

RESEARCH ARTICLE

IMPROVING GERMINATION AND STAND ESTABLISHMENT OF KIWIFRUIT (*Actinidia deliciosa* cv. Hayward) SEED THROUGH MEDIA SELECTION AND HORMONAL USE IN DOLAKHA, NEPAL

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ABSTRACT

This study was conducted at Boach, Bhimeshwor-8, Dolakha, Nepal from February to April, 2020 to analyze the effect of varied concentrations of gibberellic acid and growing media on germination of kiwifruit seeds. The experiment was done by using Factorial combination of four different concentrations: 0 ppm, 2000 ppm, 4000 ppm and 6000 ppm of gibberellic acid and different growing media: Peat moss, Cocopeat, Vermicompost and Normal Soil laid out in Completely Randomized Design (CRD); each treatment were replicated four times. Germination percentage, mean germination time, germination rate, mean germination rate, germination index, coefficient of velocity of germination and germination rate index were the parameters measured. The germination of kiwifruit seeds was significantly affected by gibberellic acid and growing media. The highest germination percentage (48.25%), germination rate (87.73%), germination index (0.88) and germination rate index (11.82) was recorded from seeds treated with 6000 ppm gibberellic acid and sown at peat moss whereas, the minimum germination percentage (13%), germination rate (23.64%) and germination index (0.24) was observed in seeds treated with 4000 ppm gibberellic acid and sown at vermicompost. The maximum time taken (46 days) to germinate was shown by seeds treated with 6000 ppm gibberellic acid and sown at normal soil whereas, the minimum time taken (35 days) to germinate was recorded in seeds treated with 6000 ppm gibberellic acid and sown at cocopeat. The highest mean germination rate (0.028 day⁻¹) and coefficient of velocity of germination (2.79%) was observed in seeds treated with 6000 ppm gibberellic acid and sown at cocopeat whereas the least mean germination rate (0.022 day⁻¹) and coefficient of velocity of germination (2.18%) was recorded in seeds treated with 6000 ppm gibberellic acid and sown at normal soil. From the findings of this experiment, it is revealed that the seeds treated with 6000 ppm gibberellic acid and sown at peat moss could be the most effective and appropriate to improve the germination and stand establishment of kiwifruit seeds.

KEYWORDS

Germination, Gibberellic Acid, Growing media, Kiwifruit

1. INTRODUCTION

Kiwifruit (*Actinidia spp.*) is a deciduous vine, originating from China, belongs to the family Actinidiaceae and genus *Actinidia* with basic chromosome number 2n=58 (Crowhurst et al., 1992). It is one of the delicious fruits which has gained its popularity worldwide in recent few years as it excludes itself from other fruits in respect of its wide climatic adaptability, its unique blend of taste and high nutritive and medicinal values. Kiwifruit, primarily produced for the fresh fruit market and its processing is typically only a way of using rejected fruits (Celik, 2006). There are more than 70 species of Kiwifruit, among them *Actinidia deliciosa* (Fuzzy kiwifruit) and *Actinidia chinensis* (Golden kiwifruit) are commercially cultivated species (Ferguson, 2007). In context of Nepal, it has been reported that kiwifruit shows the best performance at an altitude of 1200m to 2400m (Dhakal, 2018). In context of Nepal, the total area, productive area, production and yield of Kiwifruit are 1362 ha, 492 ha,

3372 mt and 6.86 mt/ha respectively (MoAD, 2020). Similarly, the total area, productive area, production and productivity of kiwifruit in Dolakha are 200.2 ha, 48.2 ha, 384.2 mt and 8 mt/ha respectively (MoAD, 2020).

