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RESEARCH ARTICLE

EFFECT OF GIBBERELLIC ACID, POTASSIUM NITRATE AND CHILLING ON SEED GERMINATION RESPONSE OF APPLE (*PYRUS MALUS* L. CV. RED DELICIOUS)

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Abstract

Premise of research: Apple (*Pyrus malus*L.cv.Red delicious) is an important cash crop which aids in socioeconomic development of the farmers in the Uttarakhand state. The delicious and its strains have gained popularity all over the world and are cultivated on a large scale in India. More than 80% of the area is under the delicious group cultivation in India. In Uttarakhand the yield of this crop is getting reduced year after year. This corresponds to many biotic and abiotic factors. One of the main reasons is Global warming. In spite of favourable weather conditions, seeds do not germinate and remain in dormant stage. So, our aim was to investigate the influence of pre-germination treatments on Apple seeds under lab conditions to find out the most promising technique.
Methodology: The effect of GA_3, KNO_3 and stratification to determine the seed germination response for various time duration was observed. Total 10 germination parameters were measured.
Pivotal results: The highest GRI, CGRI, GV1, GV2 and GS/day were recorded in seeds treated with 0.1% KNO_3 for 48hrs. Lowest MGT and highest CVG were found in combination of (250 ppmGA_3+0.1%KNO_3) in 24 hrs. Combination of (1000 ppmGA_3+0.3%KNO_3) treated seeds for 48 hrs showed maximum FGP and MDG value. GSP was highest of all in 0.1% KNO_3 treated seeds applied for 48 hrs.
Conclusion: Our study clearly depicted that germination percentage can be increased by giving a pre-sowing treatment to seeds with a combination of (1000 ppm GA_3+0.3% KNO_3) for upto 48 hrs. This is by-far the most effective and simplest method that could be easily adopted by the farmers for improving and enhancing the economic cultivation of this variety. Hence, germination of Apple seeds can be increased by inundating the seeds with GA_3 and KNO_3 before sowing.

Introduction:-

Apple is one of the most economically and culturally important fruit crop which belongs to the Pomoideae family, subfamily Rosaceae, along with some other important fruit crops such as Pear (*Pyrus communis L.*), Prune (*Prunus domestica L.*) and Cherry (*Prunus avium L.*) (**Korban and Skirvin, 1984**). Owing to its high accessibility and comparatively lower price, it marks a significant contribution in human's daily dietary consumption (**Jonsssonet al., 2010)**. India follows China in total fruit production, 12.6%, and comes at the second place (**Horticultural Statistics, 2014, Government of India**). Being the third most important fruit after Banana and Watermelon (107.1 million tons/year), Apple is widely cultivated throughout the world (76.4 million tons/year) of which more than 60% is produced in Asia.

In India, Jammu & Kashmir is the leading Apple producer and accounts for about 70% production followed by Himachal Pradesh (22%), Uttarakhand (6%) and Arunachal Pradesh (2%) (**Indian Horticulture Database, 2013, NHB, MoA, GoI, India**). Though Apple is a temperate climate fruit but there is also an increasing interest in growing Apple in subtropical and tropical countries e.g. India, Mexico, Brazil, Zimbabwe and Kenya at high altitudes (**Ashebir*et al*., 2010; Wamocho and Ombwara, 2001**).

In India, the Apple growing areas do not belong to the temperate zone of the world but the production is primarily due to the chilling requirement met by snow covered Himalayan ranges and high altitude **(www.teri.org)**. However, the global warming issues have recently caused poor yield of Apple production primarily affecting the flowering, blooming time, color, size and its shape **(Sugiura, 2007 and Slingo, 2009)**. Apple is propagated mainly by budding, grafting or by seeds. Seeds are sown for producing rootstocks because true apple varieties do not come out of seed. For producing a desirable variety budding or grafting is required **(Taylor and Whitehead, 1977).** Seeds of Apple are incompetent of germinating at the time of harvest, post-harvesting period and after removing from fruit flesh because they remain in different dormant states including deep physiological endogenous dormancy, embryonic dormancy, testa imposed dormancy and thermodormancy **(Geneve, 2003; Lewak, 2011 and Militaru *et al*., 2009).**

To overcome these problems, some earlier workers viz.,**(Pieniazek, 1967; Zarska-maciejewska 1976; Thevenot and Come, 1983; Zhang and Lespinasse, 1991; Wan, 1992; Renata, 2006; Pitera 2006; Gniazdowska, 2007; Yatoo, 2012 and Debska, 2013)** also studied the germination behaviour of Apple seeds by providing different treatments and found that stratification was essential for better germination. Similarly, application of growth regulators like GA_3, KNO_3, HCN, NO was shown to reduce dormancy of Apple seeds.

Keeping in view the above facts, the objective of the present study was to evaluate the effect of different concentrations and combinations of GA_3, KNO_3 and the effect of temperature at different time intervals on the germination behaviour of *Pyrus malus* cv. Red delicious seeds under laboratory conditions.

Material and methods:-

Study site and Seed collection:-

The present study was conducted in Department of Biotechnology, Bhimtal Campus, Bhimtal (29.35^{0}N,79.56^{0}E, 1370m asl). Fruits of cultivar Red delicious were collected from Bhowali region (29.38^{0}N,79.52^{0}E , 1654m asl) of Nainital district during August-October 2013. Fruits from healthy, disease free apple trees were taken and then seeds were extracted and stored in air tight polybags at 4^{0}C for carrying out the further investigations.

Viability test:-

Viability of seeds was checked by dipping the seeds in a glass beaker containing triple distilled water ($dddH_2O$). Seeds that sank down at the bottom of the beaker were considered as viable and selected for further study. Embryo less seeds that floated on the surface were discarded.

Pre-sowing seed treatments:-

Seeds were washed thoroughly with detergent (labolene 1% v/v) for 15 min and then washed with double distilled water (ddH_2O). Then fungicidal treatment (1% bavistin) was given for 20 min. After a thorough wash by ddH_2O, seeds were disinfected with 70% ethanol for 3-4 min. and rinsed 3 times with $dddH_2O$. After the disinfection process; the seeds were categorized into 5 treatment groups.

