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Influence of Low Storage Temperature and Gibberellic Acid Treatments on Growth and Yield of Garlic (*Allium sativum* L.) at Haramaya, Eastern Ethiopia

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

The effects of pre planting cloves treatment with Gibberellic acid (GA_3) plant hormone and cold temperature storage duration to improve growth and yield of garlic (*Allium sativum* L.), was investigated during 2013/2014 production year at Haramaya Eastern Ethiopia under rain- fed and irrigation condition. Randomized complete block design was used with four GA_3 concentration (0, 125, 250 and 375 ppm) and three cold storage (7℃) du rations (10, 20, and 30 days) with three replications. The interaction effect of GA_3 and cold storage duration were highly significantly increased on yield and growth traits. Treatment GA_3 125 ppm for 20 days of cold storage duration recorded the greatest increased in garlic plant height (53.76 cm), leaf width (1.31 cm), bulb width (4.96 cm), neck diameter (1.60 cm), mean bulb weight (25.40 g), harvest index (0.76), total bulb yield (8.38 t/ha) and marketable yield (8.05 t/ha). Bulb size distribution in weight (8.86 g) and number (4.61) on large cloves were significantly increased at treatment GA_3 125 ppm with 20 days

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of cold storage duration. Unmarketable garlic clove weight was influenced at treatments of GA_3 375 ppm in all levels of storage duration. The whole result analysis showed that optimum growth performance and good bulb yield of garlic was obtained from treatment of 125 ppm GA_3 with 20 days of cold storage duration.

Keywords: Cold temperature; gibberellic acid; growth and yield of garlic.

1. INTRODUCTION

Garlic (*Allium sativum* L.) is a member of the onion family (*Alliaceae*). It is an herbaceous perennial that is often grown as an annual bulb crop [1]. Garlic is originated on the Northwestern side of the Tien-Shan Mountains of Kirgizia in the arid and semi-arid areas of Central Asia (Kazakhstan). Garlic has been widely used for flavoring foods. Garlic's volatile oil has many sulfur containing compounds that are responsible for the strong odor, its distinctive flavor and pungency as well as for its healthful benefit [2].

Garlic has a wide area of adaptation and cultivation throughout the world. In Ethiopia, garlic is an important bulb crop produced by small and commercial growers for both local use and export [3]. According to [4], the average productivity per ha was 13 t/ha. Out of the total production, greater than 64% was used for household consumption and 22% was for commercial purposes. Now a days, garlic production is increasing throughout the country through cultivated under irrigated and rain fed condition [4]. However, production of garlic is not sufficient for the demand of the society.

In addition to poor cultural management practices, lack of pre planting clove seed treatment also cause reduction in garlic production in Ethiopia. Even though, garlic is one of the potential vegetable crops for both home consumption and commercial purpose, its production and productivity is reduced year to year due to farmers use untreated planting cloves. Cloves of garlic after harvesting are inactivate for using planting material for proper growth and accumulate optimum bulb yield due to low concentration of growth promoting hormones. Garlic cloves require more than three months of ambient temperature and relative humidity storage condition, for more uniform maturity and yield of garlic [5]. Immediately after harvest, garlic cannot grow due to dormancy that gradually diminishes during storage [6]. Thus, it causes poor growth habit of garlic due to low content of growth promoting hormones in the cloves.

Pretreatment of garlic cloves with low temperature helps to improve its ability to utilize light and improve its yield [7]. Seed cloves treated with temperature at 5 or 10℃ for 15 to 30 days before planting also accelerated initiation, development and maturity of bulbs [8]. At 10℃ for 30 days of storage duration clove weight increase [9]. Treatment of mother bulbs at 6℃ for 50 days before planting increased the bulb size and yield of garlic [10]. Thus, there is need to identify optimal cool temperature storage regimes and durations to enhance growth of shoot in different species and cultivars [11].

Growth promoting hormones have a vital role in plants and their processes which are stem elongation, leaf extension, flower and fruit set and their structure and contents are usually associated with plant growth and progress [12]. The influence of GA_3 on early growth behaviors of garlic gave an indication of promoting of garlic growth [6]. Gibberellic acid promotes plant development [13]. The highest number of leaves per plant was obtained from 100 and 200 ppm GA_3 treated cloves [14].

Considering the importance of garlic as one of the potential vegetable crops for both domestic consumption and export, it is vital to increase its productivity in addition to appropriate agronomic management, clove seed pretreatment also indubitable contribution in increasing garlic yield. Farmers are not well aware of the different pretreatment of seed cloves practices influencing yield. The use of appropriate cold stored seed clove and treating with growth promoting hormones could increase yield considerably. Therefore, due to farmer's lack of information on pre planting clove treatment with proper Gibberellic acid concentration and cold storage duration for improving growth and yield of garlic, thus, the objective of this study was to evaluate the effects of different Gibberellic acid concentrations and cold storage durations on growth and yield of local garlic variety.

