	35.33	33.33	34.33	35.58
	1500 ppm	40.66	36.66	38.66	38.33	36.66	37.49	38.07
Sulphuric acid	7.5%	53.33	51.66	52.49	48.33	45.33	46.83	49.66
	9.5%	51.66	50.33	50.99	46.66	43.33	44.99	47.99
Potassium nitrate	0.2%	43.33	41.66	42.49	41.66	38.33	39.99	41.24
	0.4%	46.66	45.33	45.99	43.33	41.66	42.49	44.24
Control		28.26	31.16	30.16	28.33	31.33	29.83	29.99
Mean		47.73	45.29	46.51	42.99	41.44	42.21	

$CD_{0.05}$ Storage (S) = 2.05; Chemical (C) = 2.11; Seed treatment (ST) = 1.01; C × S = 2.32; ST × S = 2.05, S × C × ST = 2.37

seeds. Samaan *et al*. (16) who reported that 15 days chilling and subsequent soaking in GA_3 had increased sprouting rate in stone fruits. Data also indicates that sulphuric acid (7.5 & 9.5%) and potassium nitrate (0.2 & 0.4%) significantly improved germination however sprouting rate declined when compared with different doses of gibberellic acid. Similar results were obtained by Nasir *et al.* (12) seeds of almond nuts scarified with (7.5%) concentrated H_2S0_4 for 3 min. gave good germination (50%) than 9.5 per cent for 3 min. gave only (28.60%) germination. Seedling growth seems to be more influenced by growth regulator GA_3 compared to other chemicals (Table 2). Maximum seedling height (23.24 and 20.24 cm) was observed in GA_3 100 and 50 ppm, respectively. Growth parameters like root length, number of secondary roots, number of leaves, leaflet size, number of days from germination to leaf fall, and stem diameter were similarly influenced with GA_3 100 and 50 ppm (Tables 3-7). The increase in seedling growth parameters with GA might be related to the fact that GA promotes stem, and shoot elongation through both cell division and internodal elongation in higher plants (Hopkins and Huner, 6). The results are in accordance with Negi *et al.* (13) and Rahemi and Baninasab (14) on pistachio.

Forcing seed germination in walnut can be a viable procedure in order to evaluate most reliable chemical to be used as alternative means for enhancing seed germination and generate rootstocks for epicotyl grafting, which can be performed round the year under controlled temperature and humidity. Germination rate also declined in treatment interactions indicating that minimum germination (33.33%) in soaked nuts treated with 1000 ppm ethrel under ambient storage

Table 2. Height (cm) of walnut seedlings post chemical treatments.

Chemical treatment	Conc.	Cold seed treatment		Mean	Ambient seed treatment		Mean	Overall mean
		Unsoaked	Soaked		Unsoaked	Soaked		
GA_3	50 ppm	22.85	19.72	21.28	19.21	19.20	19.21	20.24
	100 ppm	25.35	22.99	24.17	22.31	21.34	22.31	23.24
Ethrel	1000 ppm	15.02	14.83	13.92	13.37	13.20	13.25	13.50
	1500 ppm	15.62	12.52	14.07	13.43	13.49	13.38	13.70
Sulphuric acid	7.5%	19.89	18.61	19.25	19.99	19.28	19.99	19.62
	9.5%	19.40	18.06	18.73	18.23	19.02	18.23	18.48
Potassium nitrate	0.2%	18.00	17.44	17.74	17.29	16.98	17.29	17.51
	0.4%	18.86	17.96	18.40	17.62	17.54	17.62	18.01
Control		12.91	13.12	13.01	12.09	13.02	12.56	12.78
Mean		18.66	17.11	21.28	16.91	16.76	16.83	

$CD_{0.05}$ Storage (S) = 0.13; Chemical (C) = 0.17; Seed treatment (ST) = 0.26; C × S = 0.24; ST × S = 0.31; S × C × ST = 1.13

Table 3. Stem diameter (mm) of walnut seedlings post chemical treatments.