(1) Seeds of the first group were soaked in $dddH_2O$ for 24, 48 and 72 hrs, respectively. This treatment was used as control.
(2) Seeds of the second group were treated with 0.1%, 0.2%, and 0.3% KNO_3 for 24, 48 and 72 hrs, respectively.
(3) Likewise; seeds of the third group were treated with 250, 500 and 1000 ppm GA_3 for 24, 48 and 72 hrs, respectively.
(4) Seeds of the fourth group were soaked in aqueous solutions supplemented with 250 ppm GA_3+0.1% KNO_3 , 250 ppm GA_3+0.2% KNO_3 , 250 ppm GA_3+0.3% KNO_3 , 500 ppm GA_3+0.1% KNO_3 , 500 ppm GA_3+0.2% KNO_3 , 500 ppm GA_3+0.3% KNO_3 , 1000 ppm GA_3+0.1% KNO_3 , 1000 ppm GA_3+0.2% KNO_3 , 1000 ppm GA_3+0.3% KNO_3 . All these combinations of GA_3 and KNO_3 were applied for the same time duration as above.
(5) Seeds pertaining to fifth group were given moist chilling environment (stratified) by dipping in ddH_2O and kept at 4^{0}C for 24, 48 and 72 hrs, respectively.

Seed germination conditions:-

Since all the five treatment groups were tested for different time intervals, so overall there were 51 treatments consisting of three replications and each replication representing 50 seeds. Seeds were placed in sterile glass petriplates (95 x 17mm) lined with moistened No 1. Whatman filter paper and incubated at room temperature in dark conditions. dd H_2O was added each alternate day to maintain humidity and the petri plates were placed in sealed plastic bags to avoid moisture loss.

Data collection and Statistical Analysis:-

The cultures were observed daily and the germinated seeds were counted every 24 hrs upto 30 days. Seeds were considered as germinated upon radical emergence ≥0.5 mm.

The following parameters were recorded:

(1) Final Germination percentage (FGP %) **(Gashi *et al.*, 2012)**:

$$= \frac{\text{no. of germinated seeds} \times 100}{\text{Total no. of seeds}}$$

(2) Mean germination time (MGT) **(Moradi *et al.*, 2008)**:

$$= \frac{\Sigma D.n}{\Sigma n} = \frac{\Sigma Dn}{N}$$

Where D = no. of days counting from the beginning of germination.

n = no. of seeds that germinated on D day.

N = total no. of seeds that germinated.

(3) Germination rate index (GRI) **(Esechie, 1994)**:

$$= \frac{\text{germination \%}}{\text{Day 1}} + \frac{\text{germination \%}}{\text{Day 2}} + \text{-----}$$

(4) Corrected Germination rate index (CGRI) **(Gashi *et al.*, 2012)**: $= \frac{\text{GRI} \times 100}{\text{FGP}}$

(5) Germination Speed **(Rajabi and Poustini, 2005)**:$= \frac{\text{no. of germinated seeds}}{\text{no. of days}}$

$$\frac{n_1}{d_1} + \frac{n_2}{d_2} + \text{----------} + \frac{n_n}{d_n} \text{ or } \frac{\Sigma Si}{Di}$$

(6) Germination Speed % **(Shah *et al.*, 2015)**: =

$$\frac{\text{no. of seeds germinated on first count} \times 100}{\text{no. of seeds germinated on final count}}$$

(7) Mean daily germination (MDG) **(Kaifi and Goldani, 2001)**:

Index of daily germination = FGP/d,

where d is day no. to reach final germination.

(8) Germination value (GV_1) **(Djavanshir and Pourbeik, 1976)**: $GV = \frac{\Sigma DGS}{N} \times \frac{GP}{10}$

Where GP = germination % at the end of the test.

$$\Sigma DGS = \frac{\Sigma \text{ Cumulative germination \%}}{\text{No. of test days}}$$

N= total no of daily counts starting from the date of first germination.

(9) Germination value (GV_2) **(Czabator, 1962)** : GV= MDG x PV

Where Peak Value (PV) is the point at which the cumulative germination % / no of days is a maximum value.

(10) Coefficient of velocity of germination (CVG): **(Elyasi *et al.*, 2014)**

$$= \frac{100 \times \Sigma Ni}{\Sigma NiTi}$$

Where Ni = No. of germinated seeds/day
Ti = No. of days from the start of the experiment.

The experiment was conducted in a completely randomized design. Mean and one-way ANOVA were calculated using SPSS (version 22) software. The mean separations were carried out using Duncan's multiple range tests (DMRT) **(Duncan, 1955)** and significance was determined at $p \leq 0.05$. Graphs and statistical tables were drawn using Excel and Word programs.

Result and discussion:-

The propagation of plants by seeds is an easy, fast and mostly preferred because it preserves genetic variations. However, seeds of many plant species exhibit dormancy and fail to germinate even in favourable conditions. Depending on the plant species and type of dormancy, various treatments are used to break dormancy. In the present study, to break *Pyrus malus* seed dormancy, different germination experiments for different time durations were carried out to investigate the effect of plant growth regulators (GA_3, KNO_3), cold stratification on germination response of Apple seeds. Despite of being one of the most preferred and economically important fruit tree crops, so far, there is not much documentation on *Pyrus malus* seed germination, therefore, much more insights are needed on this aspect for large scale propagation of this economically important tree.

Seed germination is defined as the process associated with the progress of a seed from imbibition through radical emergence and is controlled by environmental factors such as light, temperature, moisture and the availability of oxygen as well as by physiological processes **(Holdsworth *et al*., 2008; Nonogaki *et al*., 2007; Finch-Savage and Leubner-Metzger, 2006; Bewley and Black, 1978, 1982)**.

Dormancy is considered as an important component of plant fitness and is defined as a set of internal blocks imposed upon processes cardinal for growth and development. It is a relative term as it is observed only under certain environmental conditions **(Come, 1980; Lewak, 2011 and Graeber *et al*., 2012)**. The blocks are removed by pre-treatments such as after ripening, chilling, brief exposure to light, and exogenous growth regulators. Deep physiological endogenous dormancy arises when embryos, isolated from seed coat do not germinate or form dwarf abnormalities. Long chilling, cold stratification is required to remove this.