2. MATERIALS AND METHODS

2.1 Experimental Site Description

The research was conducted at Rare research field of Haramaya University, during 2013/2014 cropping season. The study area is located in the Eastern Hararghe zone of Oromia Regional State, 14 km west of Harar town and 508 km east of Addis Ababa, Ethiopia. Geographically, it is located between latitudes of 9° 24' 53.13'' N and 9° 24' 51.34'' N, and between longitudes of 42° 01'55.69'' E and 42° 01' 56.62'' E at an altitude of 1980 masl. The study area experiences bi-modal type of rainfall distribution; short rainy season extends from March to April, whereas the long rainy season extends from June to October of the annual rainfall [15]. The mean annual maximum temperature is 24.7℃ and monthly values range between 23.1 and 25.7℃, and the mean annual minimum temperature is 11.4℃ and monthly values range between 4.6 and 14.4℃. The soil of the study area is classified as Fluvisol [16].

2.2 Treatments and Experimental Design

The experiment consisted of 4 x 3 factorial combinations of Gibberellic acid concentrations (0, 125, 250 and 375 ppm) and storage durations (10, 20, and 30 days) stored at cold temperature (7℃) (Table 1). The experiment was conducted in a field to evaluate the effects of the treatments on growth and yield parameters of garlic. The treatments were laid out as a randomized complete block design in a factorial arrangement where each treatment was replicated three times.

Table 1. Treatments used in the study

No	Treatments		
	Gibberellic acid (ppm)	Cold temperature storage (days)	Combinations
1	0	10	GA_3 0 x Day 10
2	0	20	GA_3 0 x Day 20
3	0	30	GA_3 0 x Day 30
4	125	10	GA_3 125 x Day 10
5	125	20	GA_3 125 x Day 20
6	125	30	GA_3 125 x Day 30
7	250	10	GA_3 250 x Day 10
8	250	20	GA_3 250 x Day 20
9	250	30	GA_3 250 x Day 30
10	375	10	GA_3 375 x Day 10
11	375	20	GA_3 375 x Day 20
12	375	30	GA_3 375 x Day 30

2.3 Experimental Procedures

Uniform and fresh bulbs of a local garlic variety, harvested at physiological maturity, were obtained from a farm in Haramaya University area. The bulbs were cured for one week and separated into cloves. The cloves were stored at cold temperature storage (7℃) for 10, 20, and 30 days. Then those stored cloves were soaked in all concentrations of Gibberellic acid solutions at 0, 125, 250, and 375 ppm for 24 hours. Gibberellic acid solution was made by dissolving Gibberellic acid powder in a drop of 70% ethanol enough to wet the powder for each concentration. Then it was mixed with distill water to get the required treatment concentrations.

The plot size of the experiment was 3 m^2 (2.0 m x 1.5 m) and consisted of five rows with 20 plants per row, making a total of 100 plants per plot. The spacing between plots and blocks were 1.0 m and 1.5 m, respectively. Land preparation was made by plowing at a depth of 25-30 cm, followed by pulverizing and leveling it by tractor. Then, ridges and furrows were prepared manually. Planting was done on ridges of about 20 cm height. Treated cloves were planted at a depth of 4 cm at recommended spacing of 30 cm and 10 cm between rows and plants, respectively. Cultural practices were applied uniformly for all treatments as needed [17].

Fertilizer, weeding and fungicide sprays were done as deemed necessary. Plots were supplemented with irrigation every five days from late September up to December. Plants in the middle three rows of each plot were used for data collection. The plants were harvested when 70% of their leaves fell over. The harvested bulbs were air dried in a curing room for two weeks; their tops and roots were trimmed-off after one week of curing and yield component data were recorded.

2.4 Data Collection

The following data were recorded from ten randomly selected plants within the three central rows at physiological maturity and after harvest for all treatments.

2.4.1 Days to maturity

The actual number of days from planting to fully matured was recorded.

2.4.2 Plant height (cm) and Number of leaves

Height of the plants from ground level to the top of the mature leaf was measured and the number of leaves was recorded.

2.4.3 Leaf length and diameter (cm)

The length and diameter of the longest leaf were measured by ruler.

2.4.4 Neck diameter (cm)

Neck diameter was measured from the thickness of the bulb neck.

2.4.5 Shoot fresh and dry weight (g)

The total fresh and dry weights of above ground plant biomass were recorded.

2.4.6 Bulb length and diameter (cm)

The length and the diameter of longest bulb were recorded.

2.4.7 Mean number and weight (g) of cloves

Average number and weight of cloves per bulb were recorded.

2.4.8 Mean bulb weight (g) and yield per hectare (t/ha)

The average weight of bulbs was recorded and yield was calculated from net plot.

2.4.9 Harvest index and bulb dry mater (%) per plant

Harvest index was calculated by dividing total bulb yield per plant to the biological yield and bulb dry mater was the ratio of dry weight to fresh weight of garlic and expressed as the formula suggested by [18].

2.4.10 Total dry biomass yield (g)

Total dry biomass yield was recorded from the sum total biomass weight of dried bulbs, leaves, stems and roots after drying in an oven at 70℃ until a constant weight was attained.

