Effect of different pre sowing treatments on germination and seedling growth of walnut (*Juglans regia* L.)

Thesis

Submitted to the

College of Horticulture,

VCSG Uttarakhand University of Horticulture and Forestry,

BHARSAR-246123 (Pauri Garhwal), Uttarakhand, INDIA

By

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IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Master of Science
Horticulture (Fruit Science)

July, 2016

ACKNOWLEDGEMENT

Prima facea, I am grateful to the God for the good health and wellbeing that were necessary to complete this thesis.

I would like to express the deepest appreciation to my Co-Advisor Dr. Nidhika Thakur for her valuable guidance, suggestions, constructive criticism and constant encouragement during the investigations and preparation of the manuscript. Without her guidance and persistent help this thesis would not have been possible.

I wish to express my sincere thanks to Chairman of my Advisory Committee, Dr. B. P. Nautiyal, for providing me with all the necessary facilities for the research and valuable guidance in the preparation of this manuscript.

I also want to express sincere thanks to Dr. Manju Negi and Dr. Pankaj Bahuguna , the Members of My Advisory Committee for their valuable help during investigation and statistical analysis of my research.

I place on record, my sincere thank you to Mr. Amit Awasthi, Dr. Vijay Kumar and Dr. Sandeep Upadhaya for their help in field work, disease management and weather data respectively. Mr. Bharat Singh (Supervisor fruit field) and fruit research farm workers for their regular supports.

I wish to extent warm thanks to all my classmate, friends and juniors especially Priyanka Malasi, Neelam Devrari, Seema Dobhal, Priyanka Negi, Bengia Mekap, Kanchan Chouhan, Mr. Kulveer Singh Negi, Maduri Kandwal, Neema Panwar, Hemawati and Dipika for their help in field work and also to entire batch of M.Sc. 2014-2016 for their joyous company at different phases of this research and for keeping me mentally happy.

Loyal thanks from the core of my heart are due to my loving father (Rajendra Singh Negi), mother (Sushma Devi) for their immense love, heartiest blessings and continuous moral supports during the course of my study.

Place: Bharsar
Date : *Priya Negi*

CERTIFICATE

This is to certify that the thesis entitled **"Effect of different pre sowing treatments on germination and seedling growth of walnut (*Juglans regia* L.)"** submitted in partial fulfilment of the requirements for the degree of **Master of Science (Horticulture)** with major in **Fruit Science** of the College of Horticulture, VCSG Uttarakhand University of Horticulture & Forestry, Bharsar, is a record *of bona fide* research carried out by **Miss Priya Negi Id. No. 14109**, under my supervision and no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been acknowledged.

Prof. B. P. Nautiyal
Chairman
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CERTIFICATE

We, the undersigned, members of the Advisory Committee of **Miss Priya Negi Id. No. 14109**, a candidate for the degree of **Master of Science (Horticulture)** with major in **Fruit Science** agree that the thesis entitled **"Effect of different pre sowing treatments on germination and seedling growth of walnut (*Juglans regia* L.)"** may be submitted in partial fulfillment of the requirements for the degree.

Prof. B. P. Nautiyal
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ABBREVIATIONS

@	:	At the rate
cm	:	Centimeter
CD	:	Critical Difference
°C	:	Degree centigrade
et al.	:	et alia (Co workers)
GP	:	Germination percentage
GA_3	:	Gibberellic acid
g	:	gram
i.e.	:	id est (that is)
l	:	litre
MT	:	Metric tonne
MSL	:	Mean Sea Level
μm	:	Micro mole
mm	:	Millimeter
min.	:	Minute
mg	:	Milligram
M	:	Molar
ppm	:	parts per million
%	:	per cent
KNO_3	:	Potasium nitrate
SE(m)	:	Standard error of the mean
SE(d)	:	Standard error of the difference
H_2SO_4	:	Sulfuric acid
cm^2	:	Square of centimeter
viz.	:	videlicet (namely)

CHAPTER 1
INTRODUCTION

Walnut (*Juglans regia* L.) is an important temperate nut crop with delicious kernel. It belongs to the family Juglandaceae and genus *Juglans*. The genus *Juglans* has 21 species of which *Juglans regia* is the most important. Its chromosome number is 2n = 32. Walnut is believed to have originated in Iran and the areas surroundings it. The major walnut growing countries are China, USA, Iran, Ukrain, Turkey and Mexico. In India, walnut production is confined to Jammu & Kashmir, Himachal Pradesh and Uttarakhand. Some limited variability also exists in eastern and north-eastern regions, viz. Darjeeling, Sikkim and Arunachal Pradesh. The most common vernacular name for walnut in the region is *akhrot*, but other names are also known, such as *dun* in Kashmir and *khod* in parts of Himachal Pradesh and Uttarakhand.

In India, the total area and production of walnut are estimated to 121.87(000ha) and 240.63 (000MT) (NHB, 2014). The productivity of walnut in India is about 1.2 MT/ha. Jammu & Kashmir occupy the largest share in total area and production (2.61 lakh MT) of walnut. Uttrakhand rank second in the country in production of walnut (0.21 lakh MT). In Uttarakhand major walnut producing areas are Almora, Dehradun, Chamoli, Pauri, Tehri and Uttarkashi.

Walnuts are rich in proteins (14.8 g/100g), fats (64 g/100 g), minerals and are a concentrated source of energy. Oil is rich in omega fatty acid. These contain has a good amount of vitamin B group and are the richest in vitamin B6 among all the nuts. Walnut is having very high nutritive values and good for curing heart diseases. Immature fruits of walnut can be utilized for preparing various products like pickles, chutneys, fresh juices and syrups. The fruit has excellent flavor and is mainly consumed as a dry fruit. Commercially, it is used for preparation of bakery products, chocolates, ice-creams, ornaments, oils, confectionary and salad products. Shells are used in glue and plastics and for making solutions for cleaning and polishing surfaces. Walnut oil has a pleasant aroma and is used as edible oil and in varnish and soap manufacture. Immature fruit has ascorbic acid content. Bark is used as dye and for cleaning teeth. Decolourizing charcoal and activated carbon are obtained from walnut shell.

Wood of walnut is beautifully mottled and figured and is used for high class cabinet work, piano cases and delicate carvings.

Walnut can be propagated either by seed or by vegetative methods. Walnut seed have dormancy. Seed dormancy has been defined as the condition when the viable seeds fail to germinate in the presence of favorable environmental conditions such as light, oxygen, water and chemicals due to the seed dormancy (Hilhorst, 1995). Seed dormancy has been attributed to one or more factors (Stockes, 1965) i.e. hard and impermeable seed coat, immaturity of embryo, after ripening in dry storage, inhibitors and germination stimulators and light sensitivity of seeds. In case of walnut the seed dormancy has been correlated with physiological dormancy that is controlled by seed coat and embryo dormancy. Many practices are most commonly followed to break the dormancy in walnut seeds, in order to improve or stimulate germination i.e. scarification, stratification and chemicals.

Scarification is any process of breaking, scratching or mechanically altering the seed coverings to make it permeable to water and gases. The scarification may be mechanical, hot water or chemical. The purpose of mechanical scarification is to modify hard impervious seed coats. The practices adopted for mechanical scarification are rubbing the seeds on sand paper, cutting with a file, cracking the seeds with the help of a hammer or with the help of mechanical scarifiers, specially designed for this purpose. In hot water treatment walnut seeds are dipped in to hot water @ 77-100^{0}C for 24 hours for softening the hard seed coat of walnut so that seed coat becomes permeable to water and gases.

It has long been known that the seeds of many fruit species will not germinate, if sown under warmer conditions, but will be dormant in the soil for long periods and germinate after getting low temperature. This behavior leads to horticultural practice of stratifying the seeds i.e. the process of pre-treating seeds to stimulate natural conditions that a seed must endure before germination. In nature, moist chilling occurs in wet soils combined with winter coldness. Chilling treatments is often practiced to enhance the germination of dormant seeds (Bello *et al*., 1998 and Hassan and Fetouh, 2014). It is believed that moist chilling treatment alters the inhibitor-promoter balance.

Different plant regulator treatments have also been tried to improve the seed germination in many fruit species with varying success. Gibberellins (GA_3) are the hormones proposed to control primary dormancy (the form of dormancy that is acquired during seed

development) by inducing germination (Hilhorst and Karssen, 1992). Gibberellic acid (GA_3) is an exogenous growth regulator that promotes germination by stimulating the activation of food-mobilizing enzymes (Hartman and Kester, 2009). The ability of gibberellins to break the dormancy was first observed as an empirical treatment by Khan *et al*. (1957).

Seed dormancy is due to several factors and may persist in definitely unless certain specific treatments are given. Therefore, analyzing the causes of dormancy and evaluating methods of breaking dormancy in order to increase the seed germination percentage. The present studies were done with the following objectives:

- To determine the best pre-sowing treatment combination for breaking walnut seed dormancy.
- To study the effect of various pre-sowing treatments on the shoot and root growth of walnut.

CHAPTER 2
REVIEW OF LITERETURE

The pertaining literature related to the effect of different pre-sowing treatments on germination and growth of walnut seedlings is reviewed as under:

2.1 Germination of seeds

Frankland *et al*. (1962) reported that the chilling treatments inducing an increase in the concentration of gibberellins in hazelnut seeds and suggest that the gibberellins were synthesized during the chilling treatments as a factor in breaking seed dormancy.

Mathur *et al.* (1971) reported that as the stratification proceeds GA_3 is synthesized or released from the bound form in peach seeds and the germination increases progressively until half the required stratification has been met.

Kachru *et al.* (1972) observed that when the fresh seeds of grapes were soaked in water for 96 hours, the leaching showed the presence of inhibitor. They suggested that water soluble inhibitor might be responsible for masking the effect of growth substances present in the seed, thereby disturbing the mechanism of germination. At later stage of dormancy, possibly inhibitor content is minimized with increased the level of auxin by chilling.

Dorn *et al*. (1985) studied the vacuum infiltration of gibberellic acid stimulates germination of dormant black walnut seeds. Seeds were scarified by making two small notches through the shell with a grinding wheel. Vacuum infiltration of GA_3 stimulated germination in both experiments to a level equal to or greater than germination of seeds receiving cold moist stratification for 45 days. A brief 15 day stratification period followed by GA_3 was more effective than vacuum infiltration alone. Soaking in GA_3 was effective in the first experiment but not the second. Germination rate and seedling height was increased by GA_3.

Koyuncu (2005) carried out an experiment to determine the effect of cold stratification, application of gibberellic acid (GA_3) at different concentrations and the combination (GA_3 + stratification) on seed germination of black mulberry. Seeds stratified for 100 days showed

88% germination. The combined treatment of 250 mg/l GA_3 and 100 days of stratification yielded 96% germination of seeds. The relationships between GA_3 concentration and seed germination (r = 0.93) and that between stratification duration and seed germination (r = 0.91) of black mulberry were linear.

