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Full length article

Low temperature exposure of garlic cloves: Effects on growth and yield of non-bolting and bolting genotypes

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ABSTRACT

In garlic, the main phenological stages such as clove sprouting, emergence, bulb initiation and maturation are mainly controlled by temperature. This research was conducted to evaluate the effect of low temperature treatments for different durations (0, 2, 4, 6 weeks) on the growth, bulb formation and yield of two local garlic cultivars ('Gravina' as non-bolting and 'Rosso di Sulmona' as bolting one) over two growing seasons (2016–2017 and 2017–2018). The results indicated that cultivar, chilling duration and experimental year significantly influenced garlic bulbing and yield. The highest bulb diameter (55.6 mm), bulb weight (50.2 g), bulblets weight (4.0 g), and bulb yield (11.5Mg ha^{-1}) were reached in the second year by the 'Gravina' cultivar treated for 4 weeks at low temperature before planting. In general, in the first year, production was higher with the use of bulbs refrigerated for 4 and 6 weeks, while in the second year the pre-treatments for 2 and 4 weeks were more effective. Principal component analysis (PCA) highlighted a clear separation between the two cultivars. 'Gravina' showed a stronger response in terms of morphological and productive parameters to pre-planting treatments, especially exposing cloves to longer chilling time than 'Rosso di Sulmona'. The results indicated the importance of low temperature to promote the growth of garlic and increase the bulb size and yield.

1. Introduction

Garlic (*Allium sativum* L.) belongs to the genus *Allium* within the family Amaryllidaceae. Together with onion (*Allium cepa* L.), garlic is a widely used vegetable thanks to the presence of bioactive compounds (i.e. flavonoids, phenolic compounds and nitrogen oxides) which give particular flavours to foods and health benefits (Ansary et al., 2020; Li et al., 2022). Allicin (allyl disulfide oxide) is the most abundant bioactive compound, responsible for the characteristic smell and taste of garlic, alongside its antibacterial, antifungal and therapeutic properties (reduction of triacylglycerol, anti-inflammatory and anti-cancer) (Borlinghaus et al., 2014; Parreño et al., 2023).

In 2023, the world production of garlic was 28.67 million Mg from 1689,758 ha harvested (FAOSTAT, 2023). China is the largest producer reaching about 73 % of the world's total production (20.74 million Mg), followed by India, Bangladesh, Egypt, Spain, Korea, Ethiopia, Uzbekistan, the United States of America and Myanmar (FAOSTAT, 2023). In some countries, garlic production has been reduced due to biotic and abiotic factors (i.e., climate change, diseases, inappropriate agronomic management), postharvest conditions, high cost of labour and agricultural materials (Galgaye, 2023). Europe recorded a 28 % reduction in the area cultivated with garlic over the last twenty years. In Italy, the production of garlic has undergone to a 47 % decline from 6400 ha producing 57,400 Mg in 1961–3380 ha and 26,730 Mg in 2023 (FAOSTAT, 2023). In the Russian Federation, the harvested area decreased from 25,734 ha in 2000–15,681 ha in 2023 (FAOSTAT, 2023).

Genotypes of garlic produce flowers only in specific environmental conditions and are classified as complete bolting, incomplete bolting (semi-bolting) and non-bolting (Kamenetsky and Rabinowitch, 2001; Kamenetsky et al., 2004a). Garlic propagation is conducted vegetatively using cloves, small bulbs covered by tunics of different colours (white, reddish or purple), whose size is one factor which particularly influences the garlic yield (Desta et al., 2021). The cold storage of propagation material, widely used in floriculture to force ornamental bulbous plants, to anticipate flowering and increase flower quality, has found little use in the horticultural sector for species that propagate through underground organs. Several studies have reported that low-temperature storage of garlic cloves can influence dormancy release, plant growth, bulbing and yield (Desta et al., 2022; Lu et al., 2022). In particular, low-temperature storage (5 °C) of cloves can: i) reduce the dormant

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period by anticipating the emergence of the plants, ii) lower the percentage of failures in the field, iii) stimulate inflorescence, leaf and root growth, iv) induce earlier bulb formation and maturation, v) increase the bulb diameter and bulb weight per plant, vi) enhance the polyphenolic content (Youssef, 2013; Rohkin Shalom et al., 2015; Ben Michael et al., 2018, Herrera et al., 2021). In addition, low temperatures can cause axillary bud sprouting (Kamenetsky et al., 2004b; Mojtahedi et al., 2013). Wu et al. (2015) studied the response of chilling treatment on cloves by evaluating three temperatures (5, 10, 15 °C) performed for different durations (20, 40, 60 days). This research reported that a 5 °C temperature that lasted 60 days had the best response on plant height, leaf sheath length and leaf sheath diameter. On the contrary, when the chilling duration was of 20 days, the main effects were on bulb yield and diameter. Moreover, Volk et al. (2004) highlighted that the response to cold-storage conditions depended on the cultivar, showing that the cultivars such as 'Spanish Red' and 'Polish Hardneck' were sensitive to storage at low temperatures and formed rounds, small and single-cloved garlic bulbs, instead of standard cloves when planted in the spring. Nevertheless, information on the comparative response of non-bolting and bolting genotypes to such treatments remains limited. This aspect deserves further investigation, since the two types are characterized by different developmental patterns but are both important for garlic cultivation in Mediterranean environments. In this framework, the aim of the present study was to evaluate the effects of pre-planting low--temperature treatments on: i) plant growth, ii) bulbing process and iii) yield of two Italian garlic cultivars with contrasting bolting behaviours: 'Gravina' (non-bolting) and 'Rosso di Sulmona' (bolting).

2. Material and methods

2.1. Experimental site and design

Research was carried out on garlic in 2016–17 and 2017–18 in the countryside of Matera in Southern Italy (40° 42' N; 16° 42' E; 385 m above sea level), in fine loamy soil (sand 35 %, silt 37 %, clay 28 %), with a sub-alkaline reaction (pH 8.3), $EC_{1:5}$ 0.28 dSm^{-1}, organic carbon 0.75 %, organic matter 1.29 % (Walkley and Black method), CEC 14.4 cmol ($^{+}$) kg^{-1}, with 15.6 % content of active limestone (B2 method), N 1.14 g kg^{-1} and available P 20 mg kg^{-1}. All soil analyses were performed according to Italian regulations (Ministero per le Politiche Agricole, 1999).