2.4.11 Weight (g) and number of cloves by size category

Weight (g) and number of cloves were determined by weighing and counting cloves categorized into very small or unmarketable (<1 g) cloves, small (1-1.49 g, scarcely marketable), medium (1.5- 1.9 g, acceptably marketable), large (2-2.5 g, marketable) and very large (>2.5 g, marketable) [19].

2.5 Statistical Analysis

Data collected for all parameters were subjected to analysis of variance using statistical analysis system institute package (SAS, 2010 version 9.1). Comparisons of treatment mean values were made by least significant difference (Fisher's LSD) and Duncan's Multiple Range Test (DMRT) at 5% probability.

3. RESULTS AND DISCUSSION

3.1 Vegetative Growth Parameters

3.1.1 Days to maturity

Analysis of variance showed that there was high significantly ($P<0.01$) differences among treatments for days to maturity of garlic bulbs. Treatments which received cold storage for 10 days with GA_3 375 ppm showed significant delay in maturity of garlic (Table 2). On the other hand, in treatments with well-established stand, maturity was enhanced in bulbs treated with no GA_3 but cold stored for 30 days, which also was statistically comparable to the effects of 20 days of cold storage with 125 ppm GA_3. Generally, increases in the concentration of GA_3 proportionally delayed maturity of garlic plants while cold storage duration enhanced maturity at day 30 than 10 day treated treatments.

The result is in line with the work of [6] who reported that GA_3 treatment at 250 ppm delayed bulb maturity as compared to those treated cloves with lower than 250 ppm concentrations.

Gibberellic acid cause delay maturity because of its physiological effects in stimulation of vegetative growth [20,6]. This result is in agreement to [21] finding, who reported that cold treated garlic with 5℃ was 30 days earlier in its differentiated and completed development as compared to 20℃ treated one. Seed cloves treated with cold temperatures at 5 or 10℃ for 15 to 30 days before planting had accelerated maturity of bulbs relative to those stored at 15 and 20℃ [9]. This is due to long cold storage duration for effective breaking dormancy followed by early emergence and initiation of bulbing to accelerated maturity than non-cold treated cloves [22,8,23,24].

3.1.2 Plant height

There were significant (P<0.05) differences among treatments on plant height. The tallest plants were observed when cloves were cold stored for 20 days and treated with GA_3 125 ppm. While the shortest plants were recorded from cloves stored under cold for 10 days with no GA_3 treatment or from those cold treated for 20 days with additional treatment of 375 ppm GA_3 (Table 2). All the other treatment effects had relatively intermediate plant heights that were not statically different from the highest and the lowest plant height values. When cold storage duration increases from 10 to 30 days without GA_3 combination, plant height was also increased. In agreement to the current finding, author [9] reported that garlic plant height was increased at 15℃ for 30 days of cold storage treatment. Similarly, author [22] found that bulbs that received low temperature storage had increased plant growth compared to non-treated counterparts. The increase in plant height with increase in duration of cold temperature is due to its effect on early breaking dormancy and enabling emerging seedlings to utilize reserve food in the cloves for easy field establishment and further growth and development [22,8,23, 24].

3.1.3 Number of leaves per plant

There were no significant differences in the number of leaves of garlic plant among the treatments (p>0.05) (Table 2). The result is in line with the work of [22] who reported that the effect of storage temperature at 0, 7 and 25℃ for 30 days storage duration was non-significant difference on number of leaves per plant. Author [25] also reported that cold storage for 30 and 40 days had non-significant influence on number of leaves. In contrast to the present study result, [24] recorded that pre-chilled cloves at 4℃ for three weeks produced higher number of leaves per plant. Similarly, author [9] found that the number of leaves increased in bulbs stored at 15℃ for 30 days.

Author [26] also reported that the number of leaves decreased at 200 ppm concentrations of GA_3 spry application. GA_3 enhanced rapid leaf proliferation and that the highest number of leaves per plant was obtained from 200 ppm GA_3 treated cloves than those treated with more than 200 ppm GA_3 concentrations [14].

3.1.4 Leaf length and width

Similar to plant height, there were highly significant effects of treatments on leaf length and width (P<0.01) (Table 3). Cold storage of cloves for 30 days (38.79 cm) and 20 days (38.20 cm) with GA_3 125 ppm resulted in the highest mean leaf length while the widest leaves (1.31 cm) was recorded from plants whose cloves were treated with the same concentration of GA_3 after receiving prior cold storage for 20 days. The shortest leaf length and the narrowest leaf width were obtained in cloves cold stored for 10 days without any supplemental GA_3 treatment and also statistically similar value from the treatment of 30 days of cold storage with GA_3 375 ppm. As cold storage duration increases from 10 to 30 days respectively increased leaf length and width of garlic.