Kiwifruit is propagated either sexually or asexually by different methods of grafting or cutting and grafting on seedling rootstock. But due to the low germination capacity, it makes kiwi propagation difficult. Under even favorable environmental and growing conditions, seeds find it difficult and erratic to germinate (Bradford, 2005). Seed dormancy is usually a block to the completion of germination of an intact viable seed under favorable conditions (Bewley, 1997). Two major forms of seed dormancy have been described, namely embryo and coat dormancy (Kucerna et al., 2005). Physiological dormancy is divided into three levels namely: deep, intermediate and slight dormancy. In physiological dormant seed, it is thought that gibberellic acids can both release dormancy and promote germination along with the growing medium (coco-peat, peatmoss, vermicompost) which ensures greater physical stability with optimum

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rooting environment, good water holding capacity with proper absorption, retention and supply of adequate nutrients and aeration to the seeds (Kucerna et al., 2005). A good growing medium would provide sufficient anchorage or support to the plants, serves as a reservoir for the nutrients and water, allow oxygen diffusion to the roots and permits gaseous exchange between the roots and atmosphere outside the substrate (Abad, 2002).

Gibberellic acid plays a key role in dormancy release and promotion of germination and is widely used to break dormancy of seeds of various plant species (Kucerna et al., 2005). The embryo of many seeds fails to germinate because oxygen does not diffuse through the seed coats but when seeds are released from dormancy, the receptors then initiate a signal transduction cascade, perhaps involving synthesis of or sensitization to germination-promoting GAs that lead to the completion of germination (Bewley, 1997). According to some researchers, gibberellic acid treatments (2.5-5.0 ppm for 24 h or 10-40 ppm for 24 h) improved germination rate (Lawes, 1980). Kiwifruit seeds stored at 0 degree celcius for a month and treated with 2000 or 6000 ppm gibberellic acid showed highest germination percentage (Ozcan, 2000). Seeds treated with 6000 ppm GA3 had the lowest and those treated with 2000 ppm GA3 had the highest germination rate (Celik, 2006). There are several reports which demonstrates the use of various chemicals and growth media are suitable for enhancing kiwifruits seed germination (AHN et al., 1984; Verma et al., 1998; Ynoue et al., 1999).

2. MATERIALS AND METHODS

The research was conducted from 16th February, 2020 to 6th April, 2020 at Temperate Fruits Rootstock Development Centre, Boach, Dolakha. It is situated at an altitude of 1975 masl. It is situated within 27°28' - 28°0' N latitude and 85°50' - 86°32' E longitudes.

2.1 Experimental materials

Kiwifruit seeds were obtained from the well-ripened fruits of Hayward cultivar, peeled, crushed to separate seeds from the pulp, washed thoroughly in water through fine sieve, dried and were packed in a polythene packet before treatment and left it at shade for 24 hours. GA3 solution was prepared by dissolving powder in water using ethanol and slightly heated to get the required concentrations; 2000 ppm, 4000ppm and 6000ppm.

2.2 Experimental Design

The experiment was carried out using two factor combination of factorial Completely Randomized Design (CRD) with 16 treatments, each treatment replicated 4 times. The hormonal treatment of seeds was done in the dark at 25±2°C for 24 h. After 24 h, the seeds was dried in shade at 25±2°C for 24 h to reduce the moisture content.

Factor A: Different concentrations of Gibberellic acid
1) 0 ppm 2) 2000 ppm 3) 4000 ppm 4) 6000 ppm

Factor B: Growing Medium

1) Peat moss 2) Coco-peat 3) Vermicompost 4) Normal Soil

Treatments	Treatments Combinations	Composition
T1	G1M1	0 ppm + Normal soil
T2	G1M2	0 ppm + Peat moss
T3	G1M3	0 ppm + Vermicompost
T4	G1M4	0 ppm + Coco-Peat
T5	G2M1	2000 ppm + Normal soil
T6	G2M2	2000 ppm + Peat moss
T7	G2M3	2000 ppm + Vermicompost
T8	G2M4	2000 ppm + Coco-Peat
T9	G3M1	4000 ppm + Normal soil
T10	G3M2	4000 ppm + Peat moss
T11	G3M3	4000 ppm + Vermicompost
T12	G3M4	4000 ppm + Coco-Peat
T13	G4M1	6000 ppm + Normal soil
T14	G4M2	6000 ppm + Peat moss
T15	G4M3	6000 ppm + Vermicompost
T16	G4M4	6000 ppm + Coco-Peat

Seed was directly sown in a plastic tray inside a Screen house on 16th February, 2020. The seed was treated with GA3 one day before sowing and was placed in a shade for 24 hours. In each treatment, 100 seeds were sown, thus altogether 6400 seeds were sown at growing medium.

