Studies On Apple Seed Germination And Survival Of Seedlings As Affected By Gibberellic Acid Under Cold Arid Conditions.

Rayees A. Wani, Tanveer H. Malik, A. R. Malik, Jahangir A Baba, N. A. Dar

Abstract: Ladakh, the land between earth and sky, is known for its remoteness, difficult terrain and short agriculture season. Nearly 50% of its total land lies between 16500 and 19500 feet above mean sea level. It is virtually unfit for human life and vegetation. The experiment was undertaken with an objective to investigate the effect of various concentrations of plant growth regulator (GA_3)) under cold arid conditions to standardize the procedure for seed germination of Apple (Malus x domestica Borkh.) so as to increase the dearth of apple seedling plants which can easily acclimatize under these tough conditions with better survival for maximum benefit to nursery growers and orchardists. One of the effects of cold arid conditions is the reduction in seed germination, seedling establishment (survival) and seedling growth but research reveals that pretreatment with growth regulators improves some aspects of seed germination and seedling establishment. Hence to investigate the effect of pretreatment with Gibberellic acid on the seed germination and survival of apple seedlings as well as to standardize the technique under cold arid conditions, the experiment was conducted in Horticulture research field of High Mountain Agriculture Research Institute Leh, Ladakh India. The study clearly indicated that apple seed treated with GA_3 500 ppm for 40 hours gave best response regarding seed germination, better growth of seedlings and survival of saplings.

Key words: Apple, Gibberellic acid, Growth, survival, seed germination, seedling establishment, water stress, cold arid conditions.

Introduction

Seed germination is a complex physiological process that it is response to environmental signals such as water potential, light, nitrate and other factors .Poor seed germination is the major limiting factor of Apple (Malus x domestica Borkh.) for large scale production and cultivation under cold arid conditions. Seed germination in general can be controlled by many factors like natural germination (growth) inhibitors (Taiz *et al*., 1991). These are the derivatives of benzoic acid, cinnamic acid, coumarin, naringenin, jasmonic and abscisic acid (ABA). It has been postulated that seed coat (testa) of many plant species contains considerable amount of germination inhibitor, which prevent their seed germination (EL-Barghathi *et al*., 2005). The first stage of germination consists of ingesting water and an awakening or activation of the germplasm. Protein components of the cells that were formed as the seed developed became inactive as it matured.

- *Corresponding author: Currently posted at Dry land (Krewa) Agriculture research station Budgam, SKUAST-K Srinagar India.*
- *Second author: Currently posted at Department of Agriculture, Jammu and Kashmir India.*
- *Third author: Currently posted at Faculty of Forestry Wadoora, SKUAST-K Srinagar India.*
- *Fourth author: Currently posted as High Mountain Agriculture Research Institute Leh Ladakh, SKUAST-K Srinagar India.*
- *Fifth author: Currently posted at Saffron Research Station Konibal, SKUAST-K Srinagar India.*