Before planting, the soil was ploughed at 40 cm depth, milled and fertilized with 100 kg ha^{-1} of P_2O_5 and 120 kg ha^{-1} of K_2O, and then furrowed. Bulbs of two garlic cultivars belonging to the *Sativum* group, the non-bolting 'Gravina', and the bolting 'Rosso di Sulmona', were planted on November 29th 2016 and December 5th 2017 in the first and second research year, respectively. The bulbs were collected from the Garlic Collection of the Vegetables and Floriculture section of the Department of Agricultural, Forestry, Food and Environmental Sciences (DAFE), University of Basilicata. Each year, bulbs were left to dry in the field for 3–4 days, and afterwards they were transferred to a conditioned cell (GDH-H 500, Falc Instruments s.r.l., Treviglio, Bergamo, Italy) set at a temperature of 23 ± 2 °C. A part of the bulbs remained in the aforementioned cell until the time of planting. The remainder aliquot was split in three fractions and refrigerated at 5 ± 0.2 °C for 2, 4 or 6 weeks before planting. The experimental protocol was based on the factorial combination between two garlic cultivars ('Gravina' and 'Rosso di Sulmona') and four chilling durations: i) no chilled bulbs ('0') as a control, ii) 2 weeks chilled bulbs ('2'), iii) 4 weeks chilled bulbs ('4'), iv) 6 weeks chilled bulbs ('6'). Bulblets of uniform size and weight (2.5 ± 0.2 g) were selected and planted at 7–8 cm depth, spaced 10 cm along the rows which were 40 cm apart, achieving a density of 25 plants m^{-2}. A split plot design was used for the treatment distribution in the field, with three repetitions, placing the two garlic cultivars ('Gravina' and 'Rosso di Sulmona') in the plots and the chilling duration (0, 2, 4 and 6 weeks) in the sub-plots. The cultivation was carried out following the standard agronomic techniques adopted by garlic growers in Matera. In particular, nitrogen fertilization was carried out by supplying 80 kg ha^{-1} of ammonium sulphate in two stages. During the winter and the spring weed control was carried out by hand and hoeing operations. The harvesting was carried out at the physiological maturation stage in June in both research years.

2.2. Meteorological data

Daily rainfall and mean temperature were monitored during the evaluation period (Fig. 1) by a Davis Vintage Pro weather station equipped with air temperature and rain collector probes (Davis Instruments, Hayward, CA, USA). In the first research year (Fig. 1a), the total accumulated precipitation amounted to 365.6 mm. The highest daily rainfall was recorded on 10 February 2017, with 37.2 mm. The average daily air temperature over the entire period was 12.4 °C. The minimum temperature was −3.9 °C, recorded on 7 January 2017, while the maximum reached 28.9 °C on 29 June 2017. Over the second research year (Fig. 1b), total precipitation amounted to 441.0 mm. The maximum daily rainfall was observed on 22 March 2018, with 45.6 mm. The average daily air temperature throughout the period was 12.4 °C. The lowest temperature was −2.5 °C, recorded on 27 February 2018, while the highest was 25.7 °C, reached on 11 June 2018.

2.3. Morphological traits

At each harvest time, in a test area of 3.6 m^2 per plot, the number and weight of marketable bulbs were recorded. Samples of 10 bulbs per plot were characterized according to the following parameters: mean weight, height and diameter of the bulb, mean weight and number of bulblets per bulb, diameter of the false stem ('neck'), bulbification ratio ('neck' diameter/bulb diameter), dry matter content (%). The latter parameter was calculated through drying representative samples of bulbs in a ventilated oven at 65 °C until a constant weight was reached. Furthermore, per each test area, the fresh (g m^{-2}) and dry biomass (g m^{-2}) were measured for the epigeal part (leaves, 'necks' and floral scapes if present) of the plants and for the bulbs. Therefore, the total dry biomass (g m^{-2}) was then calculated by adding the value of the bulbs to that of the aboveground components. The harvest index (HI) was also calculated as the ratio between bulb dry biomass and total dry biomass.

2.4. Statistical analysis

The data were first tested for normality and homogeneity of variance using the Shapiro-Wilk and Bartlett's tests, respectively. Then, the data were analysed with a two-way analysis of variance (ANOVA), in each research year, with 'cultivar' and 'chilling duration' as sources of variation. Mean values were separated using the Student-Newman-Keuls (SNK) post-hoc test, at $p \leq 0.05$. Furthermore, to better evaluate the correlations between the experimental treatments and some morphological traits of garlic, a principal component analysis (PCA) was performed. Statistical analyses were performed using the software RStudio: Integrated Development for R, version 4.2.2.

3. Results

3.1. Influence of cultivar and chilling duration on morphological traits

Table 1 highlights the effects of cultivar, chilling duration and their interaction during the two crop cycles on morphological parameters. In the first research year, morphological traits, except for the bulb mean weight, bulb height and bulbing, were significantly influenced by cultivar (Table 1). The highest value of bulb diameter, neck diameter and number of bulblets per bulb were reached by 'Gravina', while the highest bulblets mean weight was attained by 'Rosso di Sulmona'. In the second research year, only the bulb mean weight, bulb diameter and

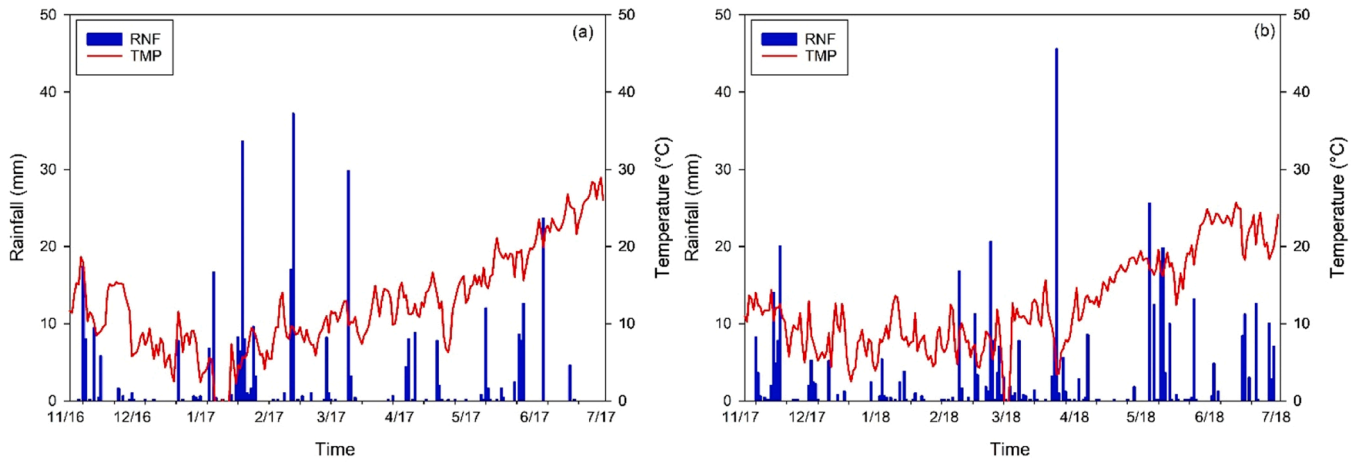


Fig. 1. Rainfall (RNF) and temperature (TMP) trends at the experimental site recorded during the first (a) and second (b) research year.

