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**PRE-PLANTING CLOVE CHILLING ENHANCED THE
SELECTION PROGRAM OF INTRODUCED GARLIC
(*Allium sativum* L.) CULTIVARS UNDER A DRIP
IRRIGATION SYSTEM**

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ABSTRACT

In a breeding program of our research in the Vegetables Branch, Horticulture Department, Faculty of Agriculture, Minia University, six foreign garlic cultivars (California Early, California Late, Lorz Italian, Inchelium Red, Early Red Italian and White Brazilian) from different regions of the world were introduced to Egypt to be evaluated under the Middle Egypt garlic growing conditions. Cloves from the selected bulbs of all genotypes were pre-planting non-chilled or chilled at 4°C for three weeks. Results revealed that the pre-planting cold treatment enhanced clove germination, plant growth, bulbs and cloves formation, bulbs quality prosperities and cloves content of the total soluble solids (TSS) and helped sustain the germplasm of the imported genotypes.

INTRODUCTION

Garlic (*Allium sativum* L.) is an annual bulb crop and ranks the second after onion in order of importance and cultivation (Purseglove, 1972; Yamaguchi, 1983; Tindal, 1986).

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The growth stages of *A. sativum* include clove sprouting, shoot growth, bulb growth and maturation (Del Pozo and González, ۲۰۰۵). Clove sprouting and emergence are mainly controlled by temperature (Takagi, ۱۹۹۰). The early growth stage of garlic is suited by exposure of cloves to low temperature. Such exposure could be achieved by using controlled temperature chambers such as refrigerators (Del Pozo and González, ۲۰۰۵) or planting in a cool growing period and this treatment is essential for proper development of shoot and good yield of bulb (Bhuiya *et al.*, ۲۰۰۳; Ade-Ademilua *et al.*, ۲۰۰۹).

The main edible part of garlic is the bulb, consisting of “cloves”, which develop from axillary buds of the foliage leaves (Rahim and Fordham, ۱۹۸۸). The bulbing and cloving of garlic are influenced by day length and the temperature to which the dormant cloves or growing plants are exposed before bulbing begins. In general, low initial temperatures, followed by long days, are essential for the formation of bulbs and cloves (Kolev, ۱۹۶۲).

The chilling requirement for improved bulbing in garlic can be supplemented by low-temperature treatment of mother bulbs prior to planting (Siddique and Rabbani ۱۹۸۵). Jones and Mann (۱۹۶۳) reported that exposure of dormant cloves or young plants to temperatures between ۰ and ۱۰°C for ۱–۲ months hastens bulbing, whereas those never exposed to temperatures below ۲۰°C failed to form bulbs or cloves. Siddique and Rabbani (۱۹۸۵) reported that treatment of mother bulbs at ۶°C for ۵۰ days before planting increased the bulb size and yield of garlic, particularly when the crop was planted late in the season. The duration of low-temperature storage or pre-plant chilling for improved cloving in garlic has not been adequately determined (Bandra *et al.*, ۱۹۹۹). Cooler temperatures are required to enhance shoot growth (Bhuiya *et al.*, ۲۰۰۳) while higher light intensity help to enhance bulb growth (Argaello *et al.*, ۱۹۹۷).

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As garlic is vegetatively propagated exclusively by bulb cloves because the garlic plant is sterile (Tindal, 1986; Novak 1990). Hence, introduction of new different garlic genotypes is a desirable behavior in garlic selection and breeding programs. However, the newly imported genotypes mostly will not grow well under the new environmental conditions and may fail to set bulbs (Moustafa *et.al.*, 2009; Osman and Moustafa, 2009). The Middle Egypt (e.g., Minia governorate) is well known for its vast garlic cultivation areas and garlic genotypes selection and evaluation programs in this area are crucial. In this study, new imported garlic genotypes from different growing environments were evaluated and pre-planting chilling clove-treatments were applied to study the effects of this treatment on plants growth and yield, bulb quality as well as conservation of the germplasm of these newly imported genotypes.