Table 2. The effects of GA_3 and cold storage treatments of seed cloves on days to maturity, plant height and leaves number of garlic

Treatments	Days to maturity (days)**	Plant height (cm)*	Leaves/plant (no) ns
GA_3 0 x Day 10	135.00c-f	41.92c	6.80
GA_3 0 x Day 20	132.66c-f	42.94bc	6.60
GA_3 0 x Day 30	122.66f	47.23abc	6.33
GA_3 125 x Day 10	144.00abc	45.46abc	7.66
GA_3 125 x Day 20	127.66ef	53.76a	7.93
GA_3 125 x Day 30	130.00def	51.80ab	7.93
GA_3 250 x Day 10	137.33b-e	51.50ab	7.66
GA_3 250 x Day 20	140.33a-e	44.52abc	6.46
GA_3 250 x Day 30	143.00a-d	51.01ab	6.53
GA_3 375 x Day 10	148.00ab	49.85abc	7.80
GA_3 375 x Day 20	142.66a-d	42.02c	6.53
GA_3 375 x Day 30	130.33def	43.04bc	6.93
CV(%)	4.86	9.03	10.69

** and ** indicate significant differences at 5% and 1% probability levels. ns = non-significant different. Means followed by the same letter within a column are not significantly different at 5% probability levels according to DMRT test. CV=Coefficient of Variation. no= Number*

Table 3. The effects of GA_3 and cold storage treatments of seed cloves on leaf length and width and shoot fresh and dry weight of garlic

Treatment	Leaf length (cm)**	Leaf width (cm)**	Shoot fresh weight (g/plant)**	Shoot dry weight (g/plant)**
GA_3 0 x Day 10	29.26c	0.86f	20.46cd	6.69cd
GA_3 0 x Day 20	31.00bc	0.98c-f	22.37bcd	7.32bcd
GA_3 0 x Day 30	36.23ab	1.14a-e	23.73abc	7.76abc
GA_3 125 x Day 10	33.63abc	1.16a-d	19.81cde	6.48cde
GA_3 125 x Day 20	38.20a	1.31a	29.76a	9.74a
GA_3 125 x Day 30	38.79a	1.28ab	22.36bcd	7.31bcd
GA_3 250 x Day 10	35.96ab	1.18a-c	21.00cd	6.87cd
GA_3 250 x Day 20	31.12bc	0.90d-f	20.46cd	6.69cd
GA_3 250 x Day 30	36.66ab	1.12a-f	18.58cde	6.08cde
GA_3 375 x Day 10	36.36ab	1.26ab	21.02cd	6.88cd
GA_3 375 x Day 20	31.78bc	1.02bcdef	12.62e	4.13e
GA_3 375 x Day 30	29.82c	0.88ef	16.83cde	5.50cde
CV(0.05)	8.69	22.68	22.43	17.39

*** indicate significant differences at 1% probability level. Means followed by the same letter within a column are not significantly different at 5% probability levels according to DMRT test. CV=Coefficient of Variation*

In agreement to the current finding, author [6] reported that pre-planting immersion of cloves in aqueous solution of Gibberellic acid enhanced initiation and development of garlic leaf growth. Such positive effect, however, appears to be under optimal cold storage condition and GA_3 concentrations. At increased concentration of GA_3 (375 ppm) with respect to increase cold storage duration, leaf length and width were reduced. The reason for reduced leaf length and width is due to increase in cold storage duration together with increased applied GA_3 concentration. Thus, it could be resulted in tissue proliferation and growth in thickness or possibly due to toxicity effect at higher levels of each factor [26].

3.1.5 Shoot fresh and dry weight

Garlic shoot fresh and dry weight were highly significantly ($p<0.01$) influenced by the effects of cold storage duration and GA_3 treatments. The highest shoot fresh weight (29.76 g/plant) and dry weight (9.74 g/plant), respectively, were recorded from the treatment of GA_3 125 ppm with 20 days of cold storage duration. The lowest shoot fresh (12.62 g/plant) and dry weight (4.13 g/plant) were observed under treatment of GA_3 375 ppm with 20 days of cold storage duration (Table 3). These results indicated that shoot fresh and dry weight were increased due to early breakdown of clove dormancy which enables proper growth in vegetative parts. Cooler temperatures storage helps to enhance shoot growth, indicating that garlic cloves treated under cold storage better enhance vegetative growth and thus increase both fresh and dry weight of garlic plant [11] and [7]. The result of the current study is in agreement with the result of [27] who reported that shoot biomass of garlic expressed either in fresh or dry weight was increased significantly under GA_3 treatment at 100 ppm application after three weeks of germination.

3.2 Bulb Traits and Yield

3.2.1 Bulb length

Length of garlic bulbs showed highly significant ($P<0.01$) variation due to the effect of different treatments. Treatment of GA_3 125 ppm with cold storage for 20 days provided the longest garlic bulbs (5.64 cm; (Table 4). However, this value was statistically similar with GA_3 250 ppm with cold storage for 10 days. The lowest bulb length (3.37 cm) was recorded from cloves cold stored for 20 days and treated with 375 ppm GA_3.

The increased bulb length due to extended cold storage and optimum concentration of GA_3 could be the result of good vegetative growth. Similarly, bulb initiation and development by pre-planting exposure of cloves to low at 5 or 10℃ for 15 to 30 days than 15 or 20℃ treatment was reported by [8] and cold treatment that raises cell gibberellin activities [28,29]. According to [21], cell size of cold-treated cloves was larger than those in non-cold treated controls.