Chemical treatment	Conc.	Cold seed treatment		Mean	Ambient seed treatment		Mean	Overall mean
		Unsoaked	Soaked		Unsoaked	Soaked		
GA_3	50 ppm	4.29	4.12	4.20	4.01	3.98	3.99	4.19
	100 ppm	5.71	4.49	5.10	4.68	4.13	4.40	4.75
Ethrel	1000 ppm	4.01	3.93	3.97	3.57	3.39	3.43	3.70
	1500 ppm	4.20	3.99	4.09	3.62	3.40	3.56	3.80
Sulphuric acid	7.5%	4.52	4.05	4.28	3.92	3.79	3.85	4.06
	9.5%	4.40	4.12	4.26	3.80	3.71	3.75	4.00
Potassium nitrate	0.2%	3.79	3.50	3.64	3.72	3.69	3.70	3.67
	0.4%	4.41	3.88	4.14	4.10	3.50	3.80	3.97
Control		2.82	3.03	2.91	2.59	2.80	2.69	2.80
Mean		4.23	3.93	4.09	3.79	3.73	3.53	

$CD_{0.05}$ Storage (S) = 0.21; Chemical (C) = 0.17; Seed treatment (ST) = 0.13; C × S = 0.19 ; ST × S = 0.24; S × C × ST = 0.32

Table 4. Root length (mm) of walnut seedlings post chemical treatments.

Chemical treatment	Conc.	Cold seed treatment		Mean	Ambient seed treatment		Mean	Overall mean
		Unsoaked	Soaked		Unsoaked	Soaked		
GA_3	50 ppm	193.33	188.66	190.99	182.66	160.33	171.49	181.24
	100 ppm	201.66	195.33	198.49	190.66	178.66	184.66	191.57
Ethrel	1000 ppm	151.66	133.66	142.66	115.33	114.66	114.99	128.82
	1500 ppm	104.66	100.33	102.49	106.66	102.33	104.49	103.50
Sulphuric acid	7.5%	171.33	169.33	170.33	167.66	162.33	164.99	167.66
	9.5%	162.66	160.33	161.49	162.33	131.33	146.83	154.16
Potassium nitrate	0.2%	136.33	130.33	133.33	126.66	114.33	120.49	126.91
	0.4%	143.33	137.66	140.49	131.66	118.33	124.99	132.74
Control		85.33	104.66	94.99	84.66	98.33	91.48	93.24
Mean		150.03	146.69	148.36	139.80	129.84	134.82	

$CD_{0.05}$ Storage (S) = 3.32; Chemical (C) = 5.42; Seed treatment (ST) = 2.02;C × S = 2.84; ST × S = 3.52, S × C × ST = 3.67

Table 5. Number of secondary roots on walnut seedling post chemical treatments.

Chemical treatment	Conc.	Cold seed treatment		Mean	Ambient seed treatment		Mean	Overall mean
		Unsoaked	Soaked		Unsoaked	Soaked		
GA_3	50 ppm	27.33	26.66	26.99	25.33	24.66	24.99	25.99
	100 ppm	29.33	27.33	28.33	26.66	26.66	26.66	27.49
Ethrel	1000 ppm	19.43	18.33	18.83	18.86	18.33	18.59	18.66
	1500 ppm	19.33	18.23	18.63	18.18	18.22	18.15	18.39
Sulphuric acid	7.5%	22.66	21.66	22.16	21.33	20.33	20.83	21.49
	9.5%	21.66	19.66	20.66	18.92	19.02	18.97	19.81
Potassium nitrate	0.2%	20.66	18.33	19.49	19.33	18.33	18.83	19.16
	0.4%	20.33	20.33	20.33	18.66	18.66	18.66	19.49
Control		14.35	15.60	14.99	15.66	15.33	15.49	15.24
Mean		21.66	20.69	21.18	20.38	19.94	20.16	

$CD_{0.05}$ Storage (S) = 0.48; Chemical (C) = 1.28; Seed treatment (ST) = 0.63; C × S = 1.55; ST × S = 1.29, S × C × ST = 1.42

Table 6. Leaflet size (cm^2) in walnut seedlings post chemical treatments.