MATERIALS AND METHODS

Six foreign garlic genotypes (California Early, California Late, Lorz Italian, Inchelium Red, Early Red Italian and White Brazilian) were imported from Brazil (by Dr. Gad El-Hak, Minia University) and the United States of America by MUCIA (Midwest Universities Consortium for International Activities) office (Giza, Egypt). These entries were classified to the Artichoke garlic group, which belongs to *Allium sativum* subsp. *Sativum*.

Horticultural Practices

Pre-planting treatment

Good garlic bulbs from all the genotypes under study were carefully selected and separated into individual cloves. Then, before plantation, the cloves were kept in a refrigerator at 4°C for three weeks (the chilling treatment), some selected bulbs were kept in the room temperature and their cloves were separated just before plantation (the non-chilled treatment). All chilled and non chilled garlic cloves of all genotypes were planted as described later.

The experimental field was plough and pulverized. Then, 10 ton/fed cattle farmyard manure, ammonium sulphate (20.0% N) at the

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rate of 100 kg/fed, and 100 kg superphosphate (10.0% P₂O₅) fertilizers along with 50 kg/fed agricultural sulphur were broadcasted during soil preparation. The soil was formed into beds and the used experimental unit was one bed (3m long and 100cm wide). The bed surface was carefully leveled and irrigation pipes were hand laid down to the end of the experiment. Planting was done in four rows per each bed. The cloves were spaced 10cm apart within each row and the distance between the double rows in each side was 30cm. Cloves were planted on the 1st of October in the two successive winter seasons of 2009/2010 and 2010/2011. Conventional other agronomic practices and pest control treatments were done as needed and were similar to those used in commercial garlic production. Drip irrigation (fertigation) was applied uniformly as recommended by the Egyptian Ministry of Agriculture and harvesting was carried out on the 1st of May both seasons. Data were collected per plot or per a representative sample of five or ten plant basis at various growth stages using the standard descriptors as reported by IPGRI (2001) for the following plant characteristics:

Germination percentage after 30 days from planting

Germinated cloves were counted and the germination percentage was estimated using the following formulas:

$$\text{Germination percentage} = \frac{\text{Number of germinated cloves per plot}}{\text{Total number of planted cloves per plot}} \times 100$$

Bulbing ratio: Bulb neck diameter and bulb diameter at the middle of the bulb of ten plants from both non-chilled and chilled treatments of each genotype after harvesting were estimated using a calipers and bulbing ratio was estimated using the following formula according to Mann (1952) as follow:

$$\text{Bulbing ratio} = \frac{\text{Neck diameter (mm)}}{\text{Bulb diameter (mm)}} \times 100$$

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Average weight of single fresh bulb (g): ten fresh bulbs from non-chilled or chilled treatments of each genotype were weighed and the average was recorded.

Average number of cloves per bulb: counted using five cured bulb from non-chilled or chilled treatments of each genotype.

Total soluble solids (T.S.S): Five bulbs of non-chilled or chilled plants from each replicate were crushed, their juice was mixed and the T.S.S. content was measured using a refract meter (Model: RR12/NR. 47.0, 30.6% /Made in Poland).

Statistical analyses

The Randomized Complete Block Design (RCBD) was used in this factorial experiment and all recorded data were subjected to the analysis of variance procedures and treatment means were compared using the LSD as described by (Gomez and Gomez, 1984). The statistical analysis was done by using the computer program MSTATC software version 4 (The software was purchased from Michigan University, USA in 1996).

RESULTS

Clove germination percentage after 30 days from plantation

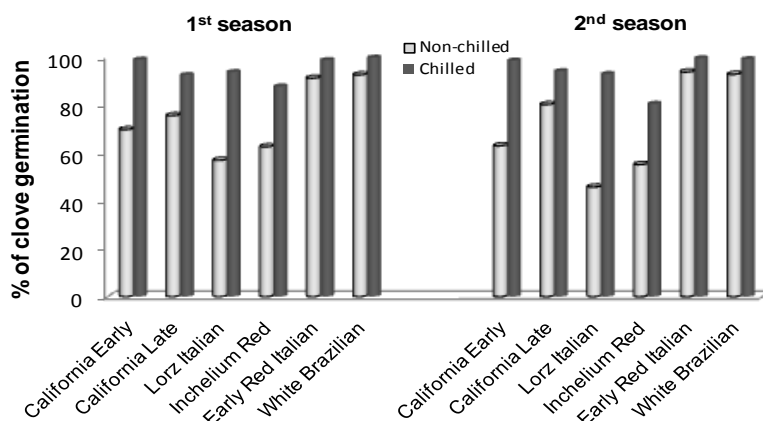
The clove pre-planting chilling treatment significantly enhanced the germination process of the garlic genotypes introduced to Egypt. Generally, the average germination percentage after 30 days from planting were (74.6% and 90.1%) and (71.6% and 94.0%) of non-chilled and chilled cloves in the first and second seasons, respectively (Fig 1 and the included Table).