3.2.2 Bulb and neck diameter

Analysis of variance showed that the effects of treatments on bulb and neck diameter ($P<0.01$) were highly significant. Treatment of cloves with cold storage for 20 days coupled with treatment with 125 ppm GA_3 resulted in the thickest (4.96

cm) bulb diameter although it was at par with those treated with cold storage for 30 days without further GA_3 treatment (4.76 cm). On the other hand, treatment of cloves with cold temperature for 20 and 30 days in combinations of 375 ppm GA_3 showed the lowest bulb diameter (Table 4). In contrast to the present study result, [26] recorded that bulb diameter reduced at 200 ppm GA_3 spry application.

As cold storage duration increases from 10 to 30 days without GA_3 application, bulb width also increased. In agreement to the current finding, author [25] reported that cold storage treated garlic for 30 and 40 days produced wider bulb diameter. Similarly, author [24] found that higher bulb diameter obtained from cloves treated with 4℃ for three weeks that increased 48% comparing to that of bulbs obtained from non-treated cloves. This result is consistent also with the finding of [22] who reported the effect of storage temperature at 0℃ and 7℃ for 30 days was higher on bulb width than stored at 25℃ for 30 days storage duration. Pre-plant chilling treatments of cloves produced significant increases bulb diameter [30,31]. Treatment of mother bulbs at 6℃ for 50 days before planting increased the bulb size of garlic that eventually reflected in bulb diameter [10]. Other author [9] also reported that bulb width resulted non-significant difference.

The highest bulb neck diameter (1.60 cm) was recorded in cloves that were cold stored for 20 days and treated GA_3 125 ppm (Table 4). The lowest (0.88 cm) bulb neck diameter was observed in plants grown from cloves stored in cold for 20 days and then treated with GA_3 250 ppm. The increase in bulb neck diameter appears to be related with vigorous vegetative growth that could accumulate high amount of photosynthetic products and leading to production of large sized bulbs with wide neck.

3.2.3 Clove number per bulb

The treatments had highly significant ($p<0.01$) influence on number of cloves per bulb. The highest mean number of cloves per bulb (13.21) was recorded from treatment of $GA_3$125 ppm with 20 days of storage duration (Table 4). On the other hand, the least number of cloves per plant (7.88) was recorded from plants that received 30 days cold storage but no GA_3. This result is in agreement to [32] finding, who reported that pre-plant treatment of cloves with Gibberellic acid produced positive impact on number of cloves.

While extending pre-plant cold treatment of cloves for over 20 days reduced number of cloves per bulb. The result is in line with the work of [9] who reported that at 10℃ for 30 days of storage duration clove number decreased. Similarly, author [33] found that pre plant cloves cold treatment reduced number of cloves per bulb. The result indicated that garlic bulbs that did not complete their dormancy period needed exposure to cold temperature to initiate indigenous production of growth hormone or exogenous application of the hormone to meet requirement for initiation, growth and development of the plant, including cloving. In contrast to the present study result, [22] recorded that the effect of storage temperature at 0℃ and 7℃ for 30 days was higher number of cloves than stored at 25℃ for 30 days storage duration. Similarly, author [24] found that increased number of cloves of garlic produced from cloves pre-treatment with 4℃ for three weeks comparing to that of bulbs obtained from non-treated cloves.

3.2.4 Mean bulb weight

The effect of treatments on mean bulb weight per plant was highly significant ($p<0.01$). The maximum average bulb weight (25.40 g) was observed from the treatment of GA_3 125 ppm with 20 days of cold storage duration. On the other hand, the least value of mean bulb weight was recorded from treatment of GA_3 375 ppm with 10 days of cold storage duration (Table 4). And also cold storage duration increment alone, resulted yield improvement. This result is consistent with the finding of [9] who confirmed that storage at 15℃ for 15 days before planting gave significant increase in bulb weight compared to storage at room temperature. This could be due to the fact that low temperature increases auxin and gibberellin production that in turn stimulates clove differentiation as well as development of larger bulbs [28]. Cell size of cold treated cloves was larger than in non-cold treated control cloves [21]. Moreover, the pretreatment of garlic cloves with low temperatures could have helped the plants to improve their ability to available growth resources, moisture and nutrients [7].

Author [24] also reported that plants obtained from the garlic cloves pre-treated with 4℃ for three weeks storage duration gave higher values of mean bulb weight than those of the non-treated ones. The effect of storage temperature at 0℃ and 7℃ for 30 days was higher mean bulb weight than stored at 25℃ for 30 days storage duration [22].