3. DATA COLLECTION

Collection of data of different growth parameters of the seeds was taken from 10th days after sowing upto 50th day at a regular interval.

3.1 Germination Percentage

The germination percentage of seeds for each treatment was computed as:

$$\text{Germination \%} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}}$$

3.2 Mean Germination Time

Mean Germination Time is the mean time taken by the overall seeds to complete the mechanism of germination and it is computed as:

Mean Germination Time (MGT) = $\Sigma Fx/\Sigma F$; where F is the number of germinated seeds on day x.

3.3 Germination Rate

It is the rate of total number of seeds germinated at a specific day and is computed as:

$$\text{Germination rate} = \frac{\text{Number of seed germinated}}{\text{Total number of days}} \times 100\%$$

3.4 Mean Germination Rate

Mean Germination Rate is a reciprocal to Mean Germination Time and is computed as:

$$\text{Mean germination rate} = \frac{1}{\text{MGT}} \quad \text{where MGT is a Mean Germination Time}$$

3.5 Germination Index

Germination Index appears to be the most comprehensive measurement parameter combining both germination percentage and speed (spread, duration and 'high/low' events). It is computed as:

GI = $(20 \times N_1) + (19 \times N_2) + \dots + (1 \times N_{20})$; where N1, N2 ...N20 are the number of seeds germinated on the first, second and subsequent days until 20th day and the multipliers (e.g. 20, 19 ...etc.) are weights given to the days of the germination.

3.6 Coefficient of Velocity of Germination

The coefficient of Velocity of Germination (CVG) was recorded in each treatment and is computed as:

$$\text{Coefficient of velocity of germination (CVG)} = \frac{N_1 + N_2 + \dots + N_i}{100 \times N_1 T_1 + \dots + N_i T_i}$$

where N is the total number of seeds germinated every day and T is the number of days corresponding to N and i is the last day of germination.

3.7 Germination Rate Index

The total Germination Rate Index was recorded and was calculated with the help of following formula:

Germination Rate Index = No. of germinated seeds / days of first count ++ No. of germinated seeds/days at final count.

4. DATA ANALYSIS

The collected data was compiled by using MS-excel program and subjected to analysis of variance as per the procedures given in R-Studio statistical computer package for the completely randomized design. Duncan's Multiple Range Test (DMRT) for the mean separations was done from the reference of Gomez and Gomez (1984). Graph and tables was constructed by using MS-excel.

5. RESULTS AND DISCUSSION

5.1 Germination Percentage (GP)

Germination percentage was found to be significantly influenced by both the varied concentrations of gibberellic acids and growing medium.

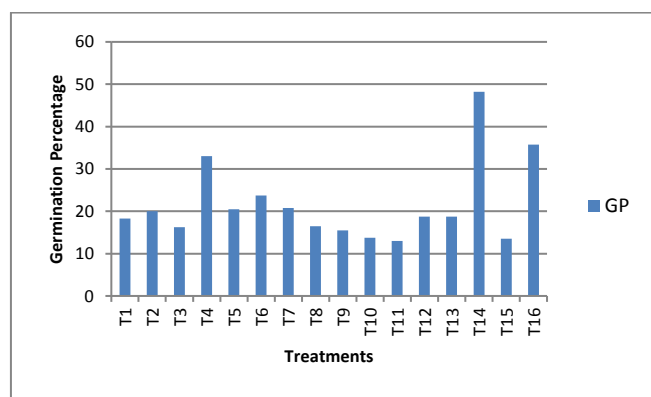


Figure 1: Germination Percentage shown by different treatments

Table 1: Germination Percentage as influenced by different concentrations of gibberellic acid and growing media on kiwifruit seeds