The Lorz Italian cv gave the highest increase percentage (47.6%) in the second season and the Inchelium Red cv showed also a high increase in clove germination caused by the chilling treatment (36.8%) in the first season. On the contrary, the least increases in the

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percentage of clove germination were obtained by the White Brazilian (7.2%) and Early Red Italian (9.8%) cultivars in the second season, respectively.

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	Clove germination % after 20 days from plantation					
	1 st season			2 nd season		
	Non-chilled	Chilled	Mean of treatments	Non-chilled	Chilled	Mean of treatments
California Early	69.6	98.8	84.2	62.8	98.4	80.6
California Late	75.4	92.4	83.9	80.0	94.0	87.0
Lorz Italian	56.8	93.6	75.2	45.6	92.8	69.2
Inchelium Red	62.4	87.6	75.0	55.0	80.4	67.7
Early Red Italian	91.0	98.6	94.8	93.6	99.4	96.5
White Brazilian	92.4	99.6	96.0	92.6	99.0	95.8
Mean of cultivars	74.6	95.1	Grand mean	71.6	94.0	Grand mean
L.S.D. at 0.05 for: Cultivars	3.45			2.79		
Treatments	1.86			1.58		
Cultivars X Treatments	4.13		84.8	5.05		82.8

Fig 1): Clove germination (%) of pre-planting non-chilled and chilled cloves of the six imported garlic cultivars grown in two successive seasons (2009/2010 and 2010/2011).

Plant growth behavior

Vigorous growth was observed in plants of all tested garlic genotypes when their cloves were pre-planting chilled at 4°C for three weeks in the refrigerator comparing to those plants grown from the non-chilled cloves which were smaller in size and contained lower number of leaves (Plate 1-A). Plants obtained from chilled cloves succeeded to form true bulbs (sometimes with branching), while, those of the non-chilled cloves didn't form true bulbs but gave bulbs similar to the onion bulbs without true cloves. These bulbs without cloves turned into spongy bulbs at the harvesting time except for the Lorz Italian cv which gave good true bulbs from both the non-chilled and chilled bulbs (Plate 1-B). The obtained true bulbs for all cultivars will be the source of clove-seeds for the next garlic cultivation seasons.