Table 1
Morphological parameters of garlic in response to cultivar and clove chilling duration in the two research years.

Treatments[1,2]	Bulb Mean Weight (g)	Bulb Height (mm)	Bulb Diameter (mm)	Neck Diameter (mm)	Bulblets per bulb (n.)	Bulblets Mean Weight (g)	Bulbing Ratio
First year (2016–2017)							
Cultivar (A)							
'Gravina'	33.0	28.4	48.4 a	7.7 a	14.7 a	2.4 b	0.16
'Rosso di Sulmona'	32.4	28.8	42.3 b	6.8 b	10.4 b	2.9 a	0.16
Significance	*ns*	*ns*	**	*	**	*	*ns*
Chilling duration (C)							
'0'	31.5 b	28.5	45.5	7.3	12.3	2.6 b	0.16
'2'	30.5 b	28.1	45.4	7.3	13.2	2.4 b	0.16
'4'	34.3 a	29.1	46.1	7.8	12.4	2.9 a	0.17
'6'	34.4 a	28.9	44.5	6.7	12.2	2.7 ab	0.15
Significance	*	*ns*	*ns*	*ns*	*ns*	*	*ns*
A×C	*	*ns*	*	*ns*	*	*	*ns*
Second year (2017–2018)							
Cultivar (A)							
'Gravina'	48.0 a	36.7	54.2 a	8.7	14.5	3.6 a	0.16
'Rosso di Sulmona'	39.9 b	34.2	48.9 b	7.8	13.1	3.1 b	0.16
Significance	**	*ns*	**	*	*ns*	*	*ns*
Chilling duration (C)							
'0'	43.1 b	35.4	51.0	7.7	15.2 a	3.1 b	0.15
'2'	44.6 a	34.8	52.3	7.8	14.5 b	3.2 b	0.15
'4'	44.2 a	36.2	51.7	8.8	13.1 bc	3.6 a	0.17
'6'	43.8 ab	35.4	51.2	8.2	12.5c	3.6 a	0.16
Significance	*	*ns*	*ns*	*ns*	**	*	*ns*
A×C	**	*ns*	**	*ns*	*ns*	*	*ns*

[1] Values followed by different letters are significantly different at $p \leq 0.05$, according to SNK test. [2] *, significance at $p < 0.05$; **, significance at $p < 0.01$; ns, no significant difference.

neck diameter were significantly influenced by cultivar and, similar to the results obtained in the first research year, 'Gravina' produced larger bulbs than 'Rosso di Sulmona'. Regarding the clove chilling duration, the 4-week treatment obtained the best performance in terms of bulb and bulblets mean weight, while the lowest values were observed in control plants (Table 1).

3.2. Influence of cultivar and chilling duration on productive traits

The effects of cultivar, chilling time and their interaction on productive traits in the two research years are shown in Table 2. Data reports that during the first crop cycle, the quantitative parameters, except for the bulb yield, bulb dry matter (%) and harvest index, were significantly influenced by cultivars. 'Gravina' showed the highest values of bulb dry matter and total dry biomass. The treatment for 6 weeks led to the highest productive parameters, followed by the 4-weeks treatment (Table 2). In the second year, the behaviour of the cultivars was the same, and regarding the chilling duration, the highest production was reached with the 4-weeks treatment (Table 2).

3.3. Interaction effects of cultivar and chilling duration on morpho-productive parameters

In the first year, significant "cultivar x chilling duration" interactions were observed for morphological (bulb diameter, bulb weight, bulblets weight) and productive parameters (bulb dry matter, total dry biomass) whereas no statistically significant differences for bulb yield were noted (Fig. 2). Specifically, 'Gravina x 4' obtained the highest bulb diameter (48.8 mm) (Fig. 2a) whilst 'Rosso di Sulmona x 4' recorded the highest weight of bulb (35.9 g) and bulblets (3.2 g) (Figs. 2b and 2c). 'Gravina x 6' showed the highest bulb dry matter (456.4 g m^{-2}) and total dry biomass (562.5 g m^{-2}) (Figs. 2d and 2e). In contrast, the lowest values

Table 2
Productive parameters of garlic in response to cultivar and clove chilling duration in the two research years.

Treatments[1,2]	Bulb Yield (t ha^{-1})	Bulb Dry Matter (%)	Bulb Dry Matter (g m^{-2})	Total Dry Biomass (g m^{-2})	Harvest Index (HI)
First year (2016–2017)					
Cultivar (A)					
'Gravina'	8.2	38.3	426.7 a	522.0 a	0.82 a
'Rosso di Sulmona'	7.8	38.6	324.0 b	422.8 b	0.77 b
Significance	*ns*	*ns*	**	**	*
Chilling duration (C)					
'0'	7.7 ab	38.1	357.2c	430.0c	0.83 a
'2'	7.4 b	38.4	368.6 b	451.0 b	0.82 a
'4'	8.4 a	38.4	382.3 ab	495.8 a	0.77 ab
'6'	8.5 a	38.9	393.3 a	512.9 a	0.76 b
Significance	*	*ns*	**	**	*
A×C	*ns*	*ns*	**	**	*ns*
Second year (2017–2018)					
Cultivar (A)					
'Gravina'	10.8 a	35.8 a	570.6 a	754.2 a	0.76 a
'Rosso di Sulmona'	8.7 b	32.2 b	407.8 b	681.9 b	0.60 b
Significance	**	*	**	**	*
Chilling duration (C)					
'0'	9.3 b	38.2	317.0 b	379.2 b	0.84 a
'2'	9.9 a	38.9	305.0 b	371.0 b	0.82 a
'4'	10.0 a	36.5	453.1 a	613.8 a	0.73 b
'6'	9.6 ab	37.0	447.0 a	602.8 a	0.74 b
Significance	*	*ns*	**	**	**
A×C	*	*ns*	**	**	*ns*