Table 4. The effects of GA_3 and cold storage treatments of seed cloves on bulb length and diameter, neck diameter, clove number, mean bulb weight of garlic

Treatments	Bulb length (cm) **	Bulb width (cm) **	Neck diameter (cm)**	Mean clove number (no.) **	Mean bulb weight (g) **
GA_3 0 x Day 10	4.72cd	4.18de	1.13b-f	12.90a-d	20.43cd
GA_3 0 x Day 20	5.35b	4.24cd	1.27bcd	12.26bcd	21.79bc
GA_3 0 x Day 30	5.40b	4.76a	1.32abc	7.88h	23.08b
GA_3 125 x Day 10	4.48de	3.98ef	1.00cdef	11.63de	22.05bc
GA_3 125 x Day 20	5.64ab	4.96a	1.60a	13.21abc	25.40a
GA_3 125 x Day 30	3.93efg	3.62g	1.22b-e	9.33fg	21.33bcd
GA_3 250 x Day 10	5.48ab	4.15de	1.07c-f	11.69de	21.81bc
GA_3 250 x Day 20	4.00ef	3.58g	0.88f	12.46a-d	15.25e
GA_3 250 x Day 30	3.44gh	2.33h	1.18b-f	8.89gh	19.48d
GA_3 375 x Day 10	4.33de	3.89f	1.18b-f	11.97cd	13.60e
GA_3 375 x Day 20	3.37h	2.07i	0.95def	10.40ef	21.34bcd
GA_3 375 x Day 30	3.69fgh	2.03i	1.06c-f	8.92gh	21.40bcd
CV(0.05)	6.49	3.48	14.41	6.42	5.85

*** indicate significant differences at 1% probability level. no. number. Means followed by the same letter within a column are not significantly different at 5% probability levels according to DMRT test. CV=Coefficient of Variation*

Table 5. The effects of GA_3 and cold storage treatments of seed cloves on clove weight, bulb dry mater, and total dry biomass yield, harvest index, total and marketable yield of garlic

Treatments	Mean clove weight (g)**	Bulb dry mater (%) ns	Total dry biomass (g/plant)**	Harvest index **	Total bulb yield (t/ha)**	Marketable yield (t/ha)**
GA_3 0 x Day 10	1.58f	29.22	12.35g	0.52bc	6.74cd	5.46c
GA_3 0 x Day 20	1.77def	24.65	37.06bc	0.55bc	7.19bc	6.19bc
GA_3 0 x Day 30	2.92a	29.72	38.08abc	0.61b	7.62b	7.56a
GA_3 125 x Day 10	1.89c-f	28.24	33.81bcd	0.53bc	7.27bc	6.53b
GA_3 125 x Day 20	2.50b	36.10	38.58abc	0.76a	8.38a	8.05a
GA_3 125 x Day 30	2.29bc	35.80	43.70a	0.57bc	7.03bcd	6.50b
GA_3 250 x Day 10	1.86def	33.11	36.49bc	0.48bc	7.19bc	6.32bc
GA_3 250 x Day 20	1.22g	23.52	24.61f	0.52bc	5.03e	4.13d
GA_3 250 x Day 30	2.19bcd	24.12	32.62cde	0.44c	6.43d	5.42c
GA_3 375 x Day 10	1.13g	25.67	35.13bcd	0.55bc	4.49e	3.14e
GA_3 375 x Day 20	2.05cde	27.81	27.56ef	0.48bc	7.04bcd	5.75bc
GA_3 375 x Day 30	1.92cf	28.73	29.41def	0.53bc	7.06bcd	5.74bc
CV(0.05)	11.60	18.85	9.64	13.28	5.84	8.28

*** indicate significant differences at 1% probability level. ns = non-significant different. Means followed by the same letter within a column are not significantly different at 5% probability levels according to DMRT test. CV=Coefficient of Variation*

3.2.5 Mean clove weight per bulb

Mean clove weight per bulb have shown highly significant difference ($p<0.01$) among the treatments. The highest (2.92 g) mean clove weight was obtained from treatment effect of non- GA_3 with 30 days of cold storage. The second highest value (2.50 g) was recorded from treatment of GA_3 125 ppm with 20 days of cold storage duration. The lowest clove weight (1.13 g) was observed from the treatments of GA_3 375 ppm with 10 days cold storage and statically similar with treatment of GA_3 250 ppm with 20 days cold storage (Table 5). As days of cold storage duration increases from10 to 30 days cold storage duration without GA_3 application, increased mean clove weight per bulb. The result is in line with the work of [9] who reported that low temperature treatments (10℃), increasing storage period to 30 days significantly increased clove weight per bulb.

Author [21] also reported that cell size of cold-treated cloves was larger than in non-cold

treated control cloves, and they suggested that gibberellin production may have been increased by the chilling treatment, proving a possible explanation for cell expansion.

3.2.6 Bulb dry matter, total dry biomass yield and harvest index

Analysis of variance indicated that bulb dry matter content was not significantly (P>0.05) affected by the treatments. On the contrary, the treatments had highly significant (P<0.01) influence on total dry biomass yield per plant. Application of 125 ppm GA_3 after cold treatment of cloves for 30 days resulted in the highest total dry biomass yield per plant (43.70 g). The lowest total dry biomass yield (12.35 g) was observed in cloves cold stored for 10 days alone (Table 5).