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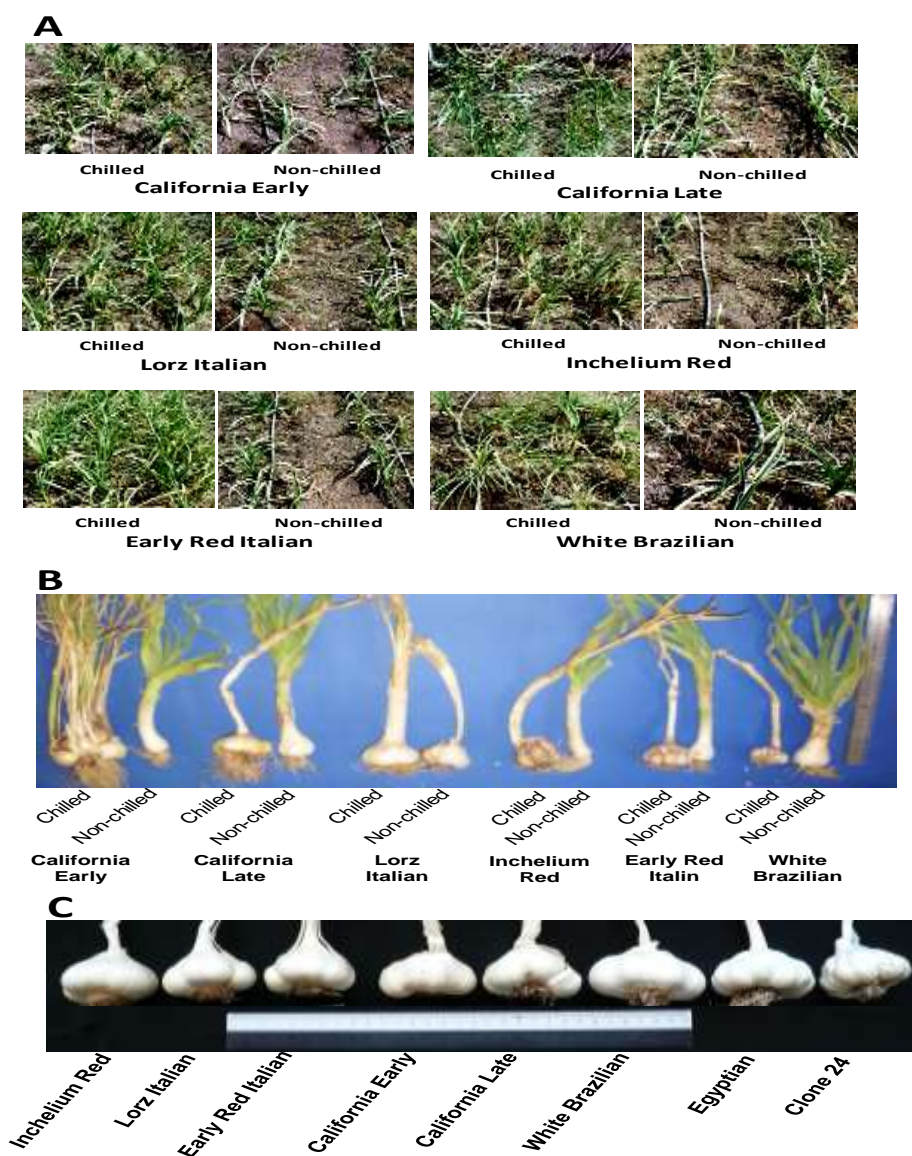


Plate 1: Photos of garlic plants of introduced cultivars obtained from pre-planting non-chilled and chilled cloves. A) Three-month-old plants from both non-chilled and chilled cloves. B) Harvested plants obtained from both non-chilled and chilled cloves. C) Photos of genotypes cultivars from which cloves were taken to be used in this experiment in addition to the Egyptian and Clone 24 “selected from a Chinese red colored cultivar grown in Egypt” (Osman and Moustafa, 2009).

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Bulb properties and qualities

Bulb diameter; neck diameter and bulbing ratio

Pre-planting chilling treatment of garlic cloves of all tested cultivars generally resulted in significant lower values of bulb neck diameter (except for the Lorz Italian cv in both seasons and the Inchelium Red cv in the second season Early) and bulbing ratio. Also, resulted in higher values of bulb diameter with an average of increase (8.1. % in the first season) and (9.1. % in the second season) comparing to that of bulbs obtained from non-treated cloves of all tested cultivars (Fig 2 and its included Table). These characteristics are desirable in garlic production, bulbs quality and bulbs storability.