Treatment	Germination Percentage
T1	18.25 ^d
T2	20 ^{cd}
T3	16.25 ^d
T4	33 ^{bc}
T5	20.5 ^{cd}
T6	23.75 ^{bcd}
T7	20.75 ^{cd}
T8	16.5 ^d
T9	15.5 ^d
T10	13.75 ^d
T11	13 ^d
T12	18.75 ^{cd}
T13	18.75 ^{cd}
T14	48.25 ^a
T15	13.5 ^d
T16	35.75 ^{ab}
SEm(±)	1.593
LSD	14.464 ^{***}
C.V.%	46.93
Grand Mean	21.64

Means followed by common letter(s) within columns are non-significantly different based on DMRT at P=0.05, ** significant at 0.01 P level, *** significant at 0.001 level, SEM: Standard error of mean, CV: Coefficient of Variance

The Germination Percentage showed significant variation among different treatments treated with varied concentrations of GA3 and growing media. The first day of germination was 10th day after sowing and the last day of germination was recorded 50th day after sowing, while some of the findings shows that in control condition it takes 52 days to complete germination (Gao XZ, 1984). It was determined in previous research studies that germination of kiwifruit seeds ranged from 35.47% (6000 ppm GA3 doses) (Ozcan, 2000) to 36.85% (2500 ppm GA3) (Mattiuz, 1996). Some researcher had also stated that the lower germination could be caused by soil mixture, inconstant moisture content and microorganisms (Ozcan, 2000). The maximum seed germination percentage (48.25%) was found in seeds treated with 6000 ppm GA3 and sown at peatmoss. Seed germination is favored by seed treatment (breaks seed dormancy and enhances germination), it acts on germination promotion as it removes impermeability of seed coat and helps in exposing macro-sclereids cells in water imbibition for initiation of germination and growth medium as it promotes water absorption, improvement in soil texture, porosity, water holding capacity, nutrient availability, oxygen supply to the germinating seeds and seedlings and activity of soil microflora fauna which helps in maintaining soil temperature and improving soil health nutrients of the medium (Mayer and Poljakoff, 1963; Nilkoleave, 1977).

The least germination percentage (13%) was found in seeds treated with 4000 ppm GA3 and sown at vermicompost. Whereas, in a control condition of GA3, seeds sown at cocopeat showed the maximum germination percentage of 33.00% and seeds sown at vermicompost showed the least germination percentage of 16.25%. Also, in a normal soil growing condition, seeds treated with 2000 ppm GA3 showed the maximum germination percentage of 20.50% than seeds treated with 6000 ppm GA3 which is in accordance to and is statistically at par with seeds treated with

0 ppm GA3 and the least germination percentage (15.50%) was found in seeds treated with 4000 ppm GA3 (Ozcan, 2000).

5.2 Mean Germination Time (MGT)

Table 2: Mean Germination Time as influenced by different concentrations of gibberellic acid and growing media on kiwifruit seeds

Treatment	Mean Germination Time
T1	40.73 ^{bcd}
T2	41.96 ^{abc}
T3	41.38 ^{abc}
T4	38.2 ^{cd}
T5	39.53 ^{cd}
T6	38.84 ^{cd}
T7	38.89 ^{cd}
T8	41.51 ^{abc}
T9	41.29 ^{abc}
T10	45.22 ^{ab}
T11	39.88 ^{bcd}
T12	39.73 ^{cd}
T13	46.34 ^a
T14	36.74 ^{cd}
T15	41.06 ^{abcd}
T16	35.86 ^d
SEm(±)	0.522
LSD	5.381 [*]
C.V.%	9.34
Grand Mean	40.45

Means followed by common letter(s) within columns are non-significantly different based on DMRT at P=0.05, ** significant at 0.01 P level, *** significant at 0.001 level, SEM: Standard error of mean, CV: Coefficient of Variance