In testa imposed or seed coat dormancy causes inhibition of O_2 and water primarily due to the structures enclosing the embryo. An inhibiting chemical present in the epidermis or adjacent interior membranes is the primary cause of this type of dormancy. Such seeds germinate on the onset of favourable weather conditions only. Embryonic dormancy described as cryogenic endodormancy by **Lang(1987)**, where the control of dormancy resides within the embryo itself. It is also removed completely by chilling conditions. When the temperature goes too high ($>25^0$C) some seeds do not germinate even if returned to their original optimum temperature. Such seeds pass into a second dormant stage called secondary dormancy also called thermodormancy after facing primary phase. Exogenous growth regulators and cold stratification is must to remove this condition **(Lewak, 2011; Baskin and Baskin, 2004; Bewley and Black, 1994;Lang, 1987; Visser, 1954).**

The results of ANOVA showed that there were significant differences (at 5% level) between effective treatments on germination characteristics and the different treatments resulted in significant differences among germination properties (**table 1**). Also, in the control none of the seeds germinated within the stipulated time (i.e. 30 days) so we discarded the control results while carrying calculations on SPSS software.Least significant differences (LSD) at $P<0.05$ level of probability was determined after analysis of variance (ANOVA) for all treatments.

Seed Viability:-

As shown in **figure 1(A)**, 95% seeds were viable.

Effect of stratification, different concentration of KNO_3, GA_3 and their combination for different time duration on FGP, MGT, GRI, CGRI, GV, MDG, GSP, GS/day and CVG:-

FGP:-

Seed germination of any plant species is checked by specific growth promoting and inhibiting compounds and there is a strong correlation among hormones used, their concentration, developmental stage and metabolic activities

(Bewley and Black, 1994; Choudhary *et al*., 1996; Hartmann *et al*., 1997; Pedroza-Manrique *et al*., 2005). **Kucera *et al*., (2005)** and **Finch-Savage W.E. *et al*., (2007)** proposed two functions of GA_3. Firstly, growth potential of seed was enhanced due to GA_3 treatment. Secondly, GA_3 weakens the tissues surrounding radicle by overcoming the mechanical barrier posed by seed outer layer coverings. In the present study, GA_3 when applied singly; a significant increase in germination percentage was seen and when it was used in combination with other components; an additive effect of both the reagents was found.

Plant growth promoters have been found to improve the germination of many other species also **(Butola and Badola, 2004; Pradhan and Badola, 2010a,2010b; Dhoran and Gudadhe, 2012; Hu *et al*., 2012).** As predicted from the results shown in **(table 2 and table 3)**, at 72 hrs there was remarkable decline in seed germination percentage on increasing KNO_3 concentration from 0.1% to 0.3% and decreasing the GA_3 concentration from 1000 ppm to 250 ppm (**table 2 and figure 2**). 48 hrs was regarded as the best time duration and proved to be effective among the other two time factors used in all the treatments. Similar is the case with different combinations of GA_3 and KNO_3 used. FGP varied from 6.667% to 83.333%. 1000 ppm GA_3 + 0.3% KNO_3 combination for 48 hrs showed maximum FGP response. The nitrates and gibberellins have been widely used to overcome seed dormancy(**Nadjafi *et al*., 2006; Çirak *et al*., 2007**).

Cold treatment was not found as effective as were GA_3 and KNO_3. But there was a sharp rise in FGP on going from 24 hrs to 72 hrs during stratification process. Breaking seed dormancy through cold pre-treatment has been confirmed in previous studies such as *Capparis ovate***(Olmez *et al*., 2006)**, *Ferula ovina***(Amooaghaie, 2009)**, *Pinus roxburghii***(Ghildiyal *et al*., 2009)** and researchers conveyed that this might be due to increase in the level of organic phosphates like fructose 2,6- bisphosphate **(Bewley and Black, 1994)**, ATP **(Noland and Murthy, 1984)**, nucleotides **(El-Dengawy, 1997)** and Gibberellic acid (GA).

Ogawa *et al*., (2003) stated that GA_3ox1, a rate limiting GA biosynthesis gene **(Yamauchi *et al*., 2004)** is induced during the process causing the expansion of radical/hypocotyl region cortex cells, thus, generating the potential for seed to enter into the germination phase. **Penfield *et al*., (2005)** further elaborate the role of GA_3ox1 gene in germination process. According to the author, SPT and PIL5 are negative regulators where SPT loses its activity after stratification and PIL5 represses germination in the dark but does not respond to lower temperature. Both SPT and PIL5 act by inhibiting the GA biosynthesis genes such as GA_3ox1 and also GA_3ox2 **(Yano *et al*., 2013).**

Similarly, **Majeed *et al*., (2010)** while studying seed dormancy in *Aesculus indica* reported that time factor was directly proportional to germination percentage if cold pre-treatment was given to seeds. This further confirm our results. **Mughal and Shoaib, (2010)** also overcomed physiological dormancy by giving a 4 week cold stratification treatment to *Fraxinus floribunda* seeds.

In this research, 0.1% KNO_3 was regarded as the best treatment for parameters like CVG, GS/Day, GSP, GV, GRI, CGRI and MGT. Several other studies also reported the similar results in *Citrullus colocynthis*, *Foeniculum vulgare*, *Cuscuta epithymum* and *Calotropis persica*. Positive effect of KNO_3 could be due to its role in decreasing the ABA sensitivity of imbibed seeds **(Bethke *et al*., 2006)** and this is achievedthrough the N-end rule pathway with the help of two components PROTEOLYSIS 6(PRT 6) and arginyl –tRNA: protein arginyltransferase(ATE) **(Holman *et al*., 2009).**
Furthermore, our findings confirmed the statement of **Shanmugavalli *et al*.,(2007)** who treated the sorghum seeds with GA_3 (100 ppm) in combination with 0.5%, 1% and 1.5% KNO_3 and obtained a germination percentage of 94%. Similarly, **Amri (2011)** observed in *Terminalia sericea* a good value of germination percentage(67%). **Liopa-Tsakalidi and Barouchas(2011)** stated that germination of Chervil seeds treated with 200, 500 and 1000 ppm GA_3 concentration is significantly higher than simply sowing in water. This further validated our results.