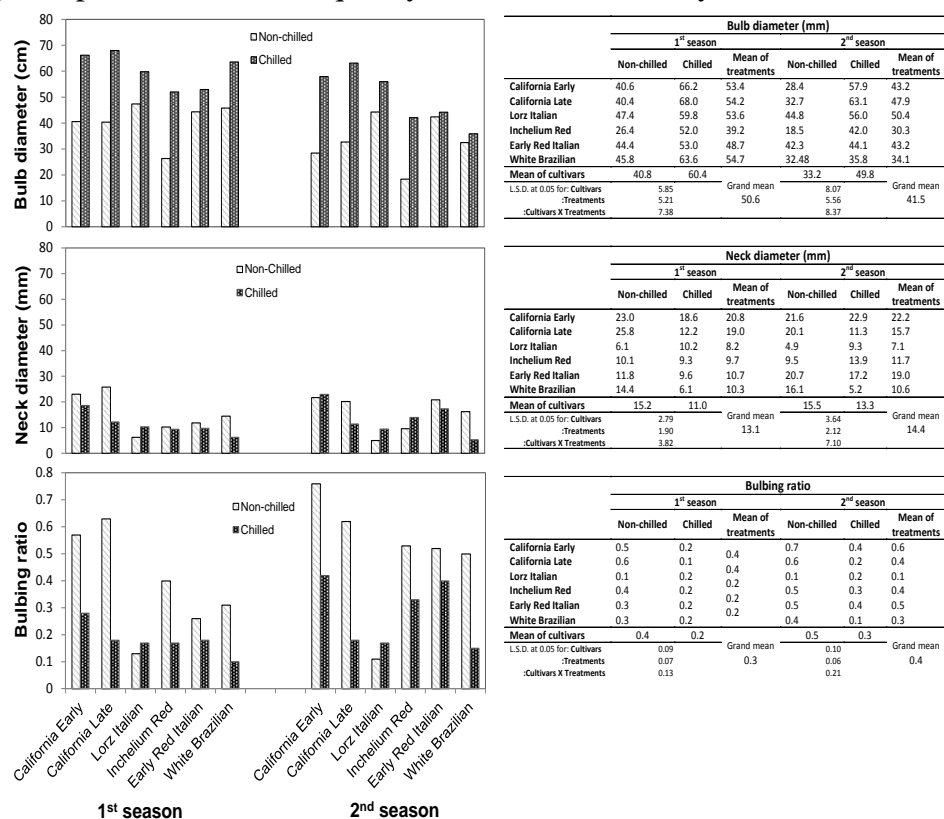
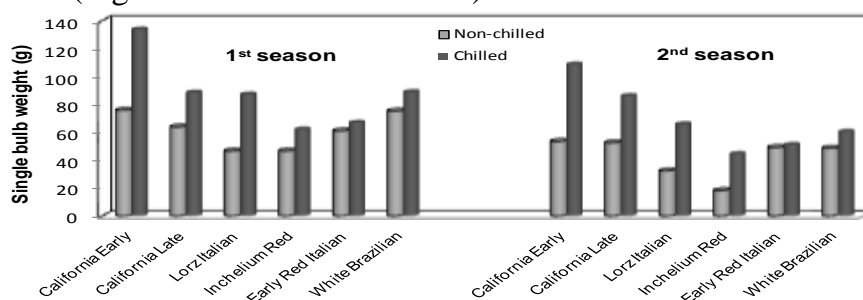


Fig 2: Plant neck diameter (mm), bulb diameter (mm) and bulbing ratio of pre-planting non-chilled and chilled cloves of the six imported garlic genotypes grown in two successive seasons (2009/2010 and 2010/2011).

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Single bulb weight (g)

Data in Fig 3 and the included Table showed that clove-pre-planting chilling treatment significantly affected the single bulb weight of all tested garlic genotypes. Generally, plants obtained from the treated cloves gave higher values of single bulb weight than those of the non-treated ones (87.9g vs 61.5 g) in the first season with 42.9% increase in bulb weights and (69.4g vs 42.3g) in the second season with 64.1% increase. In regards to the cultivars, California Early plants of the chilled cloves gave the highest values of single bulb weight in both seasons compared to the non-chilled ones (133.8g vs 75.8g) with an increase of 76.5% in the first season, respectively and (108.7g vs 53.4g) with an increase of 102.5% in the second season. On the other hand, plants obtained from the chilled cloves of the Early Red Italian cv gave the least values of single bulb weight compared to that of the non-chilled ones (67.0g vs 61.0g) in the first season with only 9.8% increase and (51.1 and 49.1g) in the second season with only 4.5% increase (Fig 3 and its included Table).