Hossain *et al.* (2005) studied the effect of different seed treatments on germination and seedling growth attributes of Horitoki (*Terminalia chebula* Retz) and highest germination percentage (66.7%) was observed in the fruit depulped and soaked in cold water for 48 hours followed by 60% in the depulped seeds soaked in cold water for 24 hours. The lowest germination percentage (48.9%) was obtained from controlled seeds. Similar trend was also observed in shoot, root and total seedling dry weight. Therefore, pre sowing treatment, i.e. depulped seeds soaked in cold water for 48 hours was more effective in germination.

Çelik *et al.* (2006) studied the germination responses to temperature, growing media and gibberellic acid (GA_3) treatments in kiwifruit (*Actinidia deliciosa*) cv. Hayward seeds. All the treatments significantly affected the kiwifruit seeds germination. Seeds sown in peat moss and subjected to the temperature of 35°C with bottom heating reached the maximum germination percentage (99.17%). Peat moss and 6,000 ppm GA_3 treatment also had a high germination rate (79%). Moreover, peat moss caused an earlier start of germination than the other mediums and shortened the germination period.

Çetinbaş *et al.* (2006) carried out an experiment to break dormancy and increase the germination of *Prunus avium* L.(mazzard cherry) seeds, various methods were tested including the removal of the seed coat after cold moist stratification and treatment with GA_3, KNO_3, or thiourea. Treatments with 7,500 ppm KNO_3 after 120 days of stratification were more effective, yielding 64.54% germination of seeds with coat. In seeds without coat, 500 ppm GA_3 treatment after 120 days of stratification gave 79.74% germination which was comparatively higher than control.

Olmez *et al.* (2006) carried out the procedures of cold stratification under greenhouse condition for eliminating seed dormancy of caper crop to find the most suitable germination conditions. The seed germination started and stopped 21 and 57 days after sowing,

respectively. While the highest germination percentage (46.6%) was obtained in seeds that were cold stratified for 60 days, the lowest germination percentage (3.67%) was determined in control seeds.

Rouhi (2006) carried out the study on effect of scarification and cold stratification on germination of *Prunus scoparia* and they reported the best germination percentage on treatment of 9-10 °C temperature for both non-scarified and scarified seeds for all durations compared to 0-1 and 4-5 °C. At 9-10 °C, non-scarified seeds had a germination percentage of 76.7%, whereas germination was 26.7% for scarified seeds after 90 days. Endocarp opening percentage (83.3%), mean germination time (37.3 days) and radicle length (9.7 cm) showed the best results for non-scarified seeds for 90 days at 9-10 °C.

Smiris *et al.* (2006) studied the effect of various treatments in seed dormancy breaking on *Arbutus uned* and *Podocytisus caramanicus* and the maximum percentage of germination for *Arbutus unedo* (85.75%) was observed when the seeds were soaked for 24h in 500 ppm GA_3 and then stratified (chilled) for a period of 3 months. The maximum percentage of germination for *Podocytisus caramanicus* (63%) was observed when the seeds were immersed in concentrated sulphuric acid (98%) for 20 min.

Abu-Qaoud (2007) reported the highest germination (60%) for *Pistacia palaestina* acid scarified plus cold stratified seeds over the control of the three *Pistacia* species Scarified seeds of *P.lentiscus* resulted in 13.3% germination, scarified plus GA_3 soak of *P.lentiscus* and *P. atlantica* Desf. (34, 39.9%). Early seed germination was obtained with seeds of *P. lentiscus* after one week of incubation with scarified plus GA_3 (6%) and scarified seeds of *P. palaestina* (5%), *P. palaestina* seeds continued with the highest germination percentage.

Cromer and Woodall (2007) observed that mechanical dormancy imposed by this endocarp was overcome in *Santalum acuminatum.* Germination may have been inhibited by a weak embryo dormancy mechanism once the endocarp was cracked; however this was overcome by gibberellic acid which increased germination to 73 % compared to 42 % in non-gibberellic acid treatments.

San and Dumanoglu (2007) investigated the effect of desiccation, cold storage, gibberellic acid (GA_3), and various combinations of desiccation with GA_3 and cold storage treatment on germination of walnut (*Juglans regia).* Desiccation was effective on germination and cold storage (4 weeks at 4°C) or GA_3 (2.9, 8.6, 14.3, 20.0, and 25.7μm) treatment together with desiccation improved the germination rate of walnut somatic embryos. The highest percentage of germination (69.1%) was determined with desiccated embryos originating from open-pollinated seeds.

Conner (2008) reported the best seed treatments and germination conditions for muscadine seed. The 90 days stratification period gave the highest germination percentage, with successively lower germination in the shorter stratification treatments. Pretreatment of seeds before stratification with three rates of hydrogen peroxide (H_2O_2) and four rate of gibberellic acid (GA_3) were used in an attempt to promote germination. Low rates of H_2O_2 (0.5 M) and GA_3 (1 g/L) were beneficial in some instances, whereas high rates of GA_3 were detrimental. Nicking the seed coats before stratification and soaking seeds in running water after stratification were ineffective in promoting germination.

Heidari *et al.*(2008) observed that nicking plus stratification for 45 days, gave the best germination in *Prunus scoparia* and *Prunus webbii* (94 and 82.5% respectivily). Immersion in H_2SO_4 for 30 minutes plus 30 days stratification increased the percentage germination of *Prunus scoparia* seeds (74.5%), but treating of endocarp from *Prunus webbii* seeds with H_2SO_4 for 60 minutes plus stratification for 45 days, increased the germination (37%). It was concluded that in both the species, before stratification, mechanical removal of seed endocarp is more effeicient than immersion in H_2SO_4.

Amooaghaie (2009) reported the best treatment was moist chilling for 6 weeks at 5 ± 1 °C or for 4 weeks of moist-chilling followed by soaking in 500 ppm GA_3 solution for 24 hours. These treatments significantly increased germination percentage and decreased time to 50% germination compared to control. Also, the characteristics of the obtained seedlings were much better than those of control. GA_3 application on unchilled seeds improved the germination process. It was concluded that treatment of moist chilling for 6 weeks or 4 weeks

followed by 500 ppm GA_3 is recommended for promoting the germination process of *Ferula ovina* seeds and improving growth characteristics of the subsequent seedlings.

Ghadikolaee *et al.* (2010) reported that stratifying kernels at 5°C for 30 days enhanced their germination capacity, whereas warm incubated (27°C) kernels turned rancid after 20 days and had reduced germination. Imbibition was sufficient to allow lipid mobilization to occur in dormant walnut kernels, although cold stratification accompanied by gluconeogenesis was essential for kernel germination.

Razavi *et al.* (2009) reported that the seeds dormancy in *Prangos ferulaceae* can be broken by cold stratification at 5°C and 12°C which induced germination up to 35 and 40%, respectively. It has also been shown that cold temperatures promote the growth of the undeveloped embryo of the plant. After 10 weeks stratification of the seeds at 5°C, the embryo length increased 200%.

Al-Absi (2010) observed that scarification with either hot water or sulfuric acid only improved germination percentage if followed by 60 days of stratification. Treating the seeds with GA_3 resulted in a significantly higher germination rate. The highest germination percentages were attained by treating seeds which had been stratified for 60 and 90 day periods with GA_3 at 1000 ppm. It was recommended that GA_3 should be used in addition to cold stratification for improving germination percentage of mahaleb cherry seeds.

Ghayyad *et al.* (2010) reported the highest percentage germination (70%) by removing the endocarp and then soaking seed for 24 hours at 1250 ppm gibberellic acid concentrate in Mahaleb (*Prunus mahaleb* L.) seeds. As for soaking seeds in gibberellic acid at 1250 ppm followed by alternate stratification only 58% seed germinated but it began germinating in a shorter period than the others (6 weeks of stratification beginning). Whereas on treating seeds with sulfuric acid for 10 minutes followed by alternate stratification was only 16.66 % seeds germinated. It was concluded that before stratification, endocarp removed seeds was more effective than immersion in sulfuric acid.

Dewir *et al.*(2011) reported that among various treatments, soaking of *Sabal* seeds in 500 ppm gibberellic acid (GA_3) for 24 hours resulted in highest final germination percentage

(FGP) of 95% in day 14 of culture and number of days lapsed to reach 50% of final germination percentage of 6.8 days. Non treated *Sabal* seeds exhibited FGP of 75% in day 16 and GT50 of 7.39 days. Soaking of *Thrinax* seeds in H_2SO_4 for 30 min resulted in a highest FGP of 90% in day 14 of culture and GT50 of 5.19 days. Non-treated *Thrinax* seeds exhibited FGP of 70% in day 16 and GT50 of 8.07 days.

Leif *et al.* (2011) reported the effect of sulfuric acid followed by a 60 days stratification, or 60 days warm, moist treatment followed by a 90days stratification on elderberry seeds (*Sambucus nigra* L.) They concluded that stratification alone was less effective in promoting germination than was acid scarification followed by stratification or warm, moist treatment followed by stratification. Germination of common elderberry seeds soaked in hot water was not significantly different from the control treatment.

Mattana *et al.* (2012) reported that gibberellic acid @ 250 mg/l and warm stratification (25^0 C for 3 months) followed by low temperature ($<15^0$ C) enhanced embryo growth rate and subsequent seed germination in *Ribes multiflorum.* Low germination occurred at warmer temperatures and cold stratification (5^0C for 3 months) induced secondary dormancy. Mean time of epicotyl emergence was affected by GA_3.

Pipinis *et al*. (2011) observed that after a period of two months of cold stratification, increasing the duration of scarification (20 to 60 min) also increased the germination percentages (31% to 65%) in *Cercis siliquastrum*. High germination percentages equal to 94%, 88%, and 98% were attained after a period of 3 months of cold stratification for seeds that had been scarified for 20, 40, and 60 min, respectively. Longer periods of stratification (4 months) of seeds scarified for 20, 40, and 60 min reduced the germination percentages (81%, 68%, and 59%, respectively). This decrease was higher in seeds that were scarified for 60 minutes.

Aboutalebi *et al.* (2012) studied the effect of various treatments on seed germination characteristics of wild Ziziphus (*Ziziphus spinachristi*). On the basis of results, the highest and lowest germination percentage was observed in 1 and 3 weeks stratification respectively, although germination percentage in treatments of scarification with sulfuric acid was in good

range. It was concluded that under the condition of this experiment, 3 week stratification (4-5° C) with 78.8 % germination was the best treatment.

Çalişkan *et al.* (2012) investigated an experiment to evaluated the effect of several treatments (priming with water for 24hours, GA_3 at 500 and 1000 ppm for 24hours, 3% KNO_3 for 24hours, and stratification at 4°C for 7, 14 and 21 days) on seed germination and they reported highest percentages of germination and emergence were obtained with GA_3 at 500 or 1000 ppm for both cultivars. The application of GA_3 at 500 ppm or 1000 ppm reduced the time to germinate and emergence from the seeds of both cultivars. In summary, the GA_3 treatments showed efficacy to overcome the dormancy of these fig seeds.