Control is exercised by four classes of plant hormones: inhibitors such as abscisic acid which block germination; auxins which control root formation and growth; the gibberellins which regulate protein synthesis and stem elongation; and cytokinins that control organ differentiation. Gibberellic acid (actually a group of related substances called gibberellins) was discovered as a metabolic byproduct of the fungus *Gibberella fujikuroi.* Gibberellic acid (GA3) is known to be concerned in the regulation of plant responses to the external environment (Chakrabarti *et al*., 2003). Recent studies have revealed that cold stratification has a direct effect on production of gibberellins (GAs) in seeds of *Arabidopsis thaliana* (Yamaguchi *et al*., 2000, 2002**)**. Exogenously applied GA_3 overcomes seed dormancy in several species (Baskin and Baskin, 1998; Hartmann et al., 1997) and promotes germination in some species that normally require cold stratification, light, or after-ripening (Bewley *et al*., 1994). GA promotes the production of enzymes such as endo-β-mannanase, which loosen cell walls in the endosperm, thereby reducing resistance to radicle emergence (Yamaguchi *et al*., 2002**)**. In some temperate species, dormancy is broken by a period of warm temperatures followed by cold stratification. This response is most often associated with morpho-physiological dormancy (*et al*., 2005); however, seeds with morpho-physiological dormancy have underdeveloped embryos (*et al*., 2005).In order to accelerate this method, it can be combined with some treatments such as chemical applications or mechanical seed coat removal (Mehanna *et al*., 1985; Martinez-Gomez *et al*., 2001). Many investigators have studied the effects of exogenous growth regulators on seed germination. Gibberellins eliminated the chilling requirements of peach and apple seeds and increased their germination (Mehanna *et al*., 1985**)**. Temperate fruit production in India is based mainly on apple. In India apple ranks 5th in production and roughly occupies 4.5% of fruit area in the country. The apple is mostly cultivated in Jammu and Kashmir followed by Himachal Pradesh and Uttaranchal and Jammu Kashmir alone accounts for 60% of its production in India .Since more than 60% area of Jammu and Kashmir state falls in Ladakh region hence this study was undertaken to extend and popularize its cultivation in this vast area with prime objective to increase the seedling rootstocks of apple so that it can establish easily and survival rate is improved for increasing the apple fruit production in this nontraditional area. Sexually raised plants are long lived, have extensive and powerful root system with wide and deep distribution in the soil which accounts for persistent and adequate annual growth of absorbing roots thus helping them acclimatize easily in the environment. Also rootstocks exhibit a great effect on production efficiency yield quality, adaptability, tree vigor, impart resistance to biotic (diseases and insects/pests) and aboitic stresses (salt tolerance and water logging) of scion cultivar with the main function of seedling rootstocks is to provide anchorage by growing deep in to the soil and also regulate uptake of moisture and nutrients. Hence this study was under taken to standardize apple seed germination with regard to different concentrations of gibberellic acid so this large number of apple fruit trees may be established in this nontraditional area of Ladakh India for commercial orcharding and get huge returns of apple fruit production in this remote area of India under cold arid conditions for the benefit of country in general and farmers in particular. Chemically induced germination of unstratified apple (*Malus x domestica* Borkh.) has two possible uses. First, by eliminating stratification one might obtain seedlings immediately after seed maturation. Second, wide familial variation in germination time might be reduced by chemically treating either partially stratified or unstratified seed. Many investigators have studied the effects of exogenous growth regulators on seed germination. The application of gibberellins increases the seed germination percentage by increase of the amino acid content in embryo and they cause release of hydrolytic enzyme required for digestion of endospermic starch when seeds renew growth at germination. Gibberellic acid acts synergistically with auxins, cytokinins and probably with the other plant hormones; what might be called a system approach, or synergism. The overall development of plant is regulated by the growth hormones, nutrient and environmental factors. They also vary in their germination requirement (Chauhan *et al*., 2009). It is not known in which concentrations these hormones will cause a response in the cell. This investigation with growth hormones will help in determining which of the hormonal concentration is suitable for seed germination and proper seedling growth. Hence the main objective of this study was carried out to find out the effect of GA_3 on germination of apple seed, their subsequent growth and survival of seedlings. In view of the above back ground, the present investigation was undertaken to study the influence of growth substances like Gibberellic acid (GA3) on seed germination, radical and plumule elongation; to draw the information of timing and control of seed germination and seedling growth of a species in their natural habitat.

Materials and Methods

The experiment was conducted in the Horticulture laboratory of High Mountain Agriculture Research Institute Leh Ladakh in 2009-10 by following completely randomized design with three replications. Three concentrations of GA_3 (450, 500 and 550 ppm) were taken into consideration by long dip method for 30 hrs, 35 hrs and 40 hrs. In this experiment, seeds of all treatments were placed on filter paper moistened with 3% fungicide solution (Captan) in Petri dishes were placed in an incubator at 21±1℃ and 70–80% humidity with darkness. Germination was measured in 3-day intervals during 30 days. All seeds with at least a 5 mm long radicle were considered as germinated. Germination percentage per treatment was calculated as the average of three replicates with 50 seeds. Percentage data of germination were subjected to the angle transformation and the analysis of variance was performed. After long dipping, seeds were sown in the pots in the month of March. Observations on germination of seeds, length of seedlings, diameter of seedling, number of leaves per seedling, length and width of leaves, length and diameter of root and survival of seedling were recorded and compared with control statistically. These were soaked for 30, 35 and 40 h in the above concentrations as per treatment combination and only double distilled water for the control set. Seed germination percentage was determined by the method given by Dahiya *et al*., (2007), after 14 days of treatment. Mean values were subjected to analysis of variance (ANOVA) to test the significance for germination percent as per the methodology advocated by Panse *et al*., (1967).