Chemical treatment	Conc.	Cold seed treatment		Mean	Ambient seed treatment		Mean	Overall mean
		Unsoaked	Soaked		Unsoaked	Soaked		
GA_3	50 ppm	74.02	73.06	73.54	71.58	70.44	71.01	72.27
	100 ppm	77.36	74.37	75.86	72.67	72.10	72.38	74.12
Ethrel	1000 ppm	62.91	62.24	62.57	59.76	54.51	57.13	59.85
	1500 ppm	60.28	58.61	59.44	57.19	51.85	54.52	56.98
Sulphuric acid	7.5%	70.59	69.71	70.15	67.96	66.63	67.29	68.72
	9.5%	68.63	67.04	67.83	61.81	63.61	62.71	65.27
Potassium nitrate	0.2%	65.25	62.51	63.88	60.30	60.04	60.17	62.02
	0.4%	63.86	62.63	63.24	61.71	62.27	61.99	62.61
Control		44.10	48.21	46.15	42.58	46.44	44.51	45.33
Mean		65.22	64.26	65.62	61.72	60.87	61.30	

$CD_{0.05}$ Storage (S) = 2.11; Chemical (C) = 1.43; Seed treatment (ST) = 1.01; C × S = 2.15; ST × S = 2.19; S × C × ST = 2.3

Table 7. Number of days from germination to leaf fall in walnut seedlings post chemical treatments.

Chemical treatment	Conc.	No. of days from germination to leaf fall						Overall mean chemical
		Cold seed treatment		Mean	Ambient seed treatment		Mean	
		Unsoaked	Soaked		Unsoaked	Soaked		
GA_3	50 ppm	225.30	223.49	224.39	224.30	222.33	223.31	223.85
	100 ppm	227.33	225.45	226.39	226.33	224.66	225.49	225.94
Ethrel	1000 ppm	208.53	209.66	208.99	209.61	208.33	208.16	208.57
	1500 ppm	210.45	209.30	209.87	213.38	211.43	212.40	211.13
Sulphuric acid	7.5%	219.60	215.66	217.63	217.66	215.66	216.66	217.14
	9.5%	212.33	210.33	213.50	215.65	214.49	215.07	214.28
Potassium nitrate	0.2%	210.38	209.61	209.99	210.66	206.33	208.49	209.24
	0.4%	215.66	212.66	212.50	212.66	210.66	211.66	212.08
Control		192.66	194.33	193.43	192.63	193.13	193.41	193.49
Mean		213.56	212.27	212.97	213.47	212.02	210.45	

$CD_{0.05}$ Storage (S) = 0.32; Chemical (C) = 1.45; Seed treatment (ST) = 0.45; C × S = 1.49; ST × S = 0.53; S × C × ST = 1.54

conditions. Results are in line with Mathur *et al*. (10). Eike *et al.* (3)considering the climatic changes, which show rise in average air temperature all chilling models have predicated substantial decrease in winter chill at all sites. Interactions (Tables 2-7) of 30-day-cold × 100 ppm GA_3 produced seedling height (24.17 cm), stem diameter (5.10 mm), root length (198.49 mm), number of secondary roots (28.33), leaflet size (75.86 cm^2) and number of days from germination to leaf fall (226.39), respectively.

In artificial seed germination maximum sprouting (58.24 and 54.23%) were produced by GA_3 100 and 50 ppm, respectively. Ethrel 1500 and 1000 ppm showed lower sprouting (38.07 and 35.58%) significantly higher than control (29.99%), respectively. Cold treated nuts showed significantly higher germination (46.51%) than ambient conditions (42.21%). Unsoaked seeds x cold also produced sprouting rate (47.73%) higher than soaked with ambient storage (42.21%). Cold-30-day × 100 ppm GA_3 interaction showed maximum sprouting rate (63.29%). Interaction between unsoaked × 30-day-cold × GA_3 100 ppm showed higher sprouting rate (65.33%) than soaked × 30-day-ambient storage × ethrel 1000 ppm (33.33%). Whereas, minimum sprouting (34.33%) was obtained in ambient-30-day × ethrel 1000 ppm. Unsoaked seeds treated with cold-30-day × 100 ppm GA_3 showed vigorous growth, *viz.*, root length (198.49 mm), height (24.17 cm), stem diameter (5.10 mm), number of secondary roots (28.33), leaflet size (75.86 cm^2) and number of days from germination to leaf fall (226.39), respectively. Results are in accordance with Ruduicki (15) who reported that growth parameters like root length, leaflet size in walnut varied according to cultivar. From this present study, it is evident that GA_3 100 ppm can be used to get maximum seed sprouting percentage and can act as alternative for chilling requirement in walnut propagation.