[1] Values followed by different letters are significantly different at $p \leq 0.05$, according to SNK test. [2] *, significance at $p < 0.05$; **, significance at $p < 0.01$; ns, no significant difference.

were obtained by 'Rosso di Sulmona x 2', followed by 'Rosso di Sulmona x 0'. The highest values for all the investigated parameters were recorded by 'Rosso di Sulmona' treated at low temperature for 4 weeks. Also in the second research year, significant "cultivar × chilling duration" interactions were observed for morphological and productive parameters (Fig. 2). Specifically, 'Gravina x 4' showed the highest bulb diameter (55.6 mm), bulb weight (50.2 g), bulblets weight (4.0 g), bulb dry matter (580.9 g m^{-2}), total dry biomass (760.5 g m^{-2}) and bulb yield (11.5 t ha^{-1}) (Fig. 2). On the contrary, the lowest values of bulb dry matter (399.3 g m^{-2}) and total dry biomass (659.8 g m^{-2}) were obtained by 'Rosso di Sulmona x 6' and 'Rosso di Sulmona x 0', respectively (Figs. 2d and 2e).

3.4. Trend of leaf area index and bulbification of 'Gravina' and 'Rosso di Sulmona'

During the first crop cycle, the leaf area index (LAI) and the number of bulblets per plant were determined for the entire crop cycle, starting from 31/01/2017 (about 63 days after planting) to 1/07/2017 (about 200 days after planting) (Fig. 3). The highest LAI (1.2) was recorded by 'Gravina x 6' on April 26th, to decrease to 0.35 on May 24th (Fig. 3a). 'Rosso di Sulmona x 4' achieved its highest LAI (1.0) on May 9th and its lowest one (0.3) on June 7th (Fig. 3c). Regarding the number of bulblets per plant, the chilling treatment for 6 weeks allowed the best results in both cultivars. In fact, on May 9th 'Gravina' and 'Rosso di Sulmona' obtained their peak bulblets for plant equal to 16.7 and 12.9, respectively. Despite that, the cultivar 'Gravina' was more precocious compared to 'Rosso di Sulmona' with the bulbification started on March 29th, about one month earlier than 'Rosso di Sulmona' (April 26th) (Figs. 3b and 3d).

During the second experimental crop cycle, the above cited parameters were determined for all the crop cycle, starting from 06/02/2018–27/06/2018 (Fig. 4). 'Gravina x 4' obtained the highest LAI with a value of 1.24 on May 16th (Fig. 4a), while for 'Rosso di di Sulmona' the highest value (1.2) was reached later (on May 30th) by bulbs treated for

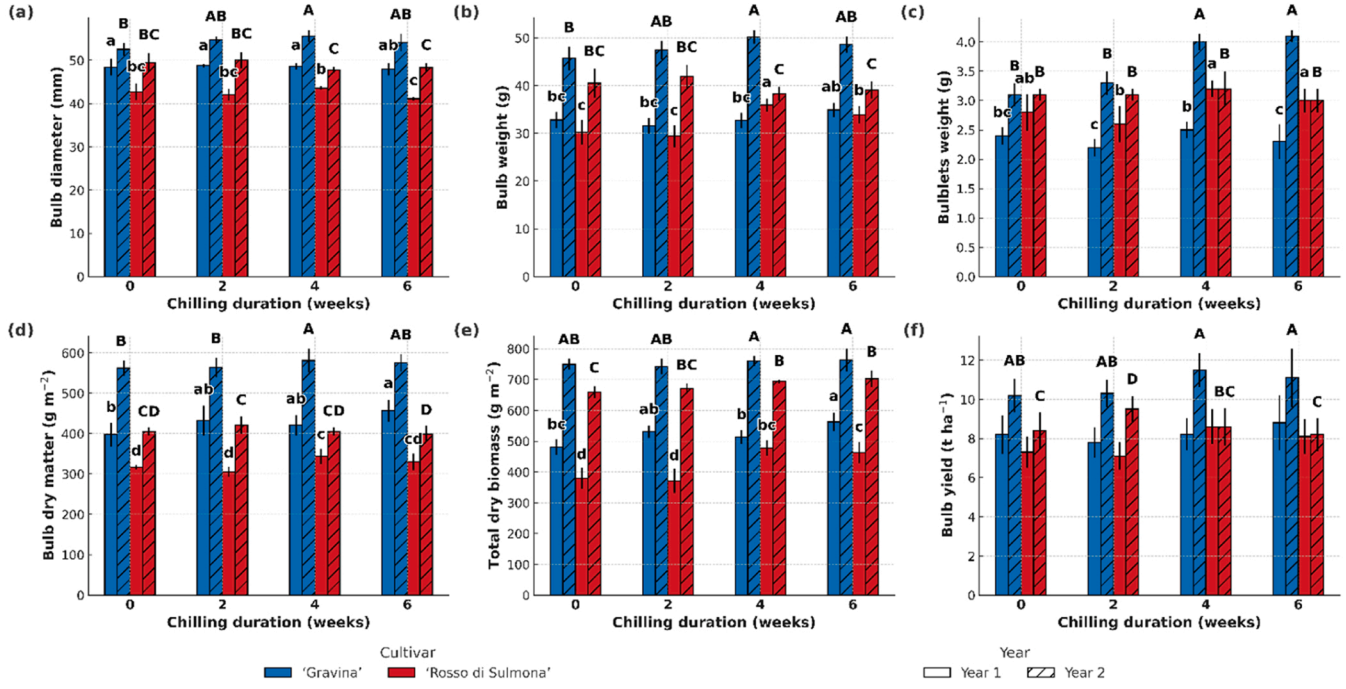


Fig. 2. Effects of "cultivar x chilling duration" interaction on morpho-productive parameters in two research years. Weeks of chilling duration = 0, 2, 4, 6. Blue = 'Gravina', red = 'Rosso di Sulmona'. Solid bars = Year 1, hatched bars = Year 2. Error bars represent means ± S.E. For each parameter and year, means followed by different letters are significantly different at $p \leq 0.05$ according to the SNK test. Lowercase and capital letters are used for the values of the 1st and 2nd research year, respectively.