Table 1. The treatments of GA3, with their different concentrations

Growth hormone	Concentration (ppm)	Seed soaked time (h)	Treatments
	-	-	T_0 (Control)
	450 ppm	30	T_1
		35	T_2
		40	T_3
GA_3	500 ppm	30	T_4
		35	T_5
		40	T_6
	550 ppm	30	T_7
		35	T_8
		40	T_9

The concentrations of the test solutions used for the treatment were 450 ppm, 500 ppm, 550 ppm and 40 ppm for Gibberellic acid with a separate control check using the distilled water treatment (Table 1).

Results and discussion

The analysis of variance (ANOVA) of germination data from growth regulators treated seeds indicated that they were all statistically significant ($P<0.05$). The significant critical difference (CD) values indicate that apple seeds *were* suitable for the treatments (see table 2). The results obtained showed that application of at all concentrations influenced germination of seeds as well as growth and development of seedlings. Maximum number of germinated seeds (9.66) was found with 500 ppm GA_3 for 40 hrs of seed treatment (table 2 and graph 1**)**. Mehanna *et al*., (1985) reported that internal GA percentage is at a high level, but the proportion of ABA is at a low level in dormant seeds. While GA in the structure increases the enzymatic activity, it slows the ABA activity. Gibberellins showed to increase germination in several species (Carrera *et al*., 1988; Giba *et al*., 1993; Karam *et al*., 2001) and to overcome physiological dormancy in seeds with dormant embryos (Hartmann *et al*., 1997). Seed dormancy may be caused by an inadequate development of embryo and/or an existence of chemical inhibitors (Karam *et al*., 2001). In addition, physiological dormancy in seeds is closely related to the proportion between inhibitors (especially ABA) and growth regulators (especially gibberellins) (Hartmann et al. 1997). This was found in consistent with the finding of Gercekcioğlu *et al*., (1999) and Carrera *et al*., (1988) and Chakrabarti *et al*., (2003). The application of plant growth regulator could increase the seed germination and other physiological activity by the reason of tolerance to the toxic effects/particles which was found in consonance with the finding of Haroun *et al*., (1991) and Hoque *et al*., (2002). Although varied in seed germination and root-shoot elongation by different treatments, the pre-soaking with different treatments was evident that soaked seed could improve in germination and seedling establishment and this observation was found equivalent the observation of Harris *et al*.. (1999). Same results were obtained by Mohanty *et al*., (2006) while working on Black gram. Treatment of seed with GA_3 500 ppm for 40 hrs before sowing also increased the length of seedlings (8.94 cm) see table 2 and graph 2, because additional 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. Increase in plant growth due to gibberellic acid treatment has also been found by Parera *et al*., (1994) and Lee *et al*., (1998). GA_3 could overcome the adverse effects in the seed physiological activity of apple seedling growth, which supports the finding of Chakrabarti *et al*., (2002), Mikulik *et al*., (2002) and Roychowdhury *et al*., (2011). The number, length and width of the leaves also increased in treatment T_6 (500 ppm GA_3 for 40 hrs) as shown in table 2. The diameter of main stem increased from 0.27 (control) to 0.44 cm by the treatment of seed with GA_3 500 ppm for 40 hrs. With the more effectiveness of low concentration of GA3 (that is ratio of growth hormone and water) could restore retardation in water content; this may be able in tolerance to water stress. This result was considered in parallel to the findings of Shnmungavelu *et al*., (1970), Powell *et al*., (1987), Lee *et al*., (1998), Parera *et al*., (1994), Hoque *et al*., (2002) and Ashraf *et al*., (2005). The length of root also increased from 9.03 cm in control to 15.03cm in the treatment with GA_3 500 ppm for 40 hrs (see table 2, graph 2).Here again linear increase in length of root was noted for every increase in concentration of GA_3 and duration of treatment of seed before sowing. It is but natural that when the treatment has boosted the growth of seedling above ground, it might have given the same effect below ground *i.e*. in the roots. The diameter of root at the base of its origin was 0.14 cm in control and 0.25 cm in the treatment T_6 (500 ppm GA_3 for 40 hrs). Here again the lower concentration and reduced duration of treatment have linearly decreased the thickness of root which is similar with findings of Copeland *et al*., (2001), Khan *et al*., (1992) and Ruan *et al*., (2002) and Pasalar *et al*., (2011). It is more important to note that survival of seedling is more important thing. Weaker seedlings are more susceptible to disease and adverse weather conditions than stronger seedlings. Survival of seedling was 4 out of 10 in control which increased to 9.66 out of 10 in T_6 (500 ppm GA_3 for 40 hrs). Here again the survival of seedlings simultaneously decreased with every decrease in the concentration of GA_3 and duration of treatment of seed before sowing which is in agreement with Singh *et al*., (1989) and Mueungu *et al*., (1989). The role of plant growth regulators in overcoming the harmful effects on growth may be due to the change in the endogenous growth regulators (Izumi *et al*., 1996) and Thomas *et al*., 2005. It has been confirmed that exogenous application of Gibberellic acid promotes seed germination and growth of the radicle and plumule of many plants (Taiz *et al*., 2010 and Roychowdhury *et al*., 2011b, Roychowdhury *et al*., 2011d).