Similarly, **Dewir *et al*.,(2011)** supports the fact that 1% KNO_3 and 500 ppm GA_3 gave the best germination percentage for the seeds of *Sabal palmetto*. Different other workers (**Ganai and Nawchoo, 2002; Shivkumar *et al*., 2006; Giri and Tamta, 2012)** while working on their respective areas reported GA_3 as a potent growth hormone regulator for breaking dormancy of seeds.

MGT:-
Plant growth regulators when applied in minute quantities show their positive effect by enhancing the germination percentage and reducing the MGT. The best treatment with the lowest value of MGT(15.324) was attributed to the

seeds treated with 250 ppm GA_3+0.1% KNO_3 for 24 hrs (**table 1and figure 3**). Highest MGT(25.533) was found in 24 hrs cold stratified seeds. Our results for this parameter were totally consistent with the results obtained by **Ganaie *et al*.,(2011)** who reported low values of MGT in seeds treated with different concentration of KNO_3 and 25 ppm GA_3. **Gashi *et al*.,(2012)** also reported low values of MGT (10.02) for *R.nathaliae* seeds treated with 500 ppm GA_3+0.1% KNO_3.

GRI, CGRI:-

The application of treatments with different concentration of GA_3 and KNO_3 as well as their combination for seeds had significant differences for GRI and CGRI between treatments.Highest value of GRI(5.269) and CGRI(6.721) was obtained in treating seeds with 0.1% KNO_3 for 48 hrs. **Dewir *et al*.,(2011)** reported the same fact that 0.1% KNO_3 significantly increased the GRI, CGRI values of *Sabal palmetto* palms.

GV_1 and GV_2:-

Germination value calculated according to Djavanshir rule was much higher than examined by Czabator's formula. Highest GV_1(16.106) and GV_2(8.985) were obtained in seeds treated with 0.1% KNO_3 for 48 hrs.

MDG:-

2.778 was assigned as the highest MDG value for seeds treated with combination of 1000 ppm GA_3+0.3% KNO_3 for 48 hrs. Lowest value of MDG(0.222) was obtained for seeds stratified for 24 hrs.

GSP:-

Highest (42.564) value found for 0.1% KNO_3 treated seeds for 24 hrs.

GS/Day:-

2.635 was assigned as the highest value to 0.1% KNO_3 treated seeds for 48 hrs.

CVG:-

250 ppm GA_3+0.1% KNO_3 combination was found as the best treatment given to seeds for 24 hrs as it gave highest value of CVG(6.859).

Correlation Analysis:-

From the correlative analysis of treatments according to Pearson (two-tailed) test, the relationship between seed germination in *P. malus* and different time intervals was determined. On the basis of correlation matrix, it was found that final germination percentage (FGP) as well as mean daily germination (MDG) had a significant positive correlation ($p<0.01$; r= 1) with the time factors (24, 48 and 72 hrs) indicating that germination in *P. malus* was favoured as the time factor increases from 24 hrs to 48 hrs but above 48 hrs a negative impact was seen as evident by the **table 4**.

This negative number clearly depicts that sowing of seeds on combination of 1000 ppm GA_3+0.3% KNO_3 for more than 48 hrs hampers the germination process thereby lowering the germination percentage and mean daily germination values. Similarly, mean germination time (MGT) and coefficient of velocity of germination (CVG) values are significant at ($p<0.05$; r= 0.99) level as shown by the correlation matrix. 250 ppm GA_3+0.1% KNO_3 gave best result at 24 hrs but above 24 hrs the value decreases slightly. In addition, a positive correlation was seen with the rest of the parameters (GV1, GS/day, GSP, GRI and CGRI) and time factors but the values were not as much significant.

Figure 1:-Effect of pre-sowing treatments on seed germination in *Pyrus malus* cv. Red delicious. (A): Viability test of seeds checked by soaking in water, arrow indicate viability difference between non-viable (1) and viable (2) seeds. (B) & (C): inoculation of seeds after pre-sowing treatments and seedling emergence response after 1 week and 30 days of incubation. (D): hardening of emerged seedling. (E) & (F): well rooted plant obtained after 45 days of inoculation.

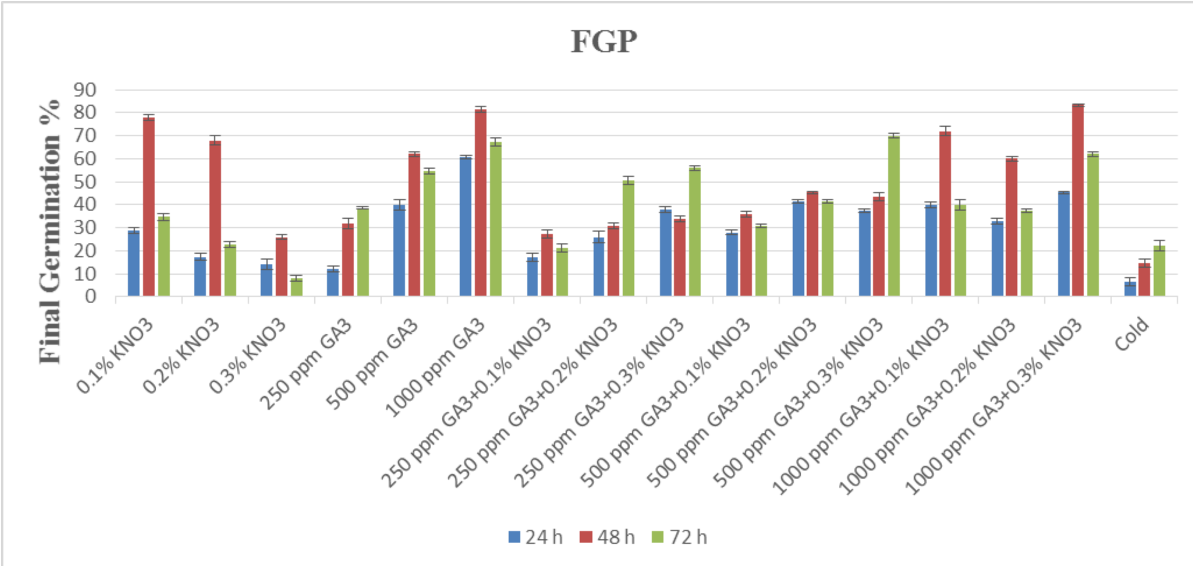

Figure 2:-Effect of different concentrations of GA_3, KNO_3 and cold on the final germination percentage.