Merou *et al.* (2012) studied the effect of various germination treatments on the germination of fresh *Carpinus orientalis* seeds. Cold stratification for three months at 5°C was found to be adequate to release dormancy and allow germination of almost all viable seeds. Acid scarification reduced germination without damaging the embryo, while mechanical scarification was lethal.

Nadeem *et al.* (2012) observed that the seed germination was improved and the dormancy of *Ochradenus arabicus* seeds was broken by subjecting them to plant growth regulators i.e.GA_3 (25-500 μM) and BAP (25-350 μM)] and two chemicals (KNO_3 and thiourea) at 0.1-0.5% concentrations and maximum germination was obtained by GA_3 at 100μM, however, decline in germination was observed at higher concentrations of GA_3. The germination of seeds was found to be improved upon storage for 6 and 12 months. In general, all treatments showed increased germination compared to that of control.

Pipinis *et al.* (2012) reported that the pre treatments of *Prunus mahaleb* seeds with exogenous GA_3, during the cold stratification (CS) period recorded significantly higher seed germination. Sulfuric acid scarification of seeds for 45 minutes prior to GA_3 (1000 ppm for 24 hours) plus CS up to 1 month pre treatment was considered to reduce the mechanical resistance of endocarp and improve germination. Seeds without endocarp, which were pretreated with GA_3 (1000 or 2000 ppm for 24 hours) and then cold stratified for 1 month were recorded to exhibit the highest germination percentages.

Vahdati *et al*. (2012) carried out the studies on stratification, chilling and heat requirements for seeds of five Persian walnut genotypes germination. Mature seeds were stratified at 4 ± 1^0 C for treatment periods ranging from 0 to 8 weeks. Stratification for 6 to 8 weeks was most appropriate to overcome walnut seed dormancy, to obtain the best germination percentage and germination rate. Data showed that the nutshell was a mechanical barrier to germination in walnut seeds with intermediate physiological dormancy.

Al-Hawezy (2013) investigated the role of the different concentrations of GA_3 on seed germination and seedling growth of loquat. GA_3 had a significant effect on germination rate as compared to control. GA_3 at 250mg/L gave best response (71.19, 86.80 and 98.75) % at 1st, 2nd and 3rd weeks but as the concentration increased above 250 mg/L the germination rates decreased rapidly but shoot and root length of seedling and vigor index increased during300 mg/L. More significant effect interactions were obtained in 250 mg/L GA_3 with different period times.

Jamwal *et al.* (2013) studied the effect of different pre sowing seed treatments and sowing dates on germination of the fourteen pre sowing treatments, highest seed germination and best seedling growth was obtained with seed soaking in water for 48 hours followed by 6 days storage in moist gunny bags and seed sowing on 15 April and seed soaking in water for 72 hours followed by 6 days storage in moist gunny bags and sowing on 15th April.

Mawalagedera *et al*. (2014) carried out the study to identify a method to break up the seed dormancy of *P. emblica.* The selected viable seeds were subjected to four different pre treatments viz., none treated seeds (i.e. control), seeds scarified and treated with 1% gibberellin and seed coat removed and followed by seeds treated with 1% gibberellin. From the four treatments, seed dormancy was overturned with germination per -centage of 43% by the seed pre treatment where the seeds were scarified and treated with 1% gibberellin and no other pre treatment methods were successful in breaking the dormancy.

Prakash *et al.* (2013) carried out an experiment on enhancement of germination in *Abrus precatorius* L. seeds by specific pre sowing treatments and the highest (95%) germination was observed just 12 days after sowing in seeds treated with concentrated H_2SO_4

for 120 minutes. 70-75% germination was achieved when the seeds were treated with acid for 105 and 135 minutes. Seeds treated with con. H_2SO_4 and also scarified by sand paper showed similar results with 60-65% germination. The seeds scarified by a mechanical scarifier and treated with hot water did not show more than 32.5% germination.

Pandey and Tamata (2013) conducted studies to evaluate the effect of different pre sowing treatments (scarification and cold stratification) on seed germination of two *Quercus* species, i.e. *Quercus serrata* (deciduous) and *Quercus semecarpifolia* (evergreen). It was observed that the pretreated seeds germinated better in comparison with control and both species responded differently to the treatments. The deciduous, lower altitude *Q. serrata* gave better results in comparison with the evergreen, high altitude *Q. semecarpifolia* in terms of percent germination and time taken for germination.

Tibugari *et al.* (2013) reported the response of *Berchemia discolor* to different scarification methods. Treating seeds with acid and nicking improved germination while seeds in the control treatments had very low germination, as did seeds scarified in hot water. Germination and early seedling vigor were significantly influenced by scarification.

El-Refaey *et al.* (2014) reported that in persimmon increasing the moist chilling period from 4 to 8 weeks progressively, significantly increased the seeds germination percentages 51.2% to 97.2% and significantly decreased the time to 50% germination from 47days to 27.3 days. The control one non moist chilled seeds gave the lowest germination percentage 22.6%. The 4 weeks chilled seeds soaked in BA gave the most satisfactory results as it yielded high germination percentage (85.2%) and saved about at least 4 weeks which would have otherwise being required for moist-chilling of the seeds.

Patel and Mankad (2014) observed that plant hormones are chemicals that regulate plant growth. These occur in extremely low concentrations and influence all developmental and physiological processes in plants. The effect of different concentrations of GA_3 on seed germination of *Tithonia rotundifolia* was studied. The maximum percentage of seed germination was with 500 ppm GA_3.

Asaadi *et al.* (2015) observed the effective treatments to stimulate seed germination were pre chilling for 150 and 30 days sulphuric acid and sandpaper scarification was done. Pre chilling for 150 and 30 days increased germination by 88.4% and 85.65%, respectively, while sulphuric acid and sandpaper scarification both increased germination by 76.1% compared to the control. The most effective treatments among the methods used for breaking seed dormancy of *Salsola arbusculiformis* were pre chilling for 150 days and mechanical scarification by hand with sandpaper.

Lilabati *et al.* (2015) reported the chemical treatment of *Emblica officinalis* seed exposed toGA_3 @ 500ppm for 24 hours resulted better germination than other treatments. Imbibition percent increased in treated seeds upto 90 % in contrast to 70% in nontreated seeds (control). The study showed that treatment of seeds with GA_3@ 500ppm and thiourea was effective in breaking seed coat dormancy.

Parvin *et al*. (2015) carried out the study on effect of gibberellic acid and chilling stratification on seed germination of eastern black walnut. They reported that the germination rate for separate application of both concentrations of GA_3 and one month chilling treatment was zero, as no seeds germinated. Highest percentage of seed germination (69.27 %) was recorded with the combined treatment of two months chilling and GA_3 application @ 400 ppm. It was found that the application of the combined treatment of chilling stratification and GA_3 was effective in increasing seed germination percentage and rate as well as improving growth parameters of eastern black walnut seedlings.

Poletto *et al*. (2015) studied the effect of seed stratification on germination rate, germination speed and initial development of seedlings of six pecan (*Carya illinoinensis*) cultivars. In the fourteenth week after sowing, the emergence speed index, total emergence, plant height, stem diameter and number of leaves were evaluated. Seed stratification significantly improved the germination potential and morphological traits of the evaluated cultivars.

Stejskalova *et al.* (2015) reported that for seeds of sycamore maple (*Acer pseudoplatanus* L.) without pericarp, the gibberellic acid improved the germination capacity to the level of stratified seeds. The data also showed positive influence of gibberellic acid on emergence rate. All variants where gibberellic acid was applied had a statistically higher emergence rate than the control. The increase was about 50% higher than in the control, i.e. without the influence of gibberellic acid.

Thakur (2015) studied the effect of growth regulator, scarification, GA_3 and thiourea on seed germination in peach. Among the treatments seeds sown after mechanical rupturing of the seed coat exhibited significantly higher percent seed germination (57.26), and minimum duration of seed germination (16.33) and mortality rate (1.9). Irrespective of the treatments the maximum percent seed germination (22.57) and minimum duration of seed germination (23.62) was also recorded on same date. The percent seed germination under GA_3, thiourea and kinetin was found to be lower.

Zulfiqar *et al.* (2015) studied the effect of pre sowing treatments on seed germination in *Quercus glauca*. They observed maximum germination rate (95.45%) in stratified seeds. With scarified treatment, maximum germination rate (100%) was observed. Scarified treatments proved to be more effective for this species as compared to stratified treatments.

Nautiyal and Bhagat (2015) studied the effect of mechanical scarification (at micropyler end, chazal end and hylum cavity) and chemical scarification (conc. H_2SO_4) on seed germination of *Abrus precatorius* L. and they reported high germination in chalazal end and hylum cavity scarification (79.5% & 74.5%) respectively while control showed lowest (6.5%) germination and in chemical scarification with H_2SO_4 the maximum germination was recorded in 135 minutes time duration which showed 90 % germination within 5 days.

2.2 Growth characteristics

2.2.1 Shoot growth

Rouhi *et al.* (2003) eported the maximum seedling lengths were observed with 250 ppm gibberellic acid and 22 °C temperature. Effects of gibberellic acid treatments on seedling lengths were significant but temperature had no effect on stem elongation. Treatment with gibberellic acid up to 250 ppm doses increased plant length.

Egharevba *et al.* (2005) studied the effects of seed treatments and soil type growing media on the germination and growth of African walnut. The factors considered included seed treatments with hot water at 90°C, warm water at 60°C, overnight soaking in cold water and scarification. Scarification and overnight soaking in cold water produced best quality seedlings.

Aatla *et al.*(2013) studied the effect of pre sowing treatments on germination, growth and vigour of mango and significantly higher values were recorded with the treatment where extracted kernel were pre treated with KNO_3 @ 0.5 per cent. They found this to be a sound integrated practice, where it recorded maximum germination percentage (64 %), seedling diameter (7.10 mm), number of leaves (10.90), leaf length (15.83 cm), leaf width (8.00 cm), root length (23.40 cm), root spread(8.66 cm), root to shoot ratio (0.807), vigor of seedling (1094.33) and vigor index (1517.30) over all the other treatments. Whereas maximum seedling height (24.13 cm) and internodal length (3.66 cm) was recorded in extracted kernel pre treated with GA3 @ 500 ppm.

Fetouh and Hassan (2014) reported that increasing cold stratification period enhanced germination parameters as well as seedling characteristics. The most effective stratification period was 90 days of cold stratification followed by 120 days cold stratification treatment. In order to enhance seed germination and seedling growth of *Magnolia grandiflora*, treating seeds with 90 or 120 days of cold stratification was recommended.

2.2.2 Root growth:

Rawat *et al.* (2010) observed stratification for 30 days showed highest germination percentage, longest radicle, maximum root and shoot length, number of leaves and highest survival of seedlings. The longest plumule, maximum collar diameter, highest shoot and root dry weight were recorded with 25 days of stratification. Thus the results of present investigation clearly revealed that 25 to 30 days stratification of *P. granatum* seeds was more suitable for uniform and faster germination as well as best growth in early stage of seedlings as compared to the control.