Conclusion

From the above discussion, it is concluded that different concentrations of Gibberellic acid (GA3) endorsed germination percentage and growth of seedlings in apple seed i.e. radical and plumule elongation. In this study, in order to remove dormancy in apple seeds, exogenous GA_3 application has been most successful in breaking dormancy at 500 ppm concentration(see table 2, graph 1 and 2). This it is recommended from this research that that the concentration of growth regulators GA_3 500 ppm for 40 hrs 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. Hence it is inferred that an adaptation and practical application of these findings in nurseries might also have an economic effect.

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Table 2: Gibberellic acid effect on germination, growth and survival of Apple seeds / seedlings.

Treatment/ Character	Hours		Germination Percent (%)	Germination of seed (N)	Growth of seedlings (cm)	No. of leaves/seedling	Length of leaf (cm)	Width of leaf (cm)	Diameter of stem (cm)	Length of root (cm)	Diameter of root (cm)	survival of seedlings (N)
Control	0	T_0	54.27	5.33	4.67	8.80	1.09	1.16	0.27	9.03	0.14	4.00
GA_3 450 ppm	30	T_1	66.64	6.33	4.95	9.00	1.69	1.58	0.31	10.03	0.15	5.00
	35	T_2	75.75	6.00	4.77	9.00	2.53	1.43	0.31	10.35	0.17	5.00
	40	T_3	62.75	6.66	5.33	8.33	1.39	1.46	0.32	10.62	0.19	6.00
GA_3 500 ppm	30	T_4	72.08	7.00	4.97	11.00	2.90	1.73	0.34	13.05	0.22	6.00
	35	T_5	87.46	7.00	4.49	11.00	2.78	1.79	0.39	13.35	0.23	7.00
	40	T_6	63.34	9.66	8.94	16.00	3.44	2.25	0.44	15.03	0.25	9.66
GA_3 550 ppm	30	T_7	69.54	7.00	5.37	9.66	1.71	1.51	0.34	11.03	0.17	7.00
	35	T_8	78.92	7.00	4.24	9.00	2.16	1.54	0.33	11.35	0.17	5.00
	40	T_9	61.28	6.00	5.61	10.00	2.36	1.45	0.34	11.55	0.15	7.00
CD>0.05			**0.648**	**1.450**	**0.038**	**1.280**	**0.034**	**0.120**	**0.016**	**0.014**	**0.017**	**1.640**

Figure 1: Schematic representation showing effect of GA_3 on germination of Apple seeds.