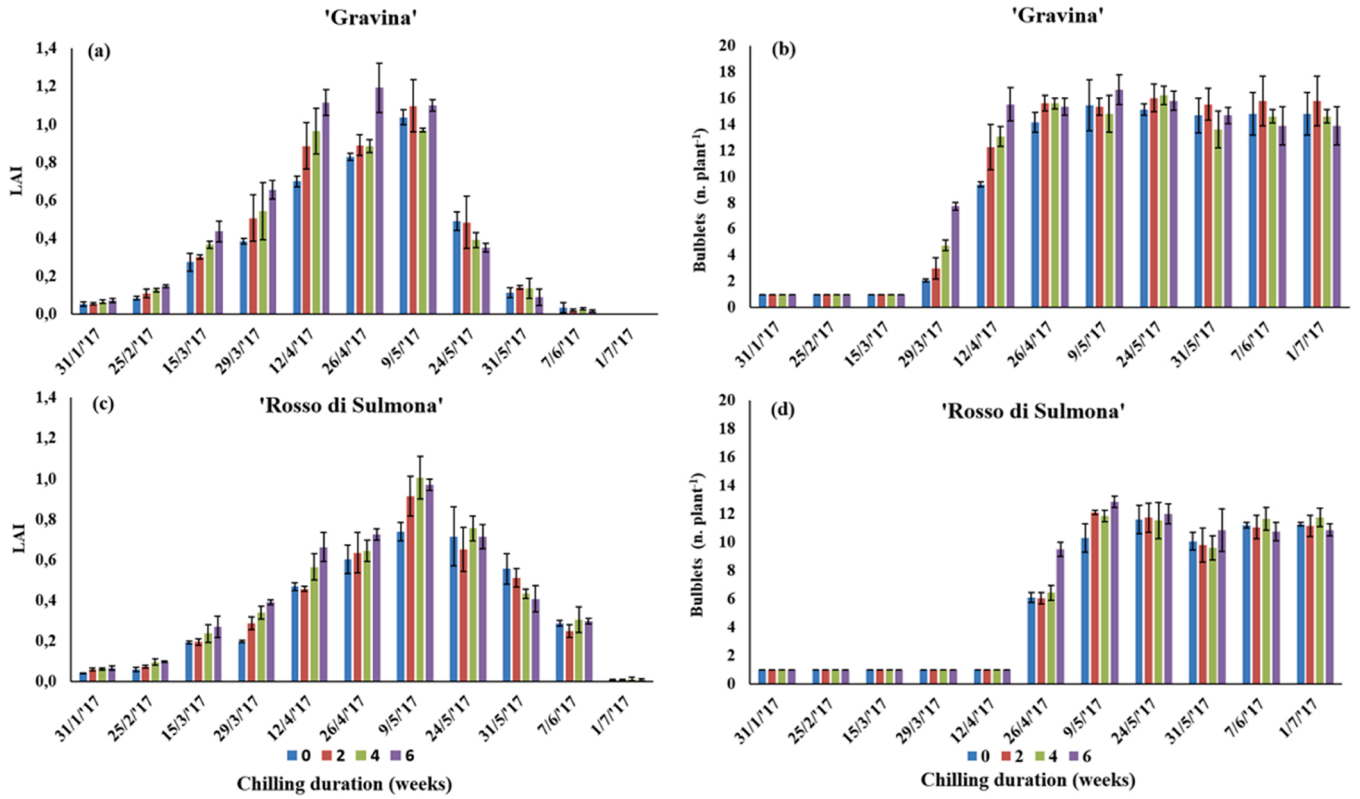


Fig. 3. Leaf area index and bulbification trends of 'Gravina' (a-b) and 'Rosso di Sulmona' (c-d) during the first crop cycle. Error bars either side of mean indicate + /- S.E.

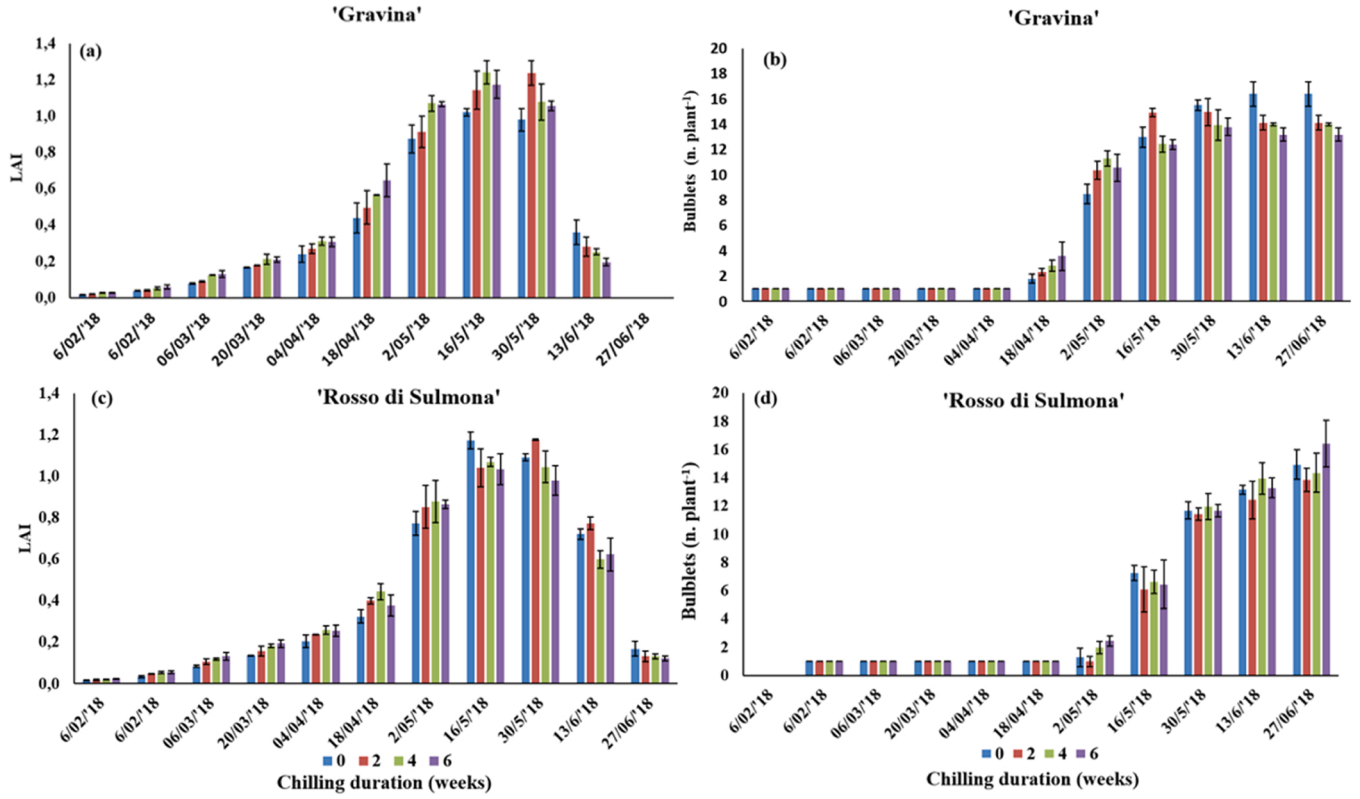


Fig. 4. Leaf area index and bulbification trends of 'Gravina' (a-b) and 'Rosso di Sulmona' (c-d) during the second year. Error bars either side of mean indicate + /- S.E.