This result indicated that as cold storage duration increases from 10 to 30 storage days without GA_3 application, consistently increased dry biomass of garlic. This result is in agreement to [26] finding, who reported that spry application below 200 ppm GA_3 significantly increased dry biomass weight while increased concentrations reduced dry biomass weight.

This could be attributed to the positive impact of optimum concentration of Gibberellic acid and cold storage temperature on vegetative growth which improves assimilate capacity of the plants and thereby resulted in high bulb yield that increased total dry biomass yield.

Harvest index was significantly (P<0.01) affected by different treatments. Treatment of cloves with125 ppm GA_3 after cold storage for 20 days produced the highest harvest index (0.76) that differed significantly from all other treatments. On the other hand, cold stored cloves for 30 days and 250 ppm GA_3 had the lowest harvest index (0.44, Table 5). This was due to production of more economical yield per biological yield of the treatments. In cloves treated with 125 ppm GA_3 after being cold storage for 20 days had improved bulb yield, harvest index, and most other plant growth parameters. As cold storage duration increases from 10 to 30 days out of GA_3 supplement, similarly harvest index also increased.

3.2.7 Total and marketable bulb yield

Both total and marketable bulb yields were highly significantly (P<0.01) affected by the different treatments. Treatment of GA_3 at 125 ppm and cold storage for 20 days gave the best total bulb yield (8.38 t/ha) while GA_3 at 375 ppm and 10 days of cold storage resulted in the lowest total bulb yield (4.49 t/ha). Similarly, GA_3 at 125 ppm with 20 days of cold storage produced the highest marketable bulb yield (8.05 t/ha). However, it was statistically at par with marketable bulb yield obtained from cloves of 30 days of cold storage alone (Table 5). The lowest marketable yield (3.14 t/ha) was also recorded from GA_3 at 375 ppm with cold storage for 10 days. Similar to total dry biomass yield and harvest index, cold storage duration increment allows improving total and marketable bulb yield. This result is consistent also with the findings of [30,31] who reported that pre-plant chilling of cloves produced significant increases in cloving resulting in a significant increase in bulb yield per plant. Likewise, [10] reported that treatment of mother bulbs at 6℃ for 50 days before planting increased yield of garlic.

The effect of storage temperature at 0℃ and 7℃ for 30 days was higher on total bulb yield than stored at 25 for 30 days storage duration means at low storage temperature increase bulb yield [22].

In agreement to the current finding, author [26] reported that at 200 ppm spry application GA_3 resulted in reduction of garlic yield.

3.3 Clove Size Category

The different treatments were high significantly affected yield and number (p<0.01) of very large sized cloves (>2.5 g, marketable cloves).

Thus, the highest yield of very large sized (7.37 g) cloves was produced from cloves stored for 30 days cold storage without any GA_3 treatment. The highest number of very large sized cloves was recorded from treatment of 125 ppm GA_3 with 10 days of prior cold storage (2.52).

The lowest yield of very large sized cloves (1.88 g) was observed in cloves stored at 7° C for 10 days and then immersed in 375 ppm GA_3. On the other hand, the lowest numbers of very large sized cloves (1.12 and 1.18 g respectively) were obtained from treatment of 375 ppm GA_3 with cold storage for 30 days (Table 6).

In this investigation, clove size were improved with respect to cold storage duration increment. This result is in agreement to [10] finding, who reported that treatment of mother bulbs at 6℃

Table 6. The effects of GA_3 and cold storage treatments of seed cloves on weight and number of very large, large, medium, small and very small cloves size category of garlic per bulb

Treatments	Very large		Large		Medium		Small		Very small	
	(No.) **	weight (g) **	(No.) **	weight (g) **	(No.) **	weight (g) **	(No.) **	weight (g) **	(No.) **	weight (g) **
GA_3 0 x Day 10	2.06a-f	3.25cde	2.55bc	4.04d	1.72cde	2.76fgh	4.04bc	6.36bcd	2.51b	4.00ab
GA_3 0 x Day 20	2.37a-e	4.24bcd	4.24a	5.61c	2.09cd	3.70def	2.85cd	5.04c-f	1.79b-e	3.19abc
GA_3 0 x Day 30	2.46abc	7.37a	2.25bcd	6.58bc	1.97cde	5.77ab	1.00e	2.97f	0.13h	0.38g
GA_3 125 x Day 10	2.52ab	4.68bc	1.39def	2.66def	2.35c	4.44cd	4.10bc	7.84ab	1.03efg	2.43cde
GA_3 125 x Day 20	2.41a-d	4.64bc	4.61a	8.86a	3.07b	5.91ab	2.49d	4.77c-f	0.61gh	1.20efg
GA_3 125 x Day 30	2.23a-e	5.10b	2.71bc	6.19bc	1.80cde	5.28bc	1.26e	2.95f	0.81fgh	1.79def
GA_3 250 x Day 10	1.51efg	2.82de	1.28ef	2.39d-g	3.60ab	6.73a	3.77bcd	7.45abc	1.50def	2.81bcd
GA_3 250 x Day 20	2.23a-e	2.73de	1.06ef	1.29fg	0.78f	0.95i	6.06a	7.05a-d	2.31bc	2.82bcd
GA_3 250 x Day 30	1.18fg	2.60de	1.09ef	2.42d-g	1.80cde	3.96def	3.64bcd	7.31abc	1.45ef	3.18abc
GA_3 375 x Day 10	1.65b-g	1.88f	2.81b	3.19de	0.85f	0.96i	2.98cd	3.38ef	3.67a	4.18a
GA_3 375 x Day 20	1.58c-g	3.21cde	1.19ef	2.58def	1.25ef	2.49gh	4.34b	8.98a	2.02b-e	4.07ab
GA_3 375 x Day 30	1.12g	2.71de	0.85f	2.10efg	1.31ef	3.12efg	3.89bc	9.30a	1.74cde	4.17a
CV(0.05)	23.23	23.32	23.31	22.68	19.58	17.03	19.60	20.96	23.90	24.98