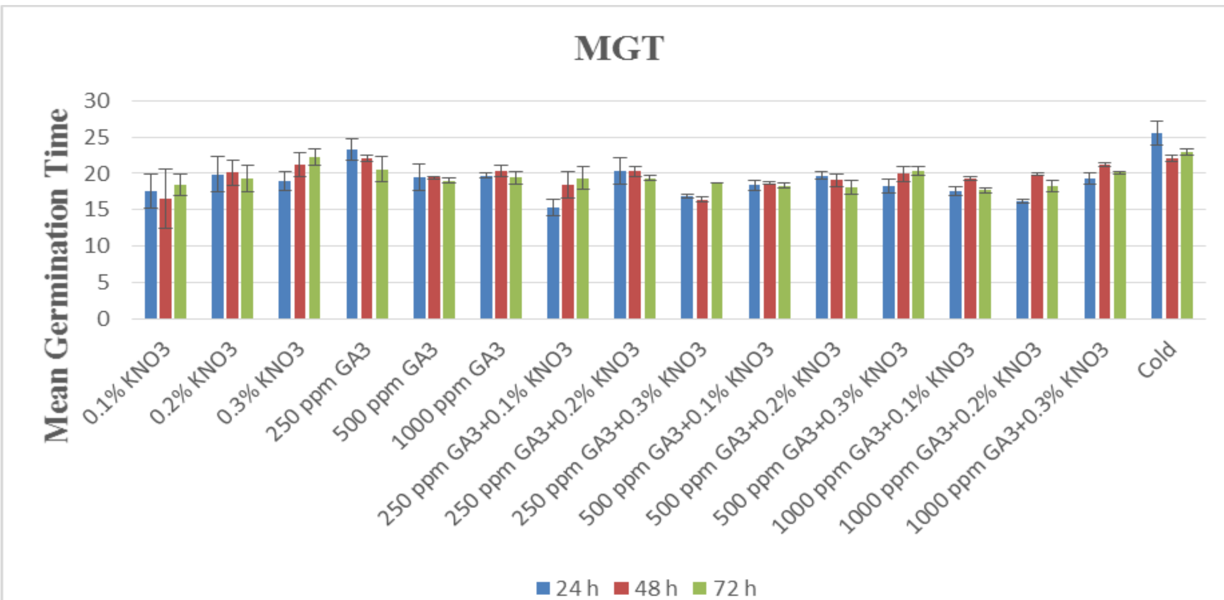

Figure 3:-Effect of different concentrations of GA_3, KNO_3 and cold on the mean germination time.

Table 1:-ANOVA Summary.

Germination Properties	Source of Variation	Degree of Freedom	Sum of Squares	Mean of Squares	F-value	LSD (p<0.05)
FGP	Between groups	47	55157.306	1173.56	183.688	8.20
	Within groups	96	613.333	6.389		
	Total	**143**	**55770.639**			
MGT	Between groups	47	510.16	10.854	2.38	0.07
	Within groups	96	437.889	4.561		
	Total	**143**	**948.049**			
GRI	Between groups	47	173.729	3.696	16.69	0.02
	Within groups	96	21.262	0.221		
	Total	**143**	**194.99**			
CGRI	Between groups	47	40.11	0.853	1.133	0.005
	Within groups	96	72.318	0.753		
	Total	**143**	**112.428**			
GV1	Between groups	47	1813.485	38.585	12.438	0.269
	Within groups	96	297.817	3.102		
	Total	**143**	**2111.302**			
MDG	Between groups	47	61.283	1.304	183.541	0.009
	Within groups	96	0.682	0.007		
	Total	**143**	**61.965**			
GV2	Between groups	47	643.108	13.683	28.08	0.095
	Within groups	96	46.779	0.487		
	Total	**143**	**689.887**			
GSP	Between groups	47	8661.748	184.293	3.448	1.288
	Within groups	96	5130.535	53.443		
	Total	**143**	**13792.283**			
GS/DAY	Between groups	47	43.434	0.924	16.69	0.006
	Within groups	96	5.316	0.055		
	Total	**143**	**48.75**			
CVG	Between groups	47	34.405	0.732	2.14	0.005
	Within groups	96	32.846	0.342		
	Total	**143**	**67.252**			

Table 2:-Mean of FGP, MGT, GRI, CGRI, GV1, MDG, GV2, GSP, GS/DAY and CVG for seeds ofRed deliciousunder varying concentration of GA_3(in ppm), KNO_3(in %) and cold at different time durations. Mean in each column followed by same letters are not significantly different at the P(0.05) level using Duncan test.