Seeds treated with 6000 ppm GA3 and sown at a normal soil showed the highest Mean Germination Time of 46.34 days and least (35.87 days) was observed in seeds treated with 6000 ppm GA3 and sown at cocopeat. The overall Mean Germination Time was found much lower than that of previously reported study of kiwi seeds takes 52 days to complete germination mechanism at controlled conditions, which states that GA3 along with suitable growing media hastens the process of germination (Gao, 1984). This finding is also supported by the result of (Lawes, 1980; Scurfield, 1963; Swan, 1961). Also, on accordance to GA3 stimulates hypocotyl growth by initiation of cell division and elongation by primarily acting on cell division, then on cell enlargement (Arney, 1966; Arney, 1965).

5.3 Germination Rate (GR)

Table 3: Germination Rate as influenced by different concentrations of gibberellic acid and growing media on kiwifruit seeds

Treatment	Germination Rate
T1	33.18 ^d
T2	36.36 ^{cd}
T3	29.55 ^d
T4	60 ^{bc}
T5	37.27 ^{cd}
T6	43.18 ^{bcd}
T7	37.73 ^{cd}
T8	31.36 ^d
T9	28.18 ^d
T10	25 ^d
T11	23.64 ^d
T12	34.09 ^{cd}
T13	34.09 ^{cd}
T14	87.73 ^a
T15	24.55 ^d
T16	65 ^{ab}
SEm(±)	2.880
LSD	26.084 ^{***}
C.V.%	46.45
Grand Mean	39.43

Means followed by common letter(s) within columns are non-significantly different based on DMRT at P=0.05, ** significant at 0.01 P level, *** significant at 0.001 level, SEM: Standard error of mean, CV: Coefficient of Variance

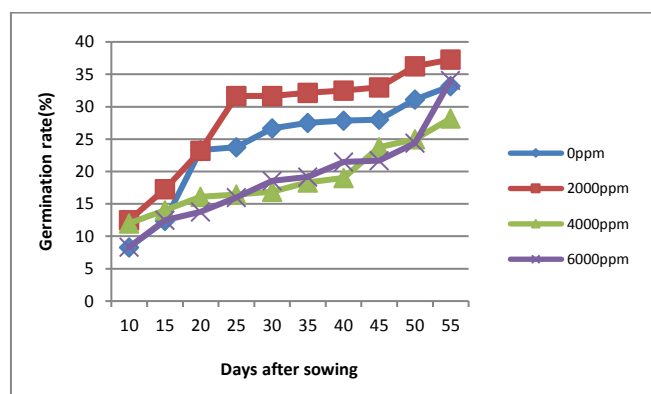


Figure 2: Germination rate as influenced by different concentrations of GA3

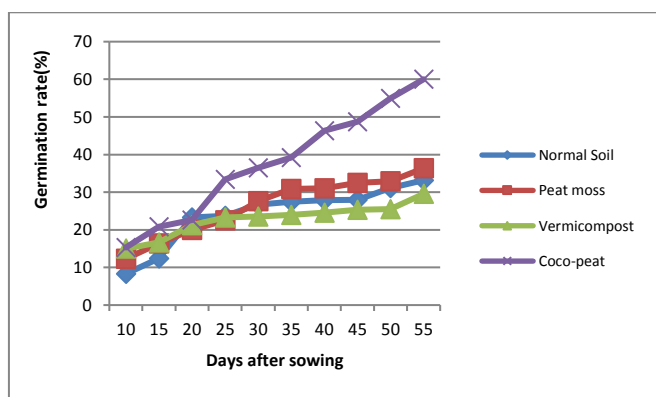


Figure 3: Germination rate as influenced by different growing media

There was a significant difference in germination rate among different treatments. Seeds treated with 6000 ppm GA3 and sown at peatmoss had the highest germination rate of 87.73% and the seeds treated with 4000 ppm GA3 and sown at vermicompost showed the lowest germination rate of 23.64%. Soil mixture reduced the rate of germination even when treated with gibberellic acid (Ozcan and Erisgin 2000).