2 chilling weeks (Fig. 4c). Regarding the phase of bulbification, in general the bulbs started the differentiation earlier than the first experimental year, on April 18th for 'Gravina' and on May 2nd for 'Rosso di di Sulmona'. 'Gravina' obtained the highest number of bulblets per plant from bulbs treated for 2 weeks, reaching a value of about 15 on May 30th (Fig. 4b), while 'Rosso di Sulmona' attained a value of 16.4 on June 27th from bulbs treated for 6 chilling weeks. Also, during the second year, 'Gravina' had the same behaviour, standing out as the earliest cultivar (Fig. 4d).

3.5. Principal component analysis (PCA)

To have an assessment of the interaction between the garlic cultivars and the chilling durations on the investigated productive and morphological parameters, a principal component analysis (PCA) was performed. The PCA was able to reduce the seven original variables in two principal components (PC1 and PC2), which together explained the 90.90 % of the total variance (Table 3).

PC1 (i.e., Dim1) is mainly determined by bulb dry matter (BDM), total dry biomass (TDB), bulb diameter (BD), bulb weight (BW), bulblets weight (BIW) and bulb yield (BY). These variables, grouped together and placed far away from the PC1 origin, are positively correlated with each other (Fig. 5). On the contrary, PC2 (i.e., Dim2) is essentially characterized by the harvest index (HI) (Fig. 5).

As shown in Fig. 6a, the major percentage contributions to PC1 are for BW, BY, TCB, BDM, and BD, that are above the red dashed line (i.e., the expected mean contribution) whilst BIW and, principally, HI are above the line. On the other hand, as reported in Fig. 6b, PC2 is mostly contributed by HI since all the other variables values are below the red line.

The score plot showed a good separation between the first and the second year of cultivation (Fig. 7a), and the two garlic cultivars (Fig. 7b) whilst, for the chilling duration, 0 and 2 weeks can be considered separately from 4 and 6 weeks (Fig. 7c). The first crop cycle was negatively linked to PC1 and positively correlated to PC2 whereas the second year was showed the opposite trend (Fig. 7a). This was because during the first year HI was in average higher than the second year. The latter, on the contrary, obtained best results in terms of all the other investigated morphological parameters and productive traits. Among the garlic cultivars, 'Gravina' was included in the upper right quadrant with positive values for PC1 and PC2 while 'Rosso di Sulmona' was placed in the opposite quadrant (Fig. 7b). This allows us to state that, between cultivars, 'Gravina' performed better than 'Rosso di Sulmona'. As reported in Fig. 7c, 0 and 2 weeks of chilling duration are in the upper left quadrant in which PC1 is negative and PC2 is positive whereas 4 and 6 weeks are in the facing quadrant therefore the latter ones are positively correlated to PC1 (Fig. 7c) leading better values of the recorded variables of garlic morphology and yield.

Table 3
Loadings of the significant variables on the two first principal components from data analysis.

Variables[1]	Principal Components	
	1	2
BDM	0.9143	0.3651
TDB	0.9458	−0.1885
HI	−0.3091	0.9279
BD	0.9091	0.2571
BW	0.9865	−0.0614
BIW	0.7908	−0.2571
BY	0.9564	0.1689
Eigenvalue	5.17	1.19
Total variance %	73.80	17.10

[1] BDM = bulb dry matter; TDB = total dry biomass; HI = harvest index; BD = bulb diameter; BW = bulb weight; BIW = bulblets weight; BY = bulb yield.

4. Discussion

The bulb development of garlic is mainly influenced by agronomic factors (i.e. clove weight, planting time, availability of nutrients, water presence in soil, growth regulators) and environmental ones such as temperature, photoperiod and storage conditions (Desta et al., 2021; Khokhar, 2023). Previous studies have indicated that low-temperature storage of cloves before planting contributes to dormancy release, early bulb formation and shorter harvest time (Del Pozo et al., 1997; Rahman et al., 2003; Rohkin Shalom et al., 2015; Guevara-Figueroa et al., 2015). Other research demonstrated the positive correlation between low temperature treatments of cloves and an increase in the production of gibberellin (Bandara et al., 2000; Wu et al., 2016) and secondary metabolites (Dufoo-Hurtado et al., 2013). In bolting genotypes, low temperature treatment decreases the transition of the apical meristem from the vegetative to the reproductive stage, enhancing the development of a flower stalk and inflorescence (Kamenetsky et al., 2004; Rotem et al., 2011).

In our research, low-temperature storage of bulbs intended for planting had positive effects on the morphological, physiological and productive parameters of garlic. However, the effectiveness of the pre-treatment was different in the two research years and varied in relation to the genotype and the duration of cold storage (Tables 1 and 2). Morphological traits such as bulb and bulblet weight proved to be particularly sensitive and positively affected by chilling duration (Table 1). The bulb size is an important characteristic for the market, as long as consumers tend to prefer garlic bulbs characterized by larger sizes and fewer cloves (Morais et al., 2023). In the present research, these parameters depended on genotype behaviour, with 'Rosso di Sulmona' showing higher productivity after shorter treatments (4 weeks) (Fig. 2). This confirms the observations of Youssef (2013), who reported an increase in the mean weight of bulbs at harvest by subjecting them to pre-planting low temperatures, 15℃ for 15 days for the cultivar "Egaseed1" and 10℃ for 21 days for "Clone17". In the first crop cycle of our study, the highest yield was obtained with the planting of refrigerated bulbs for 4 and 6 weeks, while in the second crop cycle the pre-treatments of 2 and 4 weeks were more effective (Table 2). Specifically, in the first year 'Gravina' showed a 7 % yield increase after 6 weeks of cold storage, while 'Rosso di Sulmona' responded more markedly, with an 18 % increase after 4 weeks compared to the control. In the second year, 'Gravina' exhibited an 11 % increase after 4–6 weeks of storage, whereas 'Rosso di Sulmona' benefited from shorter exposure (2 weeks), with a 13 % increase compared to the control. The positive effects of cold treatment could be due to the hydrolysis of starch and consequentially carbohydrate mobilization supplying the reserves for bulb development and photosynthetic activity (Desta et al., 2017) or the production of gibberellic acid and auxins, as reported by Kurtar and Ayan (2005). Our findings are in agreement with previous studies that confirmed the role of cold conditioning in enhancing garlic productivity (Khokhar, 2023). Ade-Ademilua et al. (2009) highlighted the importance of clove treatment at 8 °C for 15 days to obtain the highest bulb yield and clove size under open field conditions. Wu et al. (2015) suggested that the treatment of 5 °C for 20 days obtained the highest bulb yield, mean weight, diameter and height, compared to 40 and 60 days. Conversely, Dufoo-Hurtado et al. (2013) observed reduced growth, leaf number, plant weight and yield, together with enhanced synthesis of phenolic compounds and anthocyanins, when bulbs were exposed to 5 °C for 5 weeks, an effect likely linked to photosynthetic inhibition of photosystem II.