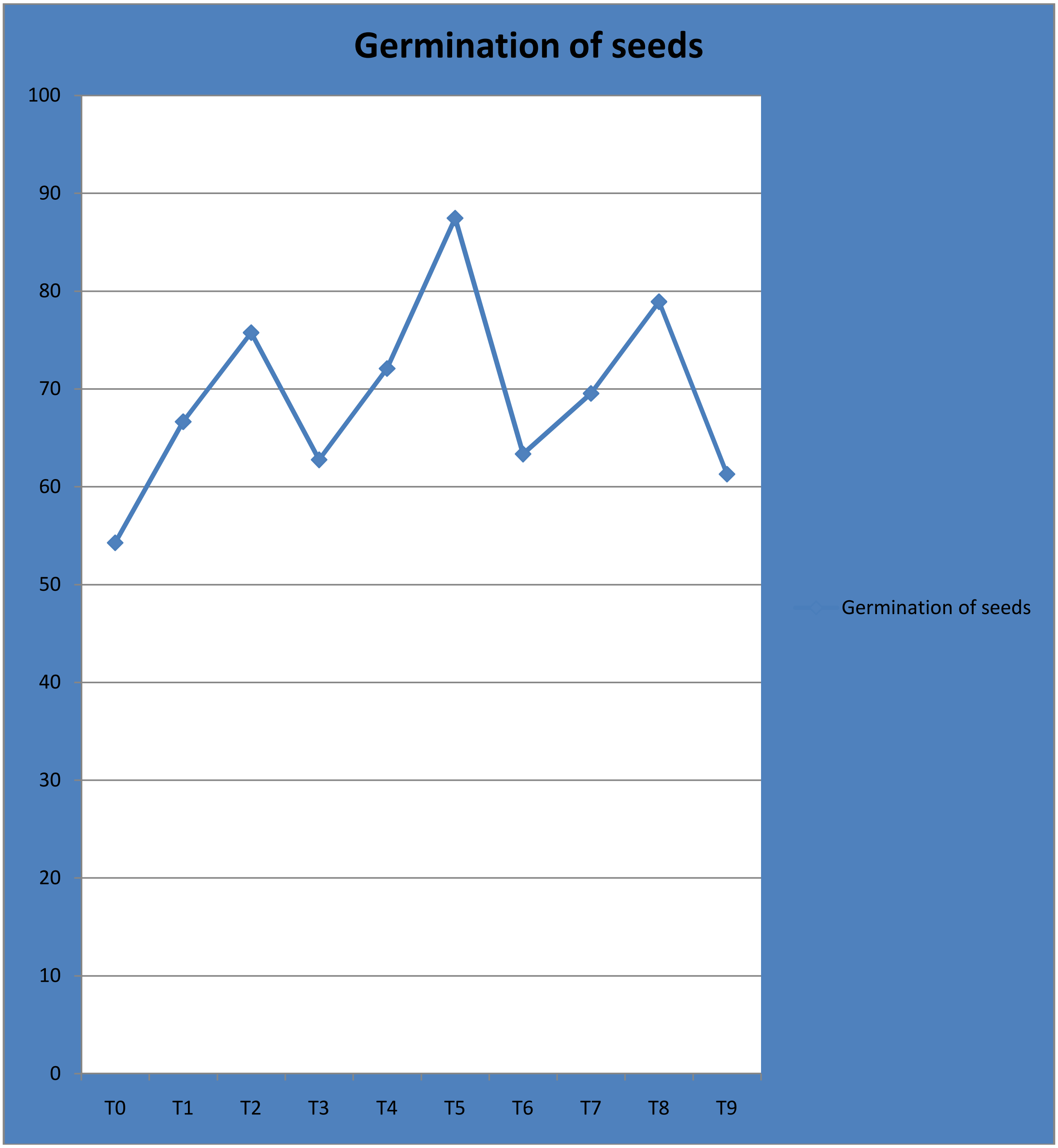

Figure 2: Schematic representation showing effect of GA_3 on growth of Apple seedlings