*** indicate significant differences at 1% probability levels. no. number. Means followed by the same letter within a column are not significantly different at 5% probability levels according to DMRT test. CV=Coefficient of Variation*

for 50 days before planting increased bulb size of garlic. Pre-plant chilling treatments of cloves produced significant increases in clove size [30] and [31]. Author [21] also found that cell size of cold-treated cloves was larger than in non-cold treated control cloves. They suggested that production of gibberellins may have been increased by chilling treatment, proving a possible explanation for cell expansion.

The data shows that the treatments had highly significant ($P<0.01$) effects on weight and number of large sized cloves (2.0-2.5 g, marketable cloves).The highest number and yield of large sized cloves 4.61 and 8.86 g were obtained from treatment of 125 ppm GA_3 and cold storage for 20 days. The lowest number of large sized cloves (0.85) was obtained from cloves treated with 375 ppm GA_3 with 30 days cold storage duration and weight (1.29 g) was from treatment of 250 ppm GA_3 for 20 days cold storage

Both number and yield of medium sized cloves (1.5-2.0 g, acceptable marketable cloves) were highly and significantly ($p<0.01$) influenced by the treatments (Table 6). The highest number and yield of medium sized cloves with the respective values of 3.60 and 6.73 g were obtained from cloves stored in cold for 10 days and treated with 250 ppm GA_3.The lowest number and yield of medium sized cloves 0.78 and 0.95 g, respectively, were obtained from cloves cold stored for 20 days and treatment of 250 ppm GA_3.

Highly significant effect of treatments ($P<0.01$) on number and yield of small sized cloves (1.0-1.49 g, scarcely marketable cloves) was also observed (Table 6). The highest yield of small sized cloves (9.30 g) was recorded in cloves treated with 375 ppm GA_3 and 30 days of cold storage. The number of cloves recorded from treatment those treated with 250 ppm GA_3 and cold stored for 20 days (6.06). On the other hand, the lowest yield of scarcely marketable cloves (2.95 g) was recorded from 30 days cold storage combined with 125ppm GA_3.The lowest number of small sized cloves (1.00) was from cloves stored in cold for 30 days but with no GA_3 treatment.

The influence of treatment on number and yield of very small cloves (<1.0 g, unmarketable cloves) was highly significant ($p<0.01$) at (Table 6). The highest unmarketable clove yield were recorded from cloves those treated with 375 ppm GA_3 with all stored duration and cold stored for 10 days alone. The lowest unmarketable number and yield of cloves were recorded from those cloves stored for 30 days without GA_3 treatment. The highest number of unmarketable cloves was recorded from cloves cold stored for 10 days and then supplemented with 375 ppm GA_3 (3.67). In contract to the present study result, [9] recorded that decreasing temperature (10℃) and increasing storage period up to 30 days increased clove weight significantly while decrease storage duration leads to decrease clove size.

4. CONCLUSION

The field experiment was conducted to determine the effect of cold storage duration and GA_3 concentration on growth and yield of garlic. Cold storage of cloves for 20 days and treating with 125 ppm resulted high significantly improved early maturity, plant height, leaf width, shoot weight, bulb width, neck diameter, mean bulb weight, harvest index, total and marketable bulb yield. However, the differences among the treatments in number of leaves and bulb dry matter yield were non-significant. Cloves treated for 30 days alone resulted in increased early maturity, mean clove weight and very large clove yield. When extending cold storage duration from 10 to 30 days alone, improved plant height, leaf length and width, shoot weight, bulb and neck diameter, mean bulb weight, mean clove weight, total dry biomass yield, harvest index, total and marketable yield and very large to medium clove size yield of garlic.

The results have strongly demonstrated that combination of GA_3 at 125 ppm and cold storage duration at 20 days could be used to treat fresh garlic cloves for early growth and higher yield, to increase production cycle year round under rain-fed and irrigation conditions at Haremaya district. But this research should be repeated at multi-locations using improved and high-yielding local varieties of garlic, inclusion of clove cutting and other growth promoting treatment methods is suggested for possibility of improving growth and yield of garlic.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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