Pratibha *et al.* (2015) studied the effect of chemical treatments on seed germination of papaya. The results revealed that among the various seed treatments maximum root length (15.30cm) and root dry weight (0.32g) were recorded in seeds treated with GA_3 @ 200 ppm for 24 hours.

2.2.3 Total Fresh weight:

Pawar *et al.* (2010) reported maximum fresh weight of shoot (30.2 g) and root (6.5 g) with application of GA3 @ 300 ppm for 8 hours as compared to control. The treatment of GA_3 @ 300 ppm for 8 hours and GA_3 @ 300 ppm for 6 hours recorded maximum dry weight of shoot as 14.3 g and 13.9 g while the treatment GA_3 @ 300 ppm for 8 hours produced maximum increase in dry weight of root (4.3 g) as compared to control.

2.2.4 Survival percentage:

Wani *et al.* (2014) were conducted an experiment with an objective to investigate the effect of various concentrations of plant growth regulator (GA_3) for seed germination of Apple (*Malus x domestica* Borkh.) The study clearly indicated that apple seed treated with GA_3 @ 500 ppm for 40 hours gave best response regarding survival of saplings.

Farhoudi *et al*. (2015) studied the effect of seed dormancy breaking treatment on *Echinacea purpurea* seed germination. Results indicated significance difference between seed dormancy breaking methods. The highest *E. purpurea* seedling fresh weight was showed in seeds treat with GA_3 (250ppm) + stratification for 7 or 4 weeks.

CHAPTER 3
MATERIALS AND METHODS

The investigation on the "Effect of different pre-sowing treatments on germination and seedling growth of walnut (*Juglans regia* L.)" carried out during 2015-16. The details of materials, techniques and methods used for studies, experimental design adopted and statistical procedures followed are presented in this chapter.

3.1 Experimental site

The investigations entitled "Effect of different pre sowing treatments on germination and seedling growth of walnut (*Juglans regia* L.)" was conducted in Poly house of Fruit Nursery Block of College of Horticulture, VCSG Uttarakhand University of Horticulture and Forestry, Bharsar.

3.2 Climate

In general, the climate of the Bharsar represents the mild summer, higher precipitation and colder or severe cold prolonged winter. The climate factors *i.e.* precipitation, temperature, relative humidity and wind, in association with elevation (valleys or mountain range from temperate zone), proximity to Great Himalaya, slope aspects, drainage, vegetation etc. are responsible for the micro-climate of this area. Major output of precipitation is in the form of rain fall, besides occasional occurrence of dew, hailstorm, fog, frost, snow fall etc. The South-East monsoon commences towards the end of June while the North-East monsoon causes occasional winter showers during November-February. During winter, snow fall is common in this region. During summer months, Bharsar has hot climate prevailing for few hours in a day, the maximum temperature during May-June is recorded between 25°C-29°C however, and nights are cool. December and January are the coldest months; the minimum temperature reaches to 1°C to -4°C. Relative humidity is normally highest during rainy season (July - August), often recorded near to saturation point (92-97%) and it gradually decreases towards winters.

Table: 3.1 List of different pre sowing treatments

Treatment	Treatments Details
T_1	Cracking
T_2	Hot water
T_3	Cracking + GA_3 (500 ppm)
T_4	Cracking + GA_3 (750 ppm)
T_5	Cracking + GA_3 (1000 ppm)
T_6	Hot water + GA_3 (500 ppm)
T_7	Hot water+ GA_3 (750 ppm)
T_8	Hot water+ GA_3 (1000 ppm)
T_9	Cracking + Stratification (30 days)
T_{10}	Hot water + Stratification (30 days)
T_{11}	Cracking + GA_3 (500ppm) + Stratification (30 days)
T_{12}	Cracking + GA_3 (750ppm) + Stratification (30 days)
T_{13}	Cracking + GA_3 (1000ppm) + Stratification (30 days)
T_{14}	Hot water + GA_3 (500 ppm) + Stratification (30 days)
T_{15}	Hot water + GA_3 (750 ppm) + Stratification (30 days)
T_{16}	Hot water + GA_3 (1000 ppm) + Stratification (30 days)
T_{17} (Control)	No pre sowing treatment

3.3 Experimental design

The seeds of local collection were subjected to various pre sowing treatments i.e. mechanical scarification (cracking), hot water treatment, stratification and GA_3 application. The details of different treatments are presented here under**:**

- Treatments : 17
- Replication : Three
- Experiment design : Randomized Complete Block Design
- Total beds : 51
- Number of seeds per bed : 50
- Spacing : (30 X 10) cm^2
- Number of seeds per treatment : 150
- Total number of seeds : 2,550

3.4 Experimental methods

3.4.1 Selection of seeds

The seeds were obtained from different places of Bharsar, Pauri Garhwal district of Uttarakhand. The seeds were cleaned by rubbing all the extraneous materials and then dipped in water. All the floating seeds were discarded and only the healthy seeds which settled down were taken for use in these studies.

3.4.2 Seed stratification

The coarse river sand was used as a medium for stratification. The stratification was done in small wooden boxes. Seeds were placed in alternate layer of moist sand in small wooden boxes and placed in cool and dark storage for 30 days period of stratification. The sand was kept moist throughout the stratification period by occasional watering. At the end of stratification the sand was separated by placing the seeds in a sieve and then washed with running water for 3 to 4 minutes.

3.4.3 Mechanical scarification (Cracking)

Walnut seeds were cracked by rubbing the seeds on sand paper or rough surface so that its seed coat was open and became permeable to water and gases. It made easy germination.

3.4.4 Hot water treatment

Walnut seeds were dipped in to hot water for 24 hours. The temperature of water was 77^0-100^0C. After immersion, they were removed from the hot water and left to cool for about 10 minutes.

3.4.5 GA_3 treatment

The solution of gibberellic acid (GA_3) was prepared by dissolving the known quantities of the chemical in a small amount of ethyl alcohol and then adding distilled water to make the volume. Walnut seeds were soaked in the solution of gibberellic acid for 24 hours and used for sowing.

3.4.6 Sowing of seeds

The seeds were sown in well prepared beds in rows at the spacing of 30X10 cm at 5 to 8cm depth. The beds were irrigated on alternate days and kept free of weeds by hand weeding at an interval of 15 days.

3.5 Observations recorded

The data on the effect of different pre sowing treatments on the germination and growth of walnut seedling were recorded and the procedures followed were described below:

3.5.1 Days taken for initial germination (days)

The data on days taken for initial germination was recorded daily after sowing till the time further emergence of seedlings. The period from the date of sowing to the emergence of first seedling was considered as the time taken for commencement of seed germination.

3.5.2 Seed germination percentage (%)

This observations were recorded when further emergence of seedlings stopped. The seeds were considered germinated when the plumule just emerged on the soil surface and radical reached 2 mm length of the seed. The seed germination was expressed in percentage on the basis of the number of seeds germinated out of the sown. At the end of germination period (eight weeks), the germination percentage (GP) was calculated using the following formula:

$$GP = \Sigma G/N*100$$

Where GP is the germination percentage, G is the numbers of germinated seeds and N is the numbers of all seeds (Copeland *et al.*, 2001).

3.5.3 Shoot height (cm)

The data on shoot length were recorded at monthly interval. Length was measured from the surface of soil to the terminal bud of the main axis and expressed as average length per shoot in cm.

3.5.4 Shoot diameter (mm)

The data on shoot diameter were recorded at monthly interval. The diameter was recorded with the help of vernier caliper. The diameter was recorded at a height of 5 cm from the ground level and was expressed as average diameter per seedling in mm.

3.5.5 Number of branches/per plant

The observation was recorded at the end of the experiment. All the branches were taken into account for this purpose and the average number of branches per plant was calculated.

3.5.6 Number of leaves

The observation was recorded at the end of the experiment. All the unfolded leaves, irrespective of their size, were taken into account for this purpose and the average number of leaves per plant was calculated.

3.5.6 Root length (cm)

The observations on root length were recorded at the end of the growing season. The plants were dug out carefully without disturbing the primary root and were washed in water. The length of the primary root was measured with the help of a meter scale and the measurement was recorded from the transition point of root and stem to the tip of the primary root.

3.5.7 Root diameter (mm)

The observations on root diameter were recorded at the end of the growing season. The diameter was recorded with the help of vernier caliper.

3.5.9 Root area (cm^2)

The observations on root area were recorded at the end of the experiment. For root area root volume was determined first. Root volume was determined by immersing root systems in a container of water placed on a balance. The displaced water (measured in grams) is equal to the volume (measured in cubic centimeters) of the root system in that 1 g of water equals 1 cm^3 at room temperature (Burdett, 1979). Root area was determined using the method of Atkinson (1980):

$$Y\ (cm^2) = 2\{(\text{root length} \times \text{root volume})\}^{0.5}$$

$Y\ (cm^2)$ = root area

3.5.10 Shoot fresh weight (g)

The observations on fresh shoot were recorded at the end of the experiment. Seedlings were cut from the point of transition of shoot and root and washed with water. Then weight of shoots was measured with the help of weigh balance machine.

3.5.11 Root fresh weight (g)

The observations on fresh root weight were recorded at the end of the experiment. Roots were cut from the point of transition of shoot and root and washed with water. Then weight of roots was measured with the help of weigh balance machine.

3.5.12 Total fresh weight (g)

The observation on total fresh weight was determined by adding total shoot and root fresh weight.

Total Fresh Weight = shoot fresh weight + root fresh weight

3.5.13 Survival percentage (%)

The observation on survival percentage was recorded at the end of the experiment by using the following formula:

$$\text{Survival\%} = \frac{\text{Total no. of germinated seedlings - Total no. of dead seedlings}}{\text{Total number of germination seedling}} \times 100$$

3.5 Statistical analysis

The statistical analysis was carried out for each observed character under the study using MS-Excel, OPSTATE. The mean values of data were subjected to analysis of variance and ANOVA was set as per Gomez and Gomez (1984) for Randomized Complete Block Design. For estimation of different statistical parameters, following procedure and formulae were adopted:

Analysis of variance

Source of variance	Degree of freedom	Sum of squares	Mean sum of squares	Variance ratio (V.R.)
Replication (r)	r-1	Sr	Sr/(r-1) = Mr	Mr/Me
Treatments (t)	k-1	Sk	Sg/(k-1) = Mk	Mk/Me
Error (e)	(r-1) (k-1)	Se	Se/(r-1) (k-1) = Me	

Where,

r = Number of replications

k = Number of treatments

Sr = Sum of squares due to replications

Sk = Sum of squares due to treatments

Se = Sum of squares due to error

Mr = Mean sum of squares due to replications

Mk = Mean sum of squares due to treatments

Me = Mean sum of squares due to error

The calculated F-value was compared with tabulated F-value. When F-test was found significant, critical difference was calculated to find out the superiority of one entry over the others.