In our study, the low-temperature storage of bulbs elicited crop precocity, and this effect was already evident in the emergence phase and was clearly recorded starting from the first destructive samples made to evaluate the growth of the crop. In particular, in both crop cycles the emergence of the two cultivars, when subjected to cold storage treatments, occurred on average one week earlier than the control plants (data not shown). In mid-March of both years, it was possible to

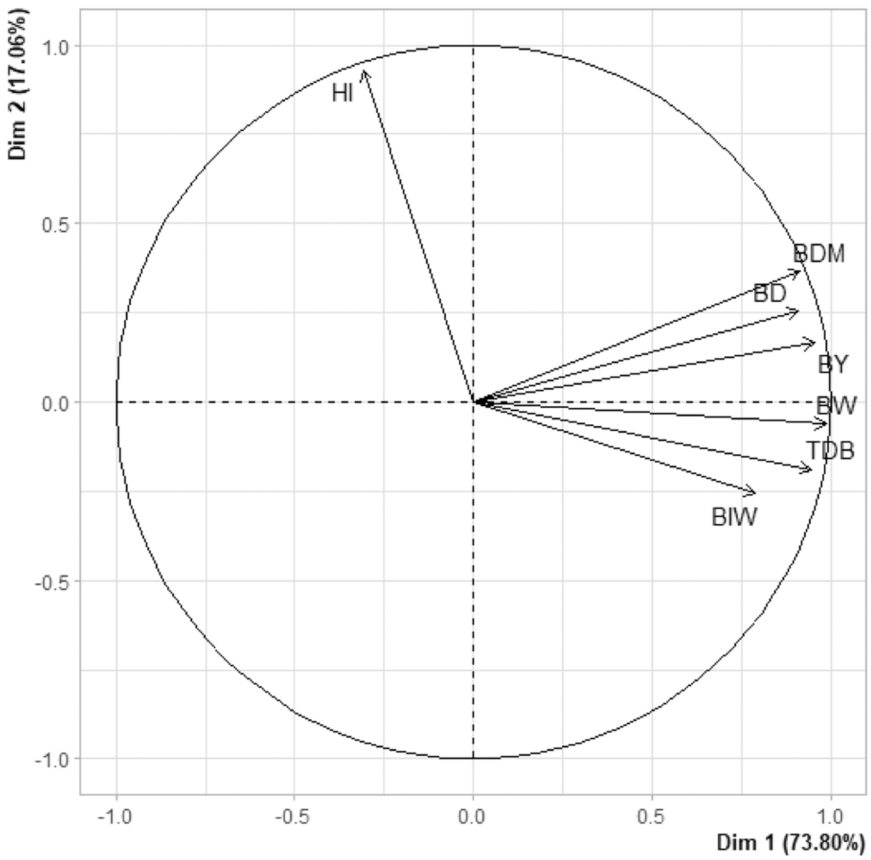


Fig. 5. Loading plot of variables contribution towards the first two principal components (BDM = bulb dry matter; TDB = total dry biomass; HI = harvest index; BD = bulb diameter; BW = bulb weight; BIW = bulblets weight; BY = bulb yield, Dim1 = PC1, Dim2 = PC2).

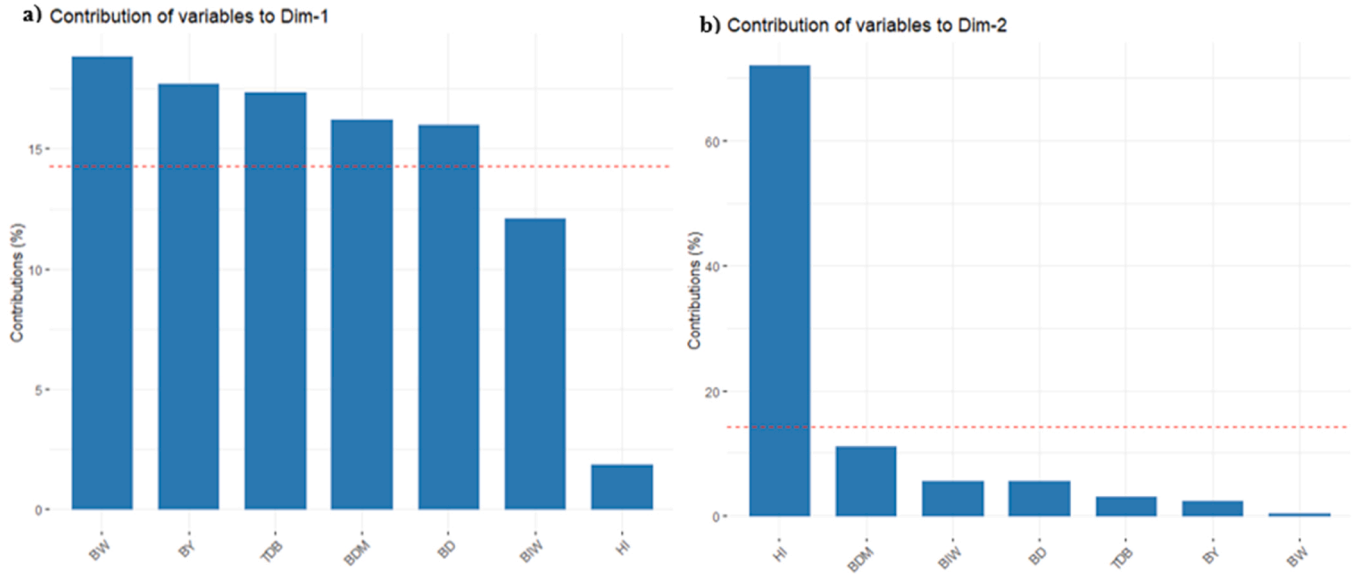


Fig. 6. a) Percentage contribution of variables to PC1; b) Percentage contribution of variables to PC2 (BDM = bulb dry matter; TDB = total dry biomass; HI = harvest index; BD = bulb diameter; BW = bulb weight; BIW = bulblets weight; BY = bulb yield; Dim1 = PC1, Dim2 = PC2; red dashed line = expected mean contribution).