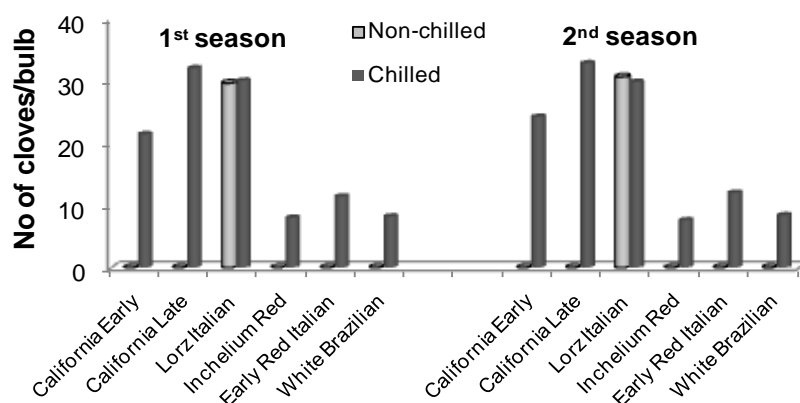
	Single bulb weight (g)					
	1 st season			2 nd season		
	Non-chilled	Chilled	Mean of treatments	Non-chilled	Chilled	Mean of treatments
California Early	75.8	133.8	104.8	53.4	108.7	81.1
California Late	63.8	88.6	76.2	52.4	86.1	69.3
Lorz Italian	46.6	87.2	66.9	32.3	65.9	49.1
Incheilium Red	46.6	62.2	54.4	18.2	44.5	31.4
Early Red Italian	61.0	67.0	64.0	49.1	51.1	50.1
White Brazilian	75.2	89.0	82.1	48.4	60.4	54.4
Mean of cultivars	61.5	87.9		42.3	69.4	
L.S.D. at 0.05 for: Cultivars	9.09		Grand mean	16.9		Grand mean
Treatments	7.96		74.7	13.4		55.9
Cultivars X Treatments	9.89			23.6		

Fig 3: Single bulb weight (g) of pre-planting non-chilled and chilled cloves of the six imported garlic genotypes grown in two successive seasons (2009/2010 and 2010/2011).

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Number of cloves/bulb

The obtained data in Fig 4 and the included Table showed that it was very crucial to apply pre-planting cold treat the cloves of all introduced and tested genotypes in order for their bulbs to form cloves. Otherwise, it could not be easy to get true garlic bulbs with cloves to produce and sustain the germplasm of these genotypes. As plants of the non-chilled planted cloves did not form true bulbs or formed spongy bulbs with no cloves except for the Lorz Italian cv which gave good true bulbs containing cloves of both its pre-planting treated and non-treated cloves with high values of number of cloves/bulb (29.6 vs 30.0) in the first season and (30.6 vs 29.8) in the second season, respectively. The other tested cultivars gave true bulbs containing cloves only from plants obtained from treated cloves.



	No of cloves/bulb					
	1 st season			2 nd season		
	Non-chilled	Chilled	Mean of treatments	Non-chilled	Chilled	Mean of treatments
California Early	0.0	21.4	10.7	0.0	24.2	12.1
California Late	0.0	32.0	16.0	0.0	32.8	16.4
Lorz Italian	29.6	30.0	29.8	30.6	29.8	30.2
Incheilium Red	0.0	8.0	4.0	0.0	7.6	3.8
Early Red Italian	0.0	11.4	5.7	0.0	12.0	6.0
White Brazilian	0.0	8.2	4.1	0.0	8.4	4.2
Mean of cultivars	4.9	18.5	Grand mean	5.1	19.1	Grand mean
L.S.D. at 0.05 for: Cultivars		1.38			0.77	
Treatments		0.84			0.83	
Cultivars X Treatments		2.14			1.32	