The standard error and critical differences were calculated as follows:

SE (m) ± = $\sqrt{Me/r}$

SE (d) = $\sqrt{2\ Me/r}$

$CD_{0.05}$ = S.E. (d) x $t_{(0.05)}$ (r-1) (k-1) df

Where,

SE (m) ± = Standard error of mean

SE (d) = Standard error of difference

$CD_{0.05}$ = Critical difference at 5 per cent level of significance

CHAPTER 4

EXPERIMENTAL RESULTS

The results obtained during the present investigation on the "Effect of different pre sowing treatments on germination and seedling growth of walnut (*Juglans regia* L.)" are presented as under:

4.1 Seed germination

4.1.1 Days taken for initial germination

It is evident from the data presented in Table 4.1 that day taken for initial germination was significantly affected by different pre sowing treatments. The minimum days taken for germination (12.67 days) was recorded with T_{12} (cracking + GA_3 750ppm + stratification for 30 days) which was statistically at par with treatment T_{14}, T_{15}, T_{13}, T_{11} and T_{16} recording 13.33, 14.00, 14.33, 14.33 and 14.67 days respectively. The maximum days taken for germination (76.67 days) was recorded with T_{17} (control).

4.1.2 Germination percentage

The data presented in Table 4.1 showed that different pre sowing treatments exerted significant influence on the seed germination. The maximum germination percentage (75.88%) was recorded with T_{11} (cracking + GA_3 @ 500ppm + stratification) which was statistically at par with treatment T_{14}, T_{12}, T_{15}, T_{16} and T_{13} recording 74.46%, 73.05%, 72.34%, 71.63% and 71.63% respectively. The minimum germination percentage (45.66%) was recorded with T_{17} (control) and was statistically at par with T_2 recording 47% which was followed by T_1 (49.89%).

Table: 4.1 Effect of different pre sowing treatments on days taken for initial germination and germination percentage of walnut seed

Treatments	Days taken for initial germination ±SE (m)	Germination percentage ± SE (m)
T_1 (Cracking)	59.33 ± 0.67	49.89 (7.13 ± 0.05)*
T_2 (Hot water)	56.33 ± 0.88	47 (6.93 ± 0.13)
T_3 (Cracking + GA_3 @500ppm)	25.67 ± 1.20	53.54 (8.07 ± 0.06)*
T_4 (Cracking + GA_3 @750ppm)	25.33 ± 1.33	64.07 (7.87 ± 0.05)*
T_5 (Cracking+ GA_3 @1000ppm)	26.00 ± 1.15	60.99 (7.38 ± 0.11)*
T_6 (Hot Water + GA_3 @500ppm)	19.67 ± 0.67	60.71(7.85 ± 0.07)*
T_7 (Hot Water+ GA_3 @750ppm)	19.00 ±1.15	55.63 (7.52 ± 0.16)*
T_8 (Hot Water + GA_3 @1000ppm)	19.33 ± 0.33	53.72 (7.39 ± 0.29)*
T_9 (Cracking + stratification for 30 days)	19.00 ± 0.58	52.05 (7.28 ± 0.09)*
T_{10} (Hot Water + stratification for 30 days)	18.00 ± 1.53	50.15 (7.15 ± 0.04)*
T_{11}(Cracking+GA_3@ 500ppm+Stratification for 30 days)	14.33 ± 0.33	75.88 (8.77 ± 0.11)*
T_{12}(Cracking+ GA_3@750ppm+Stratification for 30 days)	12.67 ± 0.33	73.05 (8.60 ± 0.04)*
T_{13}(Cracking+GA_3@1000ppm+Stratification for 30 days)	14.33 ± 0.33	71.63 (8.52 ± 0.04)*
T_{14}(Hot Water +GA_3@500ppm+Stratificationfor 30 days)	13.33 ± 0.33	74.46 (8.67 ± 0.07)*
T_{15}(Hot Water+GA_3@750ppm+Stratification for 30 days)	14.00 ± 0.58	72.34 (8.56 ± 0.07)*
T_{16}(HotWater+GA_3@1000ppm+Stratificationfor 30 days)	14.67 ± 0.33	71.63 (8.52 ± 0.04)*
T_{17} (Control)	76.67± 9.26	45.66 (6.90 ± 0.29)
SE (d)	3.38	1.89 (0.18)
$CD_{0.05}$	6.88	3.79 (0.37)

Data in parentheses are transform values.

4.2 Seedling growth

4.2.1 Shoot height (cm)

The data on the effect of different pre sowing treatments on shoot height is given in Table 4.2. It is evident from the data that the pre sowing treatments had a significant effect on shoot height. The data revealed that the maximum shoot height (37.35 cm) was recorded with T_{14} (hot water+ GA_3 500ppm + stratification for 30 days) which was statistically at par with treatment T_{15} and T_{11} recording 35.28 cm and 34.79 cm, which was followed by T_{12} (34.73 cm), T_{16} (31.08 cm) and T_{13} (30.50 cm). The minimum shoot height (18.58 cm) was recorded with T_{17} (control) which was statistically at par with treatment T_1 (20.07 cm).

4.2.2 Shoot diameter (mm)

The data on the effect of different pre sowing treatments on shoot diameter (Table 4.2) indicated that the shoot diameter was influenced significantly by the different pre sowing treatment. The data revealed that the maximum shoot diameter (3.97 mm) was recorded with T_{14} (hot water + GA_3 500 ppm + stratification for 30 days) which was statistically at par with treatment T_{12}, T_{11} and T_7 recording 3.58 mm, 3.48 mm and 3.45 mm respectively, which was followed by T_{15} (3.30 mm), T_6 (3.30 mm). The minimum shoot diameter (2.06 mm) was recorded with T_1 (cracking) which was statistically at par with treatment T_{17} (2.17 mm), T_5 (2.43mm) and T_2 (2.52 mm).

Table: 4.2 Effect of different pre sowing treatment on shoot height and shoot diameter of walnut seedling

Treatments	Shoot height (cm)±SE (m)	Shoot diameter(mm) ± SE (m)
T_1 (Cracking)	20.07 ± 0.88	2.06 ± 0.30
T_2 (Hot water)	22.21 ± 0.67*	2.52 ± 0.05
T_3 (Cracking + GA_3 @500ppm)	26.75 ± 0.99*	3.08 ± 0.32*
T_4 (Cracking + GA_3 @750ppm)	28.92 ± 0.71*	3.11 ± 0.17*
T_5 (Cracking + GA_3 @1000ppm)	25.00 ± 0.86*	2.43 ± 0.05
T_6 (Hot water + GA_3 @500ppm)	27.18 ± 1.73*	3.30 ± 0.15*
T_7 (Hot water + GA_3 @750ppm)	30.38 ± 1.18*	3.45 ± 0.18*
T_8 (Hot water + GA_3 @1000ppm)	25.12±0.39*	3.19 ± 0.14*
T_9 (Cracking + stratification for 30 days)	26.35±1.57*	3.12 ± 0.23*
T_{10} (Hot water + stratification for 30 days)	29.03±0.86*	3.05 ± 0.35*
T_{11}(Cracking+GA_3@500ppm+stratification for 30 days)	34.79±0.35*	3.48 ± 0.19*
T_{12}(Cracking+GA_3@750ppm+stratification for 30 days)	34.73±0.52*	3.58 ± 0.16*
T_{13}(Cracking+GA_3@1000ppm+stratification for30 days)	30.50±0.91*	3.09 ± 0.07*
T_{14} (Hotwater+GA_3@500ppm+stratification for 30 days)	37.35±0.34*	3.97 ± 0.31*
T_{15}(Hot water+GA_3@750ppm+stratification for 30 days)	35.28± 0.22*	3.30 ± 0.07*
T_{16}(Hot water+GA_3@1000ppm+stratification for 30 day)	31.08 ± 0.85*	3.24 ± 0.16*
T_{17} (Control)	18.58 ± 0.88	2.17 ± 0.34
SE (d)	1.28	0.31
$CD_{0.05}$	2.61	0.63

4.2.3 Number of branches per plant

The data on the number of the branches per plant as influenced by different pre sowing treatment are presented in Table 4.3. As it is evident from the data, all the treatments had non significant effect on the number of branches per plant. The maximum no. of branches per plant (8.00) was observed under T_{14} (hot water + GA_3 500ppm + stratification for 30 days), whereas the minimum number of branches per plant (4.83) was recorded in T_{17} (control).

Table: 4.3 Effect of different pre sowing treatment on number of branches and number of leaves per plant

Treatments	**Number of branches/plant ± SE (m)**	**Number of leaves/plant ± SE (m)**
T_1(Cracking)	5.17 ± 0.60	20.00 ± 0.00
T_2(Hot water)	5.83 ± 0.60	25.61 ± 1.75
T_3(Cracking + GA_3 @500ppm)	6.67 ± 0.44	31.40 ± 4.20*
T_4(Cracking + GA_3 @750ppm)	7.50 ± 0.58	34.01 ± 1.03*
T_5(Cracking + GA_3 @1000ppm)	6.67 ± 0.67	31.48 ± 1.48*
T_6(Hot water + GA_3 @500ppm)	6.83 ± 0.73	30.97 ± 0.73*
T_7(Hot water + GA_3 @750ppm)	7.33 ± 0.60	35.24 ± 1.95*
T_8(Hot water + GA_3 @1000ppm)	6.17 ± 1.30	30.87 ± 3.66*
T_9(Cracking + stratification for 30 days)	6.00 ± 0.76	30.07 ± 2.37*
T_{10}(Hot water + stratification for 30 days)	6.23 ± 0.43	25.99 ± 2.06
T_{11}(Cracking+GA_3@500ppm+stratification for 30 days)	7.57 ± 0.07	36.33 ± 0.90*
T_{12}(Cracking+GA_3@750ppm+stratification for 30 days)	7.33 ± 0.60	35.47 ± 2.24*
T_{13}(Cracking+GA_3@1000ppm+stratification for 30 days)	6.50 ± 0.58	30.74 ± 0.74*
T_{14}(Hot water+GA_3@500ppm+ stratification for 30 days)	8.00 ± 0.00	40.00 ± 0.00*
T_{15}(Hot water+GA_3@750ppm+ stratification for 30 days)	7.17 ± 0.00	35.67 ± 0.67*
T_{16}(Hot water+GA_3@1000ppm+ stratification for 30 day)	7.00 ± 0.88	32.71 ± 1.40*
T_{17} (Control)	5.00 ± 0.58	23.33 ± 4.41
SE (d)	0.98	3.10
$CD_{0.05}$	NS	6.31

4.2.4 Number of leaves per plant

The data on the effect of pre sowing treatments on number of leaves per plant are presented in Table 4.3. The data revealed that the maximum number of leaves (40.00) was recorded with T_{14} (hot water + GA_3 500 ppm + stratification for 30 days) which was statistically at par with T_{11}, T_{15}, T_{12}, T_8 and T_5 recording 36.33, 35.67, 35.47, 35.24 and 34.01 respectively, which was followed by T_{16} (32.31), T_5 (31.48) and T_3 (31.40). The minimum number of leaves (20.00) was recorded with T_1 (cracking) which was statistically at par with T_{17} (23.33), T_2 (25.61) and T_{10} (25.99).