5.4 Mean Germination Rate (MGR)

Table 4: Mean Germination Rate as influenced by different concentrations of gibberellic acid and growing media on kiwifruit seeds

Treatment	Mean Germination Rate
T1	0.0246 ^{bcd}
T2	0.0239 ^{cde}
T3	0.0243 ^{bcd}
T4	0.0263 ^{abc}
T5	0.0254 ^{abc}
T6	0.0258 ^{abc}
T7	0.0257 ^{abc}
T8	0.0246 ^{bcd}
T9	0.0245 ^{bcd}
T10	0.0222 ^{de}
T11	0.0252 ^{abcd}
T12	0.0258 ^{abc}
T13	0.0218 ^e
T14	0.0272 ^{ab}
T15	0.0244 ^{bcd}
T16	0.0279 ^a
SEm(±)	0.000
LSD	0.003 [*]
C.V.%	8.57
Grand Mean	0.025

Means followed by common letter(s) within columns are non-significantly different based on DMRT at P=0.05, ** significant at 0.01 P level, *** significant at 0.001 level, SEM: Standard error of mean, CV: Coefficient of Variance

Seeds treated with 6000 ppm GA3 and sown at cocopeat showed the highest mean germination rate of 0.028 day⁻¹ followed by seeds treated

with 6000 ppm GA3 and sown at peat moss which is statistically at par with seeds treated with 0 ppm GA3 and sown at cocopeat. The least mean germination rate (0.022 day⁻¹) was observed in seeds treated with 6000 ppm GA3 and sown at normal soil.

5.5 Germination Index (GI)

Table 5: Germination Index as influenced by different concentrations of gibberellic acid and growing media on kiwifruit seeds

Treatment	Germination Index
T1	0.33 ^d
T2	0.36 ^{cd}
T3	0.3 ^d
T4	0.6 ^{bc}
T5	0.37 ^{cd}
T6	0.43 ^{bcd}
T7	0.38 ^{cd}
T8	0.31 ^d
T9	0.28 ^d
T10	0.25 ^d
T11	0.24 ^d
T12	0.34 ^{cd}
T13	0.34 ^{cd}
T14	0.88 ^a
T15	0.25 ^d
T16	0.65 ^{ab}
SEm(±)	0.028
LSD	0.261 ^{***}
C.V.%	46.45
Grand Mean	0.39

Means followed by common letter(s) within columns are non-significantly different based on DMRT at P=0.05, ** significant at 0.01 P level, *** significant at 0.001 level, SEM: Standard error of mean, CV: Coefficient of Variance

There was a significant difference between Germination Index among different treatments treated with varied concentrations of GA3 and sown at different growing media. The maximum Germination Index (0.88) was observed in seeds treated with 6000 ppm GA3 and sown at peat moss and the least (0.24) was observed in seeds treated with 4000 ppm GA3 and sown at vermicompost.

5.6 Coefficient of Velocity of Germination (CVG)

Table 6: Coefficient of Velocity of Germination as influenced by different concentrations of gibberellic acid and growing media on kiwifruit seeds

Treatment	Coefficient of Velocity of Germination
T1	2.46 ^{bcd}
T2	2.39 ^{cde}
T3	2.43 ^{bcd}
T4	2.63 ^{abc}
T5	2.54 ^{abc}
T6	2.58 ^{abc}
T7	2.57 ^{abc}
T8	2.46 ^{bcd}
T9	2.45 ^{bcd}
T10	2.22 ^{de}
T11	2.52 ^{abcd}
T12	2.58 ^{abc}
T13	2.18 ^e
T14	2.72 ^{ab}
T15	2.44 ^{bcd}
T16	2.79 ^a
SEm(±)	0.03
LSD	0.305 [*]
C.V.%	8.57
Grand Mean	2.50

Means followed by common letter(s) within columns are non-significantly different based on DMRT at P=0.05, ** significant at 0.01 P level, *** significant at 0.001 level, SEM: Standard error of mean, CV: Coefficient of Variance

Seeds treated with 6000 ppm GA3 and sown at cocopeat showed the maximum (2.79%) Coefficient of velocity of germination (number of seeds

germinated per unit time) whereas, seeds treated with 6000 ppm GA3 and sown at normal soil showed the least coefficient of velocity of germination (2.18%).