Treatments	Conc.	hr.	FGP	MGT	GRI	CGRI	GV1	MDG	GV2	GSP	GS/DAY	CVG
KNO_3	0.001	24	28.667 ^{hij}	17.563 ^{abc}	1.819 ^{fghijkl}	6.272 ^{bcd}	2.170 ^{abcdef}	0.956 ^{hij}	1.584 ^{cdefghij}	42.564 ^{i}	0.909 ^{fghijkl}	5.871 ^{efgh}
		48	78.000 ^{x}	16.539 ^{ab}	5.269 ^{u}	6.721 ^{d}	16.106 ^{o}	2.600 ^{x}	8.985 ^{r}	10.261 ^{abcd}	2.635 ^{u}	5.844 ^{efgh}
		72	34.667 ^{klm}	18.447 ^{abcde}	2.139 ^{hijklm}	6.154 ^{bcd}	2.905 ^{abcdefgh}	1.156 ^{klm}	1.592 ^{cdefghij}	15.509 ^{abcde}	1.070 ^{hijklm}	5.505 ^{bcdefgh}
	0.002	24	17.333 ^{de}	19.900 ^{bcdefg}	1.003 ^{abcdef}	5.655 ^{abcd}	0.675 ^{abcd}	0.578 ^{de}	0.383 ^{abcd}	19.167 ^{abcdefg}	0.501 ^{abcdef}	5.195 ^{bcdefg}
		48	68.000 ^{vw}	20.130 ^{bcdefg}	3.779 ^{qrst}	5.535 ^{abcd}	9.314 ^{lmn}	2.266 ^{vw}	5.523 ^{op}	8.928 ^{abcd}	1.890 ^{qrst}	5.047 ^{abcdefg}
		72	22.667 ^{fg}	19.267 ^{abcdefg}	1.300 ^{cdefgh}	5.708 ^{abcd}	1.135 ^{abcde}	0.756 ^{fg}	0.681 ^{abcdef}	23.333 ^{cdefgh}	0.650 ^{cdefgh}	5.298 ^{bcdefg}
	0.003	24	14.000 ^{cd}	18.983 ^{abcdefg}	0.817 ^{abcde}	5.856 ^{bcd}	0.461 ^{abcd}	0.467 ^{cd}	0.295 ^{abc}	26.561 ^{efgh}	0.408 ^{abcde}	5.314 ^{bcdefg}
		48	26.000 ^{gh}	21.280 ^{cdefg}	1.348 ^{cdefghi}	5.159 ^{abcd}	1.204 ^{abcde}	0.867 ^{gh}	0.817 ^{abcdefg}	15.446 ^{abcde}	0.674 ^{cdefghi}	4.761 ^{abcde}
		72	8.000 ^{ab}	22.261 ^{efgh}	0.380 ^{ab}	4.656 ^{abc}	0.104 ^{a}	0.267 ^{ab}	0.091 ^{a}	32.777 ^{ghi}	0.190 ^{ab}	4.518 ^{abcd}
GA_3	250 ppm	24	12.000 ^{bc}	23.281 ^{gh}	0.536 ^{abc}	4.529 ^{ab}	0.172 ^{ab}	0.400 ^{bc}	0.177 ^{ab}	23.651 ^{defgh}	0.268 ^{abc}	4.336 ^{ab}
		48	32.000 ^{ijkl}	22.031 ^{defgh}	1.545 ^{defghij}	4.833 ^{abc}	1.515 ^{abcdef}	1.067 ^{ijkl}	1.150 ^{abcdefghi}	8.399 ^{abc}	0.773 ^{defghij}	4.542 ^{abcd}
		72	38.667 ^{mnop}	20.539 ^{bcdefg}	2.004 ^{ghijkl}	5.192 ^{abcd}	2.624 ^{abcdefg}	1.289 ^{mnop}	1.838 ^{efghij}	8.684 ^{abcd}	1.002 ^{ghijkl}	4.944 ^{abcdef}
	500 ppm	24	40.000 ^{nop}	19.481 ^{abcdefg}	2.195 ^{hijklmn}	5.492 ^{abcd}	3.201 ^{abcdefgh}	1.333 ^{nop}	2.039 ^{fghij}	8.367 ^{abc}	1.098 ^{hijklmn}	5.240 ^{bcdefg}
		48	62.000 ^{u}	19.405 ^{abcdefg}	3.333 ^{pqrs}	5.372 ^{abcd}	7.397 ^{jklm}	2.067 ^{u}	4.288 ^{mno}	9.684 ^{abcd}	1.667 ^{pqrs}	5.153 ^{bcdefg}
		72	54.667 ^{rs}	19.020 ^{abcdefg}	2.986 ^{mnopq}	5.456 ^{abcd}	5.944 ^{ghijk}	1.822 ^{rs}	3.453 ^{klm}	10.989 ^{abcd}	1.493 ^{mnopq}	5.262 ^{bcdefg}
	1000 ppm	24	60.667 ^{u}	19.721 ^{bcdefg}	3.218 ^{opqr}	5.307 ^{abcd}	6.827 ^{ijkl}	2.022 ^{u}	4.140 ^{lmn}	9.892 ^{abcd}	1.609 ^{opqr}	5.074 ^{abcdefg}
		48	81.333 ^{xy}	20.288 ^{bcdefg}	4.304 ^{t}	5.279 ^{abcd}	12.045 ^{n}	2.711 ^{xy}	7.504 ^{q}	8.175 ^{ab}	2.152 ^{t}	4.944 ^{abcdef}
		72	67.333 ^{v}	19.443 ^{abcdefg}	3.732 ^{qrst}	5.524 ^{abcd}	9.066 ^{klmn}	2.244 ^{v}	5.194 ^{nop}	9.876 ^{abcd}	1.866 ^{qrst}	5.166 ^{bcdefg}
Cold (4^oC) stratification		24	6.667 ^{a}	25.533 ^{h}	0.280 ^{a}	4.038 ^{a}	0.057 ^{a}	0.222 ^{a}	0.058 ^{a}	34.444 ^{hi}	0.140 ^{a}	3.947 ^{a}
		48	14.667 ^{cd}	22.013 ^{defgh}	0.685 ^{abcd}	4.679 ^{abc}	0.318 ^{abc}	0.489 ^{cd}	0.254 ^{abc}	17.725 ^{abcdef}	0.342 ^{abcd}	4.546 ^{abcd}
		72	22.000 ^{fg}	22.997 ^{fgh}	0.993 ^{abcdef}	4.496 ^{ab}	0.649 ^{abcd}	0.733 ^{fg}	0.571 ^{abcde}	9.298 ^{abcd}	0.496 ^{abcdef}	4.352 ^{abc}

Table 3:- Mean of FGP, MGT, GRI, CGRI, GV1, MDG, GV2, GSP, GS/DAY and CVG for seeds of Red deliciousunder combination of varying concentrations of GA_3(in ppm) and KNO_3(in %) at different time durations. Mean in each column followed by sameletters are not significantly different at the P(0.05) level using Duncan test.