4.2.5 Root length

The data presented in Table 4.4 showed that different pre sowing treatments averted significant influence on root length. The maximum root length (27.52 cm) was recorded with T_{14} (hot water + GA_3 500ppm + stratification for 30 days) which was followed by T_{11} (25.25 cm), T_{12} (25.04 cm), T_{15} (24.53cm), T_{13} (23.35 cm) and T_{16} (23.33 cm). The minimum root length (13.56 cm) was recorded with T_2 (hot water).

4.2.6 Root diameter

It is evident from the data as depicted in Table 4.4 that the root diameter was significantly affected by different pre sowing treatments. The maximum root diameter (5.75 mm) was observed with T_7 (hot water + GA_3 750ppm) which was statistically at par with treatment T_4, T_3, T_8, T_6 and T_{14} recording 5.53mm, 5.45mm, 5.05mm, 4.82mm and 4.59mm respectively, which was followed by T_{16} (4.56mm) and T_5 (4.54mm). The minimum root diameter (2.53mm) was recorded with T_{17} (control) and was statistically at par with T_1, T_{12}, T_2, T_{13}, $T_{11,}$ T_{10} and T_9 recording 3.14mm, 3.27mm, 3.36mm 3.37mm, 3.42mm, 3.43mm and 3.64mm respectively.

Table: 4.4 Effect of different pre sowing treatment on root length and root diameter of walnut seedlings

Treatments	Root Length(cm) ± SE(m)	Root diameter(mm) ± SE (m)	Root area (cm^2) ±SE (m)
T_1(Cracking)	15.02 ± 0.73	3.14 ± 0.42	12.40 ± 0.29
T_2(Hot water)	13.56 ± 0.95	3.36 ± 0.18	11.74 ± 0.37
T_3(Cracking + GA_3 @500ppm)	19.71 ± 0.41*	5.45 ± 0.21*	14.71±0.14*
T_4(Cracking + GA_3 @750ppm)	21.26 ± 0.51*	5.53 ± 0.12*	15.41±0.47*
T_5(Cracking + GA_3 @1000ppm)	17.70 ± 0.45*	4.54 ± 0.10*	14.19±0.82*
T_6(Hot water + GA_3 @500ppm)	21.63 ± 1.07*	4.82 ± 0.11*	16.78±0.82*
T_7(Hot water + GA_3 @750ppm)	23.65 ± 0.75*	5.75 ± 0.83*	16.03±0.20*
T_8(Hot water + GA_3 @1000ppm)	18.48 ± 1.52*	5.05 ± 0.42*	14.48±0.76*
T_9(Cracking + stratification for 30 days)	17.61 ± 0.46*	3.64 ± 0.25	14.39±0.14*
T_{10}(Hot water + stratification for 30 days)	20.23 ± 0.42*	3.43 ± 0.49	13.70±0.44*
T_{11}(Cracking+GA_3@500ppm+ stratification for 30 days)	25.25 ± 0.37*	3.42 ± 0.65	18.65±0.12*
T_{12}(Cracking+GA_3 @750ppm+stratification for 30 days)	25.04 ± 0.45*	3.27 ± 0.21	17.94±0.53*
T_{13}(Cracking+GA_3@1000ppm+stratification for 30 days)	23.35 ± 1.52*	3.37 ± 0.55	17.57±0.54*
T_{14}(Hot water + GA_3@500ppm+ stratification for 30 days)	27.52 ± 0.60*	4.59 ± 0.10*	19.62±0.32*
T_{15}(Hot water +GA_3@750ppm+ stratification for 30 days)	24.53 ± 1.15*	4.16 ± 0.53*	18.47±0.40*
T_{16}(Hot water+GA_3@1000ppm+ stratification for 30 day)	23.33 ± 0.63*	4.56 ± 0.54*	17.67±0.67*
T_{17} (Control)	14.32 ± 0.84	2.53 ± 0.30	12.04 ± 0.42
SE (d)	1.09	0.57	0.58
$CD_{0.05}$	2.23	1.16	1.18

4.2.7 Root area

The data in Table 4.4 show that the root area was significantly affected by different pre sowing treatments. The maximum root area (19.62 cm^2) was recorded with T_{14} (hot water + GA_3 500ppm + stratification for 30 days) and was statistically at par with treatment T_{11} and T_{15} recording 18.65cm^2 and 18.47 cm^2 respectively, which was followed by T_{12} (17.94 cm^2) and T_{16} (17.67 cm^2). The minimum root area (11.74 cm^2) was recorded with T_1 (cracking) and was statistically at par with treatment T_{17} and T_2 recording 12.04 cm^2 and 12.40 cm^2 respectively.

4.2.8 Shoot fresh weight (g)

It is evident from the data presented in Table 4.5 that the shoot fresh weight was significantly affected by different pre sowing treatments. The maximum shoot fresh weight (12.47g) was recorded with T_{14} (hot water + GA_3 500ppm + stratification for 30 days) and was statistically at par with T_{11} and T_{15} recording 11.89g and 11.25g respectively which was followed by T_{12} (10.51g). The minimum shoot fresh weight (5.33g) was recorded with T_{17} (control) and statistically at par with T_1, T_2, T_5 and T_8 recording 5.40g, 5.71g, 6.50g and 6.64g respectively which was followed by T_{10} (7.13g), T_4 (7.17g) and T_7 (7.77g).

4.2.9 Root fresh weight (g)

The data on the effect of different pre sowing treatments on root fresh weight are presented in Table 4.5. A perusal of data indicated that different pre sowing treatments had a significant influence on root fresh weight. The data revealed that maximum root fresh weight (12.10g) was recorded with T_{14} (hot water + GA_3 500ppm + stratification for 30 days) which was statistically at par with T_{11} recording 10.75g. The minimum root fresh weight (4.22g) was recorded with T_2 (hot water) and was statistically at par with T_{17} and T_1 recording 5.11g and 5.33g respectively.

4.2.10 Total fresh weight (g)

The data in Table 4.5 indicated that the total shoot-root fresh weight was significantly affected by different pre sowing treatments. The maximum total fresh weight (24.57g) was recorded with T_{14} (hot water + GA_3 500ppm + stratification for 30 days) which was statistically at par with treatment T_{11} recording 22.00g. The minimum total fresh weight (9.87g) was recorded with T_{17} (control) and was statistically at par with T_2 recording 9.93g and T_1 (10.73g)

Table: 4.5 Effect of different pre sowing treatments on shoot fresh weight, root fresh weight and total fresh weight

Treatments	SFW (g) ± SE (m)	RFW (g) ± SE (m)	TFW (g) ± SE (m)
T_1(Cracking)	5.40 ± 0.40	5.33 ± 0.83	10.73 ± 0.72
T_2 (Hot water)	5.71 ± 0.11	4.22 ± 0.38	9.93 ± 0.49
T_3 (Cracking + GA_3 @500ppm)	7.17* ± 1.33	6.67* ± 0.78	13.84*±1.41
T_4 (Cracking + GA_3 @750ppm)	8.58* ± 0.17	7.40* ± 0.70	15.98*±0.81
T_5 (Cracking + GA_3 @1000ppm)	6.50 ± 0.80	6.72 *± 0.75	13.22*±0.35
T_6 (Hot water + GA_3 @500ppm)	7.77* ± 0.47	7.39* ± 0.77	16.59*±1.22
T_7 (Hot water + GA_3 @750ppm)	9.19* ± 0.30	7.18* ± 0.09	14.95*±0.21
T_8 (Hot water + GA_3 @1000ppm)	6.74 ± 0.25	6.50* ± 0.49	13.23*±0.28
T_9 (Cracking + stratification for 30days)	6.93* ± 0.53	6.09* ± 0.49	13.02*±0.75
T_{10} (Hot water + stratification for 30 days)	7.13*± 0.32	6.33* ± 0.65	13.47*±0.80
T_{11}(Cracking+GA_3@500ppm+stratification for 30days)	11.89*±0.29	10.75*±0.56	22.00*±0.70
T_{12}(Cracking + GA_3 @750ppm+stratification for 30days)	10.51*±0.60	7.28* ± 0.29	17.79*±0.81
T_{13}(Cracking + GA_3@1000ppm+ stratification for 30days)	9.34* ± 0.41	6.11* ± 0.24	15.45*±0.19
T_{14} (Hot water + GA_3@500ppm+stratification for 30days)	12.47*±0.04	12.10*±0.19	24.57*±0.23
T_{15} (Hot water +GA_3@750ppm +stratification for 30days)	11.25*±0.28	7.87* ± 0.86	19.76*±1.09
T_{16}(Hot water +GA_3@1000ppm+stratification for 30days)	9.25* ± 0.25	7.12* ± 0.11	16.37*±0.33
T_{17} (Control)	5.33 ± 0.55	4.54 ± 0.13	9.87 ± 0.46
SE(d)	0.73	0.78	1.00
$CD_{0.05}$	1.48	1.59	2.04

SFW = Shoot fresh weight, RFW = Root fresh weight, TFW = Total fresh weight

4.3 Survival percentage (%)

A perusal of the data presented in Table 4.6 revealed that the survival percentage was influenced by different pre sowing treatments. The maximum survival percentage (93.24%) was recorded with T_{14} (hot water + GA_3 500ppm + stratification for 30 days) which was statistically at par with treatment T_{11}, T_{15} and T_{12} recording 91.60%, 91.19%, 90.33% respectively, which was followed by T_{16} (88.11 %), T_6 (87.42%) and T_{13} (87.30%). The minimum survival percentage (67.53%) was recorded with T_{17} (control), which was followed by T_1 (cracking).

Table: 4.6 Effect of different pre sowing treatments on survival percentage of walnut seedling

Treatments	**Survival percentage ± SE (m)**
T_1 (Cracking)	69.87 (8.42 ± 0.04)
T_2 (Hot water)	72.56 (8.58 ± 0.08)*
T_3 (Cracking + GA_3 @500ppm)	86.91 (9.38 ± 0.01)*
T_4 (Cracking + GA_3 @750ppm)	78.62 (8.92 ± 0.12)*
T_5 (Cracking+ GA_3 @1000ppm)	73.85 (8.65 ± 0.05)*
T_6 (Hot Water + GA_3 @500ppm)	87.42 (9.40 ± 0.11)*
T_7 (Hot Water+ GA_3 @750ppm)	85.26 (9.29 ± 0.04)*
T_8 (Hot Water + GA_3@1000ppm)	83.18 (9.17 ± 0.10)*
T_9 (Cracking + stratification for 30 days)	82.78 (9.15 ± 0.08)*
T_{10} (Hot Water + stratification for 30 days)	85.62 (9.31 ± 0.04)*
T_{11} (Cracking + GA_3@500ppm + Stratification for 30 days)	91.60 (9.62 ± 0.14)*
T_{12} (Cracking+ GA_3 @750ppm + Stratification for 30 days)	90.33 (9.55 ± 0.13)*
T_{13} (Cracking + GA_3 @1000ppm+ Stratification for 30 days)	87.30 (9.40 ± 0.10)*
T_{14} (Hot Water + GA_3 @500ppm + Stratification for 30 days)	93.24 (9.71 ± 0.05)*
T_{15} (Hot Water +GA_3 @750ppm + Stratification for 30 days)	91.19 (9.60 ± 0.09)*
T_{16} (Hot Water + GA_3 @1000ppm + Stratification for 30 days)	88.11 (9.43 ± 0.09)*
T_{17} (Control)	67.33 (8.26 ± 0.23)
SE (d)	2.43 (0.13)
$CD_{0.05}$	4.96 (0.28)

Data in parentheses are transform values.