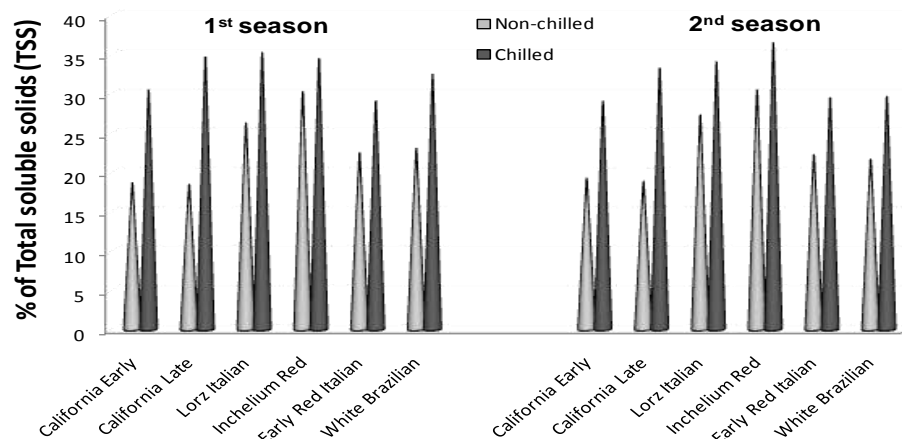
Fig 4: Clove germination % of pre-planting non-chilled and chilled cloves of the six imported garlic genotypes grown in two successive seasons of 2009/2010 and 2010/2011.

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Bulbs of the California Late cv gave the highest number of cloves/bulb obtained only from pre-planting treated plants with cold treatment (32.0 vs 32.8 cloves in the first and second season, respectively). On the other hand, bulbs of the Inchehium Red cv had the lower number of cloves/bulb and these bulbs were only those obtained from plants from pre-planting chilled cloves (8.0 and 7.6 cloves/bulb) in the first and second season, respectively (Fig 4 and its included Table).

Total soluble solids (TSS) content (%)

By determination of the TSS content in cloves of the obtained bulbs of both chilling treated and non-treated garlic plants, it was clear that the pre-planting clove chilling treatment resulted in an increase in this content for all the introduced and tested garlic cultivars with different percentages of increase (Fig 5 and its included Table).



	Total soluble solids (TSS)					
	1 st season		Mean of treatments	2 nd season		Mean of treatments
	Non-chilled	Chilled		Non-chilled	Chilled	
California Early	18.6	30.4	24.5	19.2	29.0	24.1
California Late	18.4	34.6	26.5	18.8	33.2	26.0
Lorz Italian	26.2	35.2	30.7	27.2	34.0	30.6
Inchehium Red	30.2	34.4	32.3	30.4	36.4	33.4
Early Red Italian	22.4	29.0	25.7	22.0	29.4	25.7
White Brazilian	23.0	32.4	27.7	23.6	29.6	26.6
Mean of cultivars	23.1	32.6		23.5	31.9	
L.S.D. at 0.05 for:						
Cultivars	1.56		Grand mean	1.94		Grand mean
Treatments	0.96			1.74		
Cultivars X Treatments	3.09			NS		

Fig 5: Cloves content of the total soluble solids (TSS) % of pre-planting non-chilled and chilled cloves of the six imported garlic genotypes grown in two successive seasons of 2009/2010 and 2010/2011.

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The TSS average content was 23.1% vs 32.6% of non-treated and treated bulbs in the first season (with 41.1% increase) and 23.5% vs 31.9% in the second season (with 36.3% increase). Moreover, bulbs of the California Late cv showed the highest values of TSS content (18.4% and 34.6%) of bulbs obtained from non-treated and chilling treated plants in the first season (with 86.1% increase) and (18.8% and 33.2%) of bulbs obtained from non-treated and chilling treated plants in the second season (with 76.6% increase). On the contrary, the least increase percentage in the TSS content was shown by the Inchelium Red cv (13.9% and 19.7%) in the first and second seasons, respectively as this cultivar contained the highest values of TSS content for both treated and non-treated plants (32.3% and 33.4%) in the first and second season, respectively comparing to the other cultivars (Fig 6 and its included Table).

DISCUSSION

Results showed that the pre-planting garlic clove treatment at 4°C for three weeks enhanced bulb formation and also cloving process as plants obtained from the non-treated cloves did not form bulbs or formed fleshy bulbs which turned into the spongy texture after curing without forming any cloves. Hence, it appears that pre-plant chilling is not only essential for bulb formation in garlic, but also for cloving. Therefore, the factors such as pre-chilling that are required for cloving are not necessarily the same as those required for bulbing. Further studies are needed to verify this concept. Moreover, Mann and Minges (1908) claimed that pre-plant chilling is a prerequisite for bulb formation. An in vitro study by Nagakubo *et al.* (1993) indicated that pre-plant chilling was essential for bulb formation only in late-maturing cultivars. In other studies, phenological and physiological changes in garlic in response to pre-plant chilling and photoperiod have previously been studied (Mann and Minges 1908; Del Pozo *et al.* 1997).