Treatments	Conc.	hr.	FGP	MGT	GRI	CGRI	GV1	MDG	GV2	GSP	GS/DAY	CVG
GA_3 + KNO_3 (250 ppm+ KNO_3)	0.001	24	17.333 ^{de}	15.324 ^{a}	1.171 ^{bcdefg}	6.638 ^{d}	0.863 ^{abcde}	0.578 ^{de}	0.406 ^{abcd}	30.264 ^{fghi}	0.585 ^{bcdefg}	6.589 ^{h}
		48	27.333 ^{h}	18.483 ^{abcde}	1.654 ^{efghijk}	5.965 ^{bcd}	1.724 ^{abcdef}	0.911 ^{h}	1.004 ^{abcdefghi}	19.206 ^{abcdefg}	0.828 ^{efghijk}	5.528 ^{bcdefgh}
		72	21.333 ^{ef}	19.385 ^{abcdefg}	1.211 ^{bcdefg}	5.602 ^{abcd}	0.969 ^{abcde}	0.711 ^{ef}	0.582 ^{abcde}	12.289 ^{abcde}	0.605 ^{bcdefg}	5.226 ^{bcdefg}
	0.002	24	26.000 ^{gh}	20.385 ^{bcdefg}	1.402 ^{cdefghi}	5.400 ^{abcd}	1.252 ^{abcde}	0.867 ^{gh}	0.819 ^{abcdefg}	10.381 ^{abcd}	0.701 ^{cdefghi}	4.988 ^{abcdefg}
		48	30.667 ^{hijk}	20.307 ^{bcdefg}	1.645 ^{efghij}	5.350 ^{abcd}	1.752 ^{abcdef}	1.022 ^{hijk}	1.051 ^{abcdefghi}	8.631 ^{abc}	0.822 ^{efghij}	4.937 ^{abcdef}
		72	50.667 ^{r}	19.353 ^{abcdefg}	2.752 ^{lmnop}	5.437 ^{abcd}	4.853 ^{fghij}	1.689 ^{r}	2.886 ^{jkl}	13.160 ^{abcde}	1.376 ^{lmnop}	5.170 ^{bcdefg}
	0.003	24	38.000 ^{mno}	16.931 ^{abc}	2.271 ^{ijklmn}	5.959 ^{bcd}	3.357 ^{abcdefgh}	1.267 ^{mno}	1.747 ^{defghij}	17.758 ^{abcdef}	1.135 ^{ijklmn}	5.909 ^{efgh}
		48	34.000 ^{klm}	16.398 ^{ab}	2.085 ^{ghijklm}	6.110 ^{bcd}	2.805 ^{abcdefgh}	1.133 ^{klm}	1.507 ^{bcdefghij}	19.880 ^{bcdefg}	1.042 ^{ghijklm}	6.103 ^{fgh}
		72	56.000 st	18.740 ^{abcdef}	3.101 ^{nopq}	5.528 ^{abcd}	6.235 ^{hijkl}	1.867 st	3.530 ^{klm}	14.424 ^{abcde}	1.551 ^{nopq}	5.336 ^{bcdefg}
GA_3 + KNO_3 (500 ppm +KNO_3)	0.001	24	28.000 ^{hi}	18.360 ^{abcde}	1.611 ^{efghij}	5.723 ^{abcd}	1.700 ^{abcdef}	0.933 ^{hi}	0.957 ^{abcdefgh}	12.113 ^{abcde}	0.806 ^{efghij}	5.465 ^{bcdefgh}
		48	36.000 ^{lmn}	18.655 ^{abcdef}	2.043 ^{ghijkl}	5.677 ^{abcd}	2.723 ^{abcdefg}	1.200 ^{lmn}	1.481 ^{bcdefghi}	9.282 ^{abcd}	1.021 ^{ghijkl}	5.362 ^{bcdefg}
		72	30.667 ^{hijk}	18.314 ^{abcde}	1.772 ^{fghijk}	5.764 ^{abcd}	2.041 ^{abcdef}	1.022 ^{hijk}	1.111 ^{abcdefghi}	10.972 ^{abcd}	0.886 ^{fghijk}	5.464 ^{bcdefgh}
	0.002	24	41.333 ^{opq}	19.710 ^{bcdefg}	2.251 ^{ijklmn}	5.458 ^{abcd}	3.250 ^{abcdefgh}	1.378 ^{opq}	1.918 ^{efghij}	9.683 ^{abcd}	1.125 ^{ijklmn}	5.080 ^{abcdefg}
		48	45.333 ^{q}	19.047 ^{abcdefg}	2.591 ^{klmnop}	5.705 ^{abcd}	4.258 ^{efghij}	1.511 ^{q}	2.395 ^{ijk}	14.624 ^{abcde}	1.295 ^{klmnop}	5.270 ^{bcdefg}
		72	41.333 ^{opq}	18.158 ^{abcde}	2.448 ^{jklmnop}	5.912 ^{bcd}	3.783 ^{cdefghi}	1.378 ^{opq}	2.062 ^{fghij}	19.365 ^{abcdefg}	1.224 ^{jklmnop}	5.538 ^{cdefgh}
	0.003	24	37.333 ^{mno}	18.327 ^{abcde}	2.200 ^{hijklmn}	5.917 ^{bcd}	3.024 ^{abcdefgh}	1.245 ^{mno}	1.814 ^{efghij}	18.031 ^{abcdef}	1.100 ^{hijklmn}	5.486 ^{bcdefgh}
		48	43.333 ^{pq}	19.913 ^{bcdefg}	2.398 ^{jklmno}	5.581 ^{abcd}	3.552 ^{abcdefghi}	1.444 ^{pq}	2.116 ^{ghij}	14.262 ^{abcde}	1.199 ^{jklmnop}	5.047 ^{abcdefg}
		72	70.000 ^{vw}	20.362 ^{bcdefg}	3.758 ^{qrst}	5.369 ^{abcd}	9.014 ^{klmn}	2.333 ^{vw}	5.447 ^{op}	4.765 ^{a}	1.879 ^{qrst}	4.922 ^{abcdef}
GA_3 + KNO_3 (1000 ppm+ KNO_3)	0.001	24	40.000 ^{nop}	17.570 ^{abc}	2.405 ^{jklmno}	6.011 ^{bcd}	3.689 ^{bcdefghi}	1.333 ^{nop}	2.036 ^{fghij}	11.692 ^{abcd}	1.203 ^{jklmnop}	5.703 ^{defgh}
		48	72.000 ^{w}	19.266 ^{abcdefg}	4.047 ^{rst}	5.635 ^{abcd}	10.340 ^{mn}	2.400 ^{w}	5.808 ^{p}	11.128 ^{abcd}	2.024 ^{rst}	5.192 ^{bcdefg}
		72	40.000 ^{nop}	17.698 ^{abcd}	2.481 ^{jklmnop}	6.152 ^{bcd}	3.802 ^{cdefghi}	1.333 ^{nop}	2.133 ^{ghij}	16.616 ^{abcdef}	1.241 ^{jklmnop}	5.654 ^{defgh}
	0.002	24	32.667 ^{jkl}	16.216 ^{ab}	2.082 ^{ghijklm}	6.356 ^{cd}	2.728 ^{abcdefg}	1.089 ^{jkl}	1.434 ^{abcdefghi}	16.471 ^{abcdef}	1.041 ^{ghijklm}	6.171 ^{gh}
		48	60.000 ^{tu}	19.866 ^{bcdefg}	3.262 ^{opqr}	5.438 ^{abcd}	6.772 ^{ijkl}	2.000 ^{tu}	4.008 ^{lmn}	8.896 ^{abcd}	1.631 ^{opqr}	5.034 ^{abcdefg}
		72	37.333 ^{mno}	18.212 ^{abcde}	2.151 ^{hijklm}	5.758 ^{abcd}	2.968 ^{abcdefgh}	1.245 ^{mno}	1.607 ^{cdefghij}	14.327 ^{abcde}	1.075 ^{hijklm}	5.511 ^{bcdefgh}
	0.003	24	45.333 ^{q}	19.370 ^{abcdefg}	2.478 ^{jklmnop}	5.464 ^{abcd}	3.945 ^{defghi}	1.511 ^{q}	2.303 ^{hijk}	11.792 ^{abcd}	1.239 ^{jklmnop}	5.179 ^{bcdefg}
		48	83.333 ^{y}	21.153 ^{cdefg}	4.169 st	5.001 ^{abcd}	11.187 ^{n}	2.778 ^{y}	7.717 ^{q}	6.407 ^{ab}	2.084 st	4.729 ^{abcde}
		72	62.000 ^{u}	20.102 ^{bcdefg}	3.268 ^{opqr}	5.272 ^{abcd}	6.924 ^{ijkl}	2.067 ^{u}	4.274 ^{mno}	8.609 ^{abc}	1.634 ^{opqr}	4.976 ^{abcdefg}