CHAPTER 5
DISCUSSION

Walnut (*Juglans regia* L.) is one of the most important temperate nut crop. It possesses dormancy within the seeds. Dormancy is a condition where seeds do not germinate even when the environmental conditions (water, temperature and aeration) are permissive for germination (Nikolaeva, 1977; Bewley & Black, 1994). Walnut seeds have exogenous physical dormancy (seed coat dormancy). In this dormancy seeds fail to germinate because the seed is impermeable to water and poor aeration (i.e. limit oxygen to the embryo) due to hard seed covers.

Seed germination is a complex process that started with the absorption of water and after a short pause; the enzyme is activated (Matilla and Matilla-Vazquez, 2008). Successful seed germination depends on numerous internal and external factors. Thus, in order to germinate, they require dormancy breaking treatments. There are several treatments to break exogenous physical seed dormancy and initiate early growth. These treatments are given before the seed sowing hence, these treatments are called pre sowing treatments. These include cold and warm stratification, scarification, growth regulator application etc. Stratification is the process of pre-treating seeds to simulate natural conditions that a seed must endure before germination. Successful stratification requires seeds to be stored in a moist, aerated medium at chilling temperatures for a certain period of time. Scarification is any process of breaking, scratching or mechanically altering the seed coat or endocarp to make it permeable to water and gases. Growth regulators, such as gibberellic acid has the potential to remove seed dormancy. Objectives of different pre sowing treatments are to break the seed dormancy, to help early germination and to increase the seedling growth. The different pre sowing treatments help in breaking seed dormancy and early germination. These treatments are applied either alone or in combination with other pre sowing treatments. The combination of different pre sowing treatments gives best result in germination and seedling growth.

Therefore, present investigations were undertaken to evaluate the effect of different pre sowing treatments on germination and seedling growth of walnut (*Juglans regia* L.). The results obtained during the course of present study have been discussed as under:

5.1 Effect of different pre sowing treatments on germination

The results indicated that the seed germination and days taken for initial germination were influenced by duration of stratification, scarification as well as GA_3 treatments. Most of the treatments had a beneficial effect on germination. However, the best treatment was the combination of cracking + gibberellic acid @ 500 ppm + stratification for 30 days, as it recorded the highest germination (75.88%) and minimum days taken for germination (12.67 days) was obtained with the combination of cracking + gibberellic acid @ 750 ppm + stratification for 30 days. This is in agreement with findings of Dorn *et al*. (1985), Çetinbaş (2006), Conner (2008), Al-Absi (2010), Çalişkan *et al*. (2012) and Pipinis *et al*. (2012) who observed that cracking and hot water with gibberellic acid when used in conjunction with stratification increased germination in walnut, mazzard cherry, muscadine grapes, mahaleb cherry and fig. These finding are also corroborated by the reports of several workers who found that application of gibberellic acid with stratification was effective in breaking the dormancy and promoting germination of black mulberry (Koyuncu, 2005), *Arbutus unedo* (Smiris *et al*. 2006) and walnut (San, 2007).

Similarly results were obtained by Pratibha *et al*. (2015) who reported that the gibberellic acid @ 300ppm increased the germination and decreased the days to germination of papaya seeds. The maximum germination might be due to the fact that GA_3 involved in the activation of cytological enzymes which stimulates α – amylase enzyme that converts insoluble starch into soluble sugars (Babu *et al*., 2010) and early germination might be due to the fact that, GA_3 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). The above results are in conformity with Barche *et al.* (2010), Anburani and Shakila (2010) in papaya.

In the present studies, it has been observed that gibberellic acid was required in relatively lower concentration with stratification and scarification for the maximum germination. Cracking and hot water treatment with gibberellic acid @ 750 ppm have given the maximum germination under 30 days of stratification. This is supported by

Amooaghaie (2009), Mavi *et al*. (2012), Patel *et al*. (2014), Lilabati *et al*. (2015) and Parvin (2015) who reported that gibberellic acid 500 ppm and 400ppm concentration were more effective on the germination of *Ferula ovina*, fig, *Tithonia rotundifolia* and aonla and black walnut seeds, when used in different concentrations. It is also supported by Conner (2008) who reported that increasing the GA_3 concentration resulted in a decrease in germination. In grapes concentrations of GA_3 of 2 g/L were also found to cause the death of a percentage of seed germination in some seed lots (Ellis et al., 1983)

The inability of walnut seeds to germinate may be due to the hard seed coat. As the scarification treatment given to the seed helped in uptake of water, growth hormones and air which was required for seed germination (Koyuncu *et al*., 2006; Conner, 2008; Al-Absi, 2010 and Mavi *et al.,* 2012).

Since gibberellic acid concentration can be shortened by stratification it seems possible that chilling treatments may be effective because they lead to synthesis of endogenous gibberellins (Conner, 2008; Amooaghaie, 2009 and Parvin *et al*., 2015). Mathur *et al*. (1971) suggested that as the stratification proceeds gibberellic acid is synthesized or released from the bound form in the peach seeds. Frankland *et al*. (1962) also reported that the chilling treatment induced an increase in the concentration of gibberellins in hazelnut seeds and suggested that the gibberellins were synthesized during the chilling treatment as a factor in breaking dormancy.

Prechilling stratification had a significant effect on 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, 1992). Pre-chilling stratification is a standard procedure used to enhance the germination of dormant seeds. The chilling process appears to enhance the production of some types of growth promoting substances such as GA_3 (Powell, 1987). Giba *et al*. (1993) reported that the inhibitory effect of retardants was overcome by gibberellic acid. Treatment with GA_3 + stratification was found to be successful for mahaleb (Gercekcioglu and Cekic, 1999) and plum (Ozvardar and Ozcagiran, 1991). These reports accord with our results showing that GA_3 + stratification enhance germination of *M. nigra* seeds.

5.2 Effect of different pre sowing treatments on growth characteristics

5.2.1 Shoot growth characteristics

The shoot growth increased markedly with the combined application of different pre sowing treatments i.e. cracking, gibberellic acid and stratification. The highest shoot length (37.35 cm), shoot diameter (3.97mm) and number of leaves (40.00) were observed with the application of hot water + gibberellic acid @ 500ppm + stratification for 30 days. Hence, the present findings revealed that the combined application of pre sowing treatments was the best treatment for better shoot growth. Similar results were obtained by Parvin *et al*. (2015) who reported better growth of walnut seedlings from gibberellic acid and stratification combination. It might be due to the effect of GA_3 and stratification on enhancing growth due to the solubility of fats and sugars caused by stratification plus the increase in gibberellin synthesis. In addition, the improving effect of GA_3 and stratification on seed germination might have reflected on enhancing the shoot parameters. These results are in agreement with Dahkaei (2009) on *Danae racemosa*, Rawat *et al*. (2010) on *Punica granatum* and Hassan and Fetouh (2014) on seeds of *Magnolia grandiflora*.

Aatala *et al*. (2013) also observed maximum shoot height with GA_3 which might be attributed to the cell multiplication and cell elongation in the cambium tissues of the internodal region (Dohono and Walker, 1957). GA_3 in all the concentrations used in the study, resulted in longer shoots with long and thin internodes. The increase in seedling height by application of gibberellic acid was also reported by Rajamanickam *et al.* (2004) in amla, Shirol *et al.* (2005), Rao *et al.* (2006) and Kumar *et al.* (2007) in mango.

The GA_3 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. GA_3 is inactivated after growth and calcium returns to the cell wall to stiffen it. After the absorption of water by the seed and following the active absorption stage, the embryo produces GA_3 and stimulates aleuronic cells to produce hydrolytic enzymes such as α- and β-amylase that hydrolyze starch to glucose, which can be absorbed by the embryo. GA_3 activated α- amylase which digested the available carbohydrate in to simpler sugars, so that energy and nutrition were easily available to faster growing seedlings. GA_3 affects the proteins that produce mRNA and thereby increases DNA replication and induces analysis of endospermic materials in the seed (Al- Hawezy, 2013).

In stratification, endosperm is disrupted permitting embryo growth. On the other hand, low temperatures stimulate the breakdown of proteins into soluble nitrogenous compounds and formation of the amino acids glycine and argentine, which are beneficial for embryo growth (Baskin and Baskin, 2001 and Razavi *et al*., 2009)

Maximum numbers of leaves (39.90) were produced under the same treatment combination of hot water + gibberellic acid @ 500ppm and stratification for 30 days. It might be due to the seedling which was raised from this treatment combination attained more height which suggests that as the height of seedlings increased, there was a simultaneous increase in the number of leaves. Similar observations were recorded by Mathur (1964) in peach and apricot seedlings.

5.2.2 Root growth characteristics

It is evident from the present investigation that the different pre sowing treatments influenced the root characteristics. The highest root length (27.52 cm) as well as root area (19.62 cm^2) was recorded with the application of hot water + gibberellic acid @ 500 ppm + stratification for 30 days. These finding indicate that the combined application of hot water, gibberellic acid and stratification for 30 days was successful in increasing in root growth characteristics. The present findings of increase in root length and root area by combined application of hot water, gibberellic acid @ 500 ppm and stratification for 30 days are in congruence with findings of Pravin *et al*. (2015) who reported maximum root length and root area of walnut with combination of gibberellic acid and stratification. The effect of GA_3 and stratification on root parameters followed the same trend as on the shoots. The positive effect of GA_3 and stratification on root parameters might be explained through the role of GA_3 and stratification in enhancing gibberellin synthesis which also leads in increase the growth and root branching and overall increase in root fresh weight (Penfield *et al.,* 2005). The results of the present experiment are in agreement with Rawat *et al.* (2010) on *Punica granatum* and Hassan and Fetouh (2014) on *Magnolia grandiflora* seeds.

Pratibha *et al*. (2015) also reported maximum root length under the gibberellic acid @ 300 ppm for 24 hours. It might be due to the reason that the shoot growth resulted in production of photosynthatates which were translocated through phloem to the root zone and was

responsible for increase in root length. Similar results were obtained in accordance with the results of earlier worker Palaniswamy and Ramamoorthy (1987), Ananthakalaiselvi and Dharmalingam (1998) and Anburani and Shakila (2010) in papaya.