5.7 Germination Rate Index (GRI)

Table 7: Germination Rate Index as influenced by different concentrations of gibberellic acid and growing media on kiwifruit seeds	
Treatment	Germination Rate Index
T1	2.79 ^d
T2	2.88 ^d
T3	2.25 ^d
T4	7.02 ^{bc}
T5	3.73 ^{cd}
T6	4.05 ^{cd}
T7	3.66 ^{cd}
T8	3.23 ^d
T9	1.98 ^d
T10	1.17 ^d
T11	2.09 ^d
T12	3.41 ^d
T13	1.9 ^d
T14	11.8 ^a
T15	1.85 ^d
T16	7.9 ^b
SEm(±)	0.436
LSD	3.602 ^{***}
C.V.%	65.56
Grand Mean	3.86

Means followed by common letter(s) within columns are non-significantly different based on DMRT at P=0.05, ** significant at 0.01 P level, *** significant at 0.001 level, SEM: Standard error of mean, CV: Coefficient of Variance

Seeds treated with 6000 ppm GA3 and sown at peat moss was observed having higher Germination rate index (11.82). Whereas, seeds treated with 4000 ppm GA3 and sown at peat moss showed the least Germination rate index (1.17).

Table 8: Germination characteristics as influenced by different concentrations of Gibberellic acid and growing media on kiwifruit seeds								
Treat ment	GP	MGT	GR	MGR	GI	CVG	GRI	Remarks
T1	18.25	40.73	33.18	0.0246	0.33	2.46	2.79	
T2	20	41.96	36.36	0.0239	0.36	2.39	2.88	
T3	16.25	41.38	29.55	0.0243	0.3	2.43	2.25	
T4	33	38.2	60	0.0263	0.6	2.63	7.02	III
T5	20.5	39.53	37.27	0.0254	0.37	2.54	3.73	
T6	23.75	38.84	43.18	0.0258	0.43	2.58	4.05	
T7	20.75	38.89	37.73	0.0257	0.38	2.57	3.66	
T8	16.5	41.51	31.36	0.0246	0.31	2.46	3.23	
T9	15.5	41.29	28.18	0.0245	0.36	2.45	1.98	
T10	13.75	45.22	25	0.0222	0.25	2.22	1.17	
T11	13	39.88	23.64	0.0252	0.24	2.52	2.09	
T12	18.75	39.73	34.09	0.0258	0.34	2.58	3.41	
T13	18.75	46.34	34.09	0.0218	0.34	2.18	1.9	
T14	48.25	36.74	87.73	0.0272	0.88	2.72	11.8	I
T15	13.5	41.06	24.55	0.0244	0.25	2.44	1.85	
T16	35.75	35.86	65	0.0279	0.65	2.79	7.9	II
SEm(±)	1.593	0.522	2.88	0.000	0.028	0.03	0.436	
LSD	14.46	5.381*	26.08	0.003*	0.26***	0.30	3.60	
C.V.%	46.93	9.34	46.45	8.574	46.45	8.57	65.56	
Grand Mean	21.64	40.45	39.43	0.025	0.39	2.50	3.86	

Means followed by common letter(s) within columns are non-significantly different based on DMRT at P=0.05, ** significant at 0.01 P level, *** significant at 0.001 level, SEM: Standard error of mean, CV: Coefficient of Variance

6. CONCLUSION

Based on the result of this experiment, it can be inferred that seeds treated with 6000 ppm GA3 and sown at peat moss is most effective and

appropriate to improve and hasten germination and stand establishment of kiwifruit seeds.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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