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Furthermore, seedling emergence, leaf and root growth, and clove initiation have all been enhanced by low temperature treatment (Rahim and Fordham 1988; Del Pozo et al. 1997; Rahim and Fordham, 2001; Rahman et.al., 2003).

Also, results in this study showed that garlic cloves pre-planting cold treatment resulted in vigorous plant growth and bulbs with higher values of bulb diameters and this could be explained that the cold treatment increased the cell size of cold-treated cloves than that of the non-cold treated (control) cloves (Rahim and Fordham, 1988) and also, suggested that gibberellin production may have been increased by the chilling treatment, proving a possible explanation for cell expansion. Furthermore, reports have shown that cooler temperatures are required to enhance shoot growth (Bhuiya *et al.*, 2003; Ade-Ademilua *et al.*, 2009) while higher light intensity help to enhance bulb growth (Argaello *et al.*, 1997). Results of this experiment showed that cold pre-treatment of garlic enhanced shoot growth, bulbing and cloving formation, bulb quality and bulb yield.

Some Egyptian garlic genotypes (e.g., Egyptian, Sids², and Egaseed¹) cultivars were pre-planting clove chilling treated or non-treated in this experiment but the treated cloves germinated faster and their plants formed bulbs very early than usual and gave smaller bulbs when compared with those of the non-treated ones (Data not shown).

From the aforementioned results, it could be concluded that the pre-planting chilling treatment of garlic cloves at 4°C for three weeks enhances the introduction and breeding program of introducing novel garlic genotypes from different regions of the world with different environmental conditions. The cold treatment applied in this study enhanced the garlic cloves of the genotypes used in this study to form bulbs and make true cloves, otherwise, it could not be easy to continue the introduction and evaluation program of these genotypes and sustain their germplasm along with the progress obtained in getting good garlic cured bulbs with high yield and good storability. This will

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enhance the adaptation and future utilization of these newly introduced garlic genotypes and spread their cultivations in the Egyptian garlic plantations.

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Pre-planting clove chilling enhanced

تحسين برنامج إنتخاب أصناف الثوم المستوردة بواسطة معاملة الفصوص قبل الزراعة بدرجات الحرارة المنخفضة تحت نظام الري بالتنقيط

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فى برنامج لتربية وتحسين الثوم تم إستيراد ستة أصناف أجنبية من الثوم وهى: (California Early, California Late, Lorz Italian, Inchelium Red, Early Red Italian and White Brazilian) والتي تنتج فى مناطق مختلفة من العالم لزراعتها وتقييمها تحت ظروف منطقة مصر الوسطى وعلى وجه الخصوص فى المزرعة البحثية والتعليمية بكلية الزراعة بجامعة المنيا. وتم عدم تعريض أو تعريض بعض الفصوص من الأبصال المنتخبة من كل الأصناف التى تم إستيرادها لدرجة حرارة 4°م لمدة ثلاثة أسابيع متواصلة فى الثلاجة، فأظهرت النتائج أن عملية تعريض الفصوص للمعاملة بدرجات الحرارة المنخفضة قبل زراعة الفصوص أدت الى تحسين إنبات الفصوص، تحسين النمو الخضرى للنباتات، تكوين الأبصال وتحسين خواصها وتكوين الفصوص بداخلها الى جانب زيادة محتوى الفصوص من المواد الصلبة الذائبة الكلية بالمقارنة بالنباتات الناتجة من الفصوص الغير معاملة والتي لم يتم الحصول منها على أبصال أو فصوص. وساعدت أيضاً هذه المعاملة بدرجات الحرارة المنخفضة على نجاح برنامج الإستيراد لهذه الأصناف الجديدة المستوردة من الثوم والمحافظة على التقاوى اللازم زراعتها فى الأعوام القادمة من أجل إستكمال برنامج أقرمتها وتقييمها تحت ظروف المنيا ونشر زراعتها فى مصر.