Table 4:- Pearson's correlation coefficients for seed germination parameters among different time durations. CGRI(corrected germination rate index), GRI(germination rate index), GSP(germination speed percentage), GV(germination value), GS/Day(germination speed per day), FGP(final germination percentage), MGT(mean germination time), MDG(mean daily germination) and CVG(coefficient of velocity of germination). *Correlation is significant at the 0.05 level(two-tailed), **Correlation is significant at the 0.01 level(two-tailed).

CGRI				
0.1% KNO3				
		24 hours	48 hours	72 hours
0.1% KNO3	24 hours	1		
	48 hours	0.503	1	
	72 hours	0.402	0.994	1

GRI				
0.1% KNO3				
		24 hours	48 hours	72 hours
0.1% KNO3	24 hours	1		
	48 hours	0.479	1	
	72 hours	0.645	0.98	1

GSP				
0.1% KNO3				
		24 hours	48 hours	72 hours
0.1% KNO3	24 hours	1		
	48 hours	0.015	1	
	72 hours	-0.711	0.692	1

GV1				
0.1% KNO3				
		24 hours	48 hours	72 hours
0.1% KNO3	24 hours	1		
	48 hours	0.473	1	
	72 hours	0.643	0.979	1

GS/Day				
0.1% KNO3				
		24 hours	48 hours	72 hours
0.1% KNO3	24 hours	1		
	48 hours	0.479	1	
	72 hours	0.645	0.98	1

FGP				
1000 ppm GA3+ 0.3% KNO3				
		24 hours	48 hours	72 hours
1000 ppm GA3+ 0.3% KNO3	24 hours	1		
	48 hours	1.000**	1	
	72 hours	-0.866	-0.866	1

MGT				
250 ppm GA3 + 0.1% KNO3				
		24 hours	48 hours	72 hours
250 ppm GA3 + 0.1% KNO3	24 hours	1		
	48 hours	0.891	1	
	72 hours	0.865	0.998*	1

MDG				
1000 ppm GA3 + 0.3% KNO3				
		24 hours	48 hours	72 hours
1000 ppm GA3 + 0.3% KNO3	24 hours	1		
	48 hours	1.000**	1	
	72 hours	-0.866	-0.866	1

CVG				
250 ppm GA3+0.1% KNO3				
		24 hours	48 hours	72 hours
250 ppm GA3+0.1% KNO3	24 hours	1		
	48 hours	0.924	1	
	72 hours	0.906	.999*	1

Conclusion:-

To ensure a maximum number of quality seedlings with minimum cost, time and labor, considerable efforts are being adopted by nursery practicers and farmers before the seeds are sown in fields. Despite these efforts choosing the right tools can often be a great challenge as the selection of wrong choices may cost significant amount of money. Since the seeds of *Pyrus malus* cultivar Red delicious exhibit dormancy and require a lot of time to germinate with low germination percentage so, pre-sowing treatments would be an added advantage in the practical fields. Our study clearly depicted that germination percentage can be increased by giving a pre-sowing treatment to seeds with a combination of 1000 ppm GA_3+0.3% KNO_3 for upto 48 hrs. This is by-far the most effective and simplest method that could be easily adopted by the farmers for improving and enhancing the economic cultivation of this variety.

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Authors' contributions:-

CJ: carried out the whole experimental study and interpretation of data. **RSK**: helped in sample collection. **TKN**: participated in the designing of study and drafting of manuscript.All authors read and approved the final manuscript.

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