5.2.3 Effect of different pre sowing treatments on shoot, root fresh and total fresh weight

The results revealed that shoot fresh weight was markedly increased by the combination of different pre sowing treatment i.e. hot water, gibberellic acid and stratification for 30 days during the period of study. The maximum shoot fresh weight (12.47 g), root fresh weight (12.10g) and total fresh weight (24.57g) were recorded with the combined application of hot water, gibberellic acid @ 500 ppm and stratification for 30 days. This promotion could be explained through the role of stratification in enhancing gibberellin synthesis which also leads to increase in the growth and root branching and overall increased roots fresh weight (Penfield *et al*., 2005). These results are in agreement with Harrington and Kraft (2004) on *Arbutus menziesii Pursh*, Tilki and Cicek (2005) on *Fraxinus angustifolia* and Rawat *et al.* (2010) on *Punica granatum.*

Fetouh *et al.* (2014) reported the effect of stratification on enhancing shoot growth and fresh weight could be attributed to the solubility of fats and sugars due to stratification plus the increase of gibberellin synthesizing which enhanced the growth. In addition, the promotional effect of cold stratification on seed germination reflected on enhancing the shoot parameters which increased the shoot fresh weight. These results are in agreement with Lohengrin and Arroyo (2000) on *Phacelia secunda* and Dahkaei (2009) on *Danae racemosa*

The combination of hot water + gibberellic acid + stratification increased the shoot – root weight and total fresh weight might be due to the production of maximum shoot, root height, diameter and maximum number of leaves which suggests that as the all the growth parameters of shoot and root of seedlings increased, there was a simultaneous increase in the shoot and root fresh weight. Similar observations were recorded and confirmed by Pawar *et al*. (2010) in Jatropa, Farhoudi *et al*. (2015) in *Echinacea purpurea* and Parvin *et al*. (2015) in black walnut.

Gulzar *et al*. (2001) suggested GA_3 treatment led to the disappearance of ABA, and attributed to mobilization of stored reserved and weakening of the mechanical resistance of the endosperm cells around radical tip, which increased the seedling growth and seedling fresh weight. Application of gibberellic acid to dormant seeds can eliminate their natural chilling requirement (Fang *et al*., 2006).

5.3 Effect of different pre sowing treatments on survival percentage

Survival percentage was significantly influenced by application of different pre sowing treatments. The maximum survival percentage (93.24%) was recorded with combination application of hot water + gibberellic acid @ 500 ppm + stratification for 30 days. The results are in conformity with the findings of Wani (2014) who observed increase in the survival percentage with the application of gibberellic acid @ 500 ppm for 40 hours. It might be as GA_3 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.

Rawat *et al*. (2010) reported that application of stratification for 30 days increased the survival percentage. It might be because stratification increased the early germination which resulted into longest radicle, which helps in early establishment of new seedling to produce maximum food material with the helped in photosynthesis that resulted into the maximum survival of seedlings. The results are in close in conformity with the results of Bose and Mitra (1991) in apricot and Bhatt *et al.* (2000) in *Myrica esculenta*.

CHAPTER 6
SUMMARY AND CONCLUSION

The results obtained in the present investigations on the "Effect of different pre sowing treatments on germination and seedling growth of walnut (*Juglans regia* L.)" are summarized as under:

6.1 The result obtained on effect of different pre sowing treatments on seed germination characteristics of walnut exhibited the best performance with cracking + gibberellic acid @ 500 ppm + stratification for 30 days, whereas highest germination percentage (75.88%) were observed while minimum days taken for germination (12.67 days) were observed with cracking + gibberellic acid @ 750 ppm + stratification for 30 days.

6.2 Different pre sowing treatments had a significant effect on the shoot characteristics. The highest shoot length (37.35 cm), maximum shoot diameter (3.97 mm) and number of leaves (40.00 leaves /plant) were observed with treatment combination of hot water + gibberellic acid @ 500 ppm + stratification for 30 days.

6.3 Application of different pre sowing treatments in combination significantly increased the root growth characteristics. The application of hot water + gibberellic acid @ 500 ppm + stratification for 30 days recorded the highest root length (27.52 cm) and root area (19.62 cm^2), while maximum root diameter (5.75 mm) was recorded with application of hot water + gibberellic acid @ 750 ppm.

6.4 The combine application of different pre sowing treatments had significant effect on shoot-root fresh weight. The maximum shoot fresh weight (12.47 g), root fresh weight (12.10 g) and total fresh weight (24.57 g) were recorded with treatment combination of hot water + gibberellic acid @ 500 ppm + stratification for 30 days.

6.5 The survival percentage was significantly influenced by different pre sowing treatments. The highest survival percentage (93.24 %) was recorded with treatment combination of hot water + gibberellic acid @ 500 ppm + stratification for 30 days.

Conclusion

The results obtained in the present studies showed that among different pre sowing treatments, the best results in terms of 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 obtained with hot water + gibberellic acid @ 500 ppm + stratification for 30 days. The maximum germination percentage was recorded with cracking + gibberellic acid @ 500 ppm + stratification for 30 days while the minimum days taken for germination were recorded with cracking + gibberellic acid @ 750 ppm + stratification for 30 days. Thus, the combination of different 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 30 days) which was highly effective in improving the overall growth of walnut seedlings.

CHAPTER 7

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APPENDIX – I

ANALYSIS OF VARIANCE FOR DIFFERENT CHARACTERS UNDER STUDY

Source of variation	Degree of freedom	Mean sum of squares					
		X1	**X2**	**X3**	**X4**	**X5**	**X6**
Replication	2	4.13	17.02	3.03	0.01	0.95	30.46
Treatment	16	337.56	1073.71	86.16	0.77	1.87	82.98
Error	32	10.66	17.12	2.46	0.14	1.44	27.19

X1. Germination percentage (%) X2. Days taken for initial germination X3. Shoot height (cm) X4. Shoot diameter (mm) X5. Number of branches per plant X6. Number of leaves per plant

APPENDIX – II

ANALYSIS OF VARIANCE FOR DIFFERENT CHARACTERS UNDER STUDY

Source of variation	Degree of freedom	Mean sum of squares						
		X7	**X8**	**X9**	**X10**	**X11**	**X12**	**X13**
Replication	2	7.13	2.79	4.37	0.63	1.22	3.32	27.40
Treatment	16	51.22	0.90	17.98	15.21	11.31	48.12	191.27
Error	32	1.79	0.49	0.50	0.79	0.91	1.50	8.88

X7. Root length (cm) X8. Root diameter (mm) X9. Root area (cm^2) X10. Shoot fresh weight (g)
X11. Root fresh weight (g) X12. Total fresh weight (g) X13. Survival percentage (%)

ABSTRACT

Name of the student: Priya Negi ID Number: 14109

Semester and year of admission: First and 2014 Degree: M.Sc. Horticulture (Fruit Science)

Major: Fruit Science Department: Fruit Science

Thesis title: "Effect of different pre sowing treatments on germination and seedling growth of walnut (*Juglans regia* L.)"

Advisor: Prof. B. P. Nautiyal

The present investigations entitled "Effect of different pre sowing treatments on seed germination and seedling growth of walnut (*Juglans regia* L.)" was carried out in the poly house of Fruit Nursery, College of Horticulture, VCSG, UUHF, Bharsar (Uttarakhand) during the year 2015-16. In this study walnut seeds were treated with different pre sowing treatments and planted in Randomized Complete Block Design with three replications and were assessed to know the effect of different pre sowing treatments on seed germination and seedling growth.

The results obtained in the present studies showed that among different pre sowing treatments, the best results in terms of 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 obtained with T_{14} (Hot water + gibberellic acid @ 500 ppm + stratification for 30 days). The maximum germination percentage was recorded with T_{11} (Cracking + gibberellic acid @ 500 ppm + stratification for 30 days) while the minimum days taken for germination were recorded with T_{12} (Cracking + gibberellic acid @ 750 ppm + stratification for 30 days).

Thus, the combination of different 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 30 days) which was highly effective in improving the overall growth of walnut seedlings.

Prof. B. P. Nautiyal Name and signature of Advisor	Dr. Nidhika Thakur Co-Advisor	Priya Negi Name and Signature of Author

सारांश

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मुख्य	फल विज्ञान	विभाग	फल विज्ञान

शोध का शीर्षक "अखरोट (जुगलेंस रीजिआ एल.) के बीज अंकुरण और अंकुर विकास पर विभिन्न पूर्व बुवाई उपचार के प्रभाव"

सलाहकार प्रो. बी. पी. नौटियाल

वर्तमान शोध जिसका नाम " अखरोट (जुगलेंस रीजिआ एल.)) के बीज अंकुरण और अंकुर विकास पर विभिन्न पूर्व बुवाई उपचार के प्रभाव" 2015-16 के दौरान औद्यानिकी महाविद्यालय, वी. च. सिं. ग. उत्तराखंड औद्यानिकी एवं वानिकी विश्वविद्यालय, भरसार (उत्तराखंड), फल नर्सरी ब्लॉक के पॉलीहाउस में लगाया गया था । इस अध्ययन में अखरोट के बीजो को विभिन्न पूर्व बुवाई उपचार के साथ इलाज किया गया और तीन अनुकरण के साथ पूर्ण ब्लॉक डिजाइन यादृच्छिक में लगाया और बीज अंकुरण और विभिन्न अंकुर पात्रों पर विभिन्न पूर्व बुवाई उपचार के प्रभाव का पता करने के लिए मूल्यांकन किया गया।

अध्ययन के परिणाम तहत अखरोट अंकुरण और अंकुर के विभिन्न पात्रों पर विभिन्न पूर्व बुवाई उपचार का प्रभाव महत्वपूर्ण दिखाया गया। जिब्रेलिक एसिड (500 पीपीएम और 750 पीपीएम) के दो एकाग्रता के साथ 30 दिनों के लिए स्तरीकरण के तहत खुर के संयोजन मे क्रमश: अधिकतम बीज अंकुरण तथा शीघ्र अंकुरण प्राप्त हुआ । T_{14} (गर्म पानी + जिब्रेलिक एसिड @ 500 पीपीएम + 30 दिनों के लिए स्तरीकरण के संयोजन) मे तना ऊंचाई, तना मोटाई, पत्तियों की संख्या, जड की लंबाई, जड़ क्षेत्र, तने का ताजा वजन, जड़ का ताजा वजन, कुल ताजा वजन और अस्तित्व प्रतिशत अधिकतम प्राप्त किया गया।

अत: इस अध्ययन से यह निष्कर्ष निकलता है कि अधिकतम बीज अंकुरण तथा अंकुर के विभिन्न पात्रों पर विभिन्न पूर्व बुवाई उपचार के संयोजन का प्रभाव रहा और सबसे अच्छा उपचार **T_{14}** (गर्म पानी + जिब्रेलिक एसिड @ 500 पीपीएम + 30 दिनों के लिए स्तरीकरण) था जोकि अखरोट के अंकुर कि अच्छी बढत लिए सबसे प्रभावी था ।

प्रो. बी पी नौटियाल	डॉ निधिका ठाकुर	प्रिया नेगी
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Whether sponsored by some state/ Central Govt./Univ./SAARC : No

Scholarship/ Stipend/ Fellowship, any other financial assistance received during the study period : No

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