REFERENCES

1. Aslamarz, A.A., Vahdati, K. and Rahemi, M. 2007. *Proceedings of VIth International Walnut Symposium* (Ed. D.L. McNeil). *Acta Hort.* **861**: 269-72.
2. Anonymous, 2010. Food and Agriculture Organization of United Nations c.f: htpp://Foastat.foa.org/site/567/desktopdefault.aspx
3. Eike, L., Minghua, Z., Volker, L. and Evan, H. 2009. Sensitivity of Winter chill models for fruit and nut trees to climatic changes expected in California's central *Agric. Ecol. Env. Sci.* **133**: 3-4. (http://dx.doi.org.).
4. Frutos, D. 1993. Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA), La Alberca, Murcia, Spain (diego.frutos @carm.).
5. Garcia-Gusano, M., Martinez-Gomez, P. and Dicenta, F. 2004. Breaking seed dormancy in almond. *Scientia Hort.* **99**: 363-70.
6. Hopkins, W.G. and Hüner, N.P. 2004. *Introduction of Plant Physiology* (3rd Edn.), John Wiley and Sons, Inc. USA.
7. Ji, J.P. and Wang, Y.L. 1987. Effects of stratification on hormones and its relationship

with dormancy in seeds of peach. *J. Nanjing Agri. Univ*. **1**: 25-30 {c.f. Horticultural Abstracts **59**:7264}.

8. Kumar, A., Nantiyal, M.C. and Prakash, S. 1988. Enhancement of pear (*Pyrus pashia* L.) seed germination by GA_3 and ethanol. *Curr. Sci.* **57**: 964-66.

9. Lesile, C.A. and McGranahan, G. 1998. The origin of walnut. **In**: *Walnut Production Manual*, Ramos, D.E. (Ed.) VCANR. Pub, 320 p.

10. Mathur, D.D., Couvillon, G.A., Vines, H.M. and Hendershoot, C.H. 1979. Stratification effects on endogenous gibberellic acid in peach seed. *HortSci.* **6**: 538-39.

11. Nabil, M. and Al-Ameen, A. 2007. Effect of soaking periods, gibberellic acid and benzyladenine on *Pistachio s*eed germination and subsequent seedling growth (*Pistacia vera* L.). *Mesopotamia J. Agric.* **35**: 187-97.

12. Nasir, M.A., Summrah, M.A., Nawaz, M.Z. and Mohammad, N. 2010. Effect of different scarification methods on germination of almond nuts*. Sarhad J. Agri*c. **17**: 179-82.

13. Negi, N.D., Sharma, S.K. and Pathania, A. 2010. Studies on the effect of various dormancy breaking treatments on pistachio (*Pistacia vera* L.) seed germination and seedling growth. *Himachal J. Agri. Res.* **37**: 181-86.

14. Rehemi, M. and Baninasab, B. 2000. Effect of gibberellic acid on seedling growth in two wild species of pistachio. *J. Hortic. Sci. Biotech.* **75**: 336-39.

15. Ruduicki, R.M. 1977. The physiology and biochemistry of seed dormancy and germination in Persian walnut. North Holland Pub. No. **97**: 41-44.

16. Samaan, L.G., El-Baz, E.E., Iraqi, M.A. and El-Dengawy, E.A. 2000. Effect of gibberellic acid treatments on seed dormancy, germination and subsequent seedling growth of apricot (*Prunus armeniaca* L.). *Egyptian J. Hort*. **27**: 141-56.

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