note that 'Gravina' showed a greater growth rate than 'Rosso di Sulmona'. For both cultivars, cold storage pre-treatments allowed higher values of the growth from emergence to the advanced bulbification phase than the control ones. Therefore, the results are consistent with the findings of other authors (Rahim and Fordham, 1988) who demonstrated the effectiveness of low-temperature storage of bulbs to achieve anticipated emergence and improved plant growth. Also, Del Pozo et al. (1997) reported that the storage temperature of bulbs was genotype dependent, finding that 4 °C for 4 weeks reduced the time from planting to sprout emergence in 13 clones in a glasshouse experiment. The application of the treatment with low temperature is also recommended to stimulate the development of the plants when non-optimal

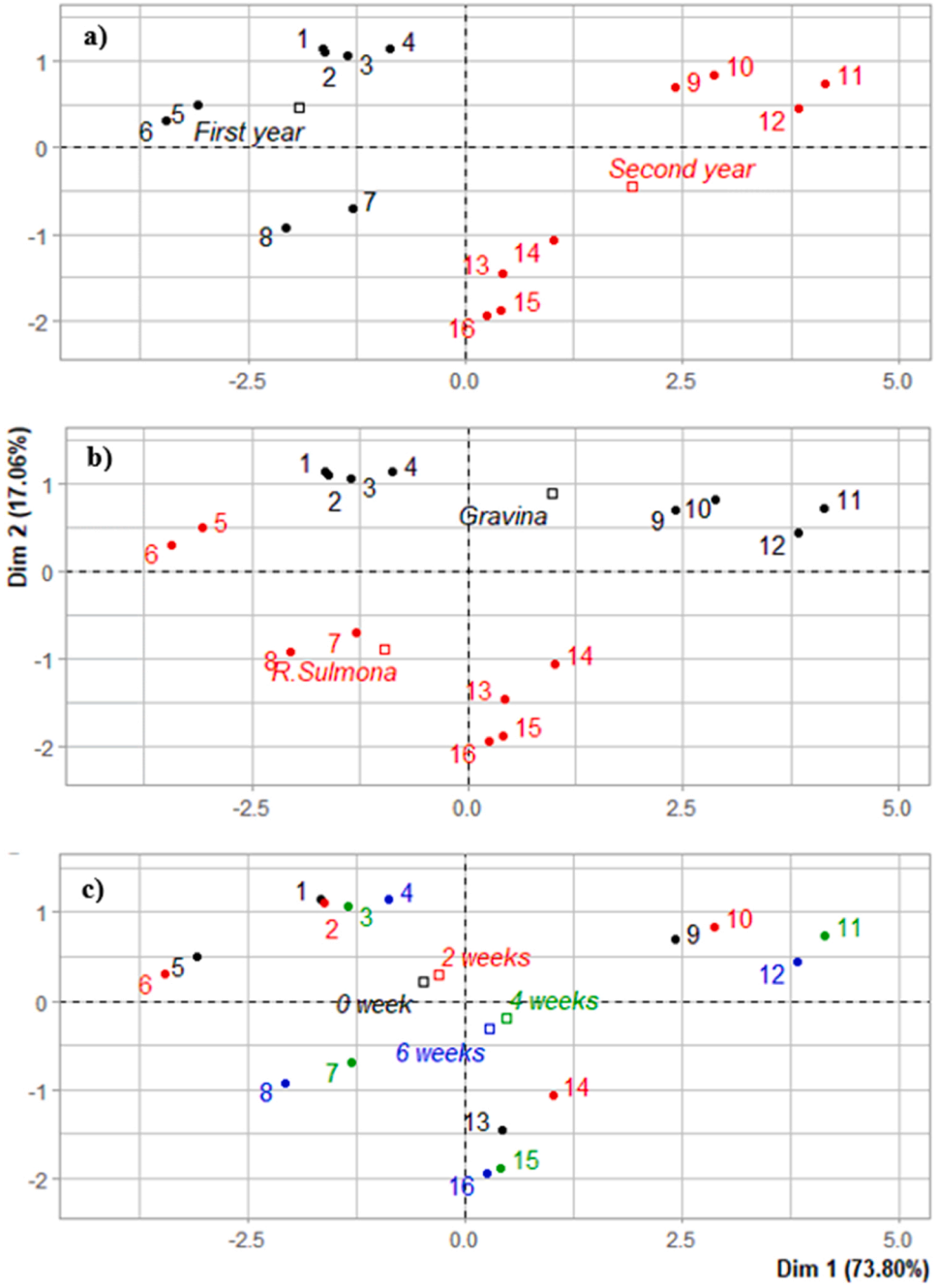


Fig. 7. Score plot showing the projection of the variables grouped by a) year of cultivation, b) cultivar type, c) chilling duration (1 = "Gravina x 0" first year; 2 = "Gravina x 2" first year; 3 = "Gravina x 4" first year; 4 = "Gravina x 6" first year; 5 = "Rosso di Sulmona x 0" first year; 6 = "Rosso di Sulmona x 2" first year; 7 = "Rosso di Sulmona x 4" first year; 8 = "Rosso di Sulmona x 6" first year; 9 = "Gravina x 0" second year; 10 = "Gravina x 2" second year; 11 = "Gravina x 4" second year; 12 = "Gravina x 6" second year; 13 = "Rosso di Sulmona x 0" second year; 14 = "Rosso di Sulmona x 2" second year; 15 = "Rosso di Sulmona x 4" second year; 16 = "Rosso di Sulmona x 6" second year).

growing conditions occur, such as high temperatures and semi-arid environments (Ade-Ademilua et al., 2009; Morais et al., 2023). However, other authors have reported negative effects of prolonged chilling on marketable yield and bulb size. Guevara-Figueroa et al. (2015) highlighted that treatment at 5 °C for 5 weeks caused a reduction of crop cycle (32–42 days), early harvest time, smaller bulb size and lower crop yields compared to the control (bulbs stored at room temperature, 19 °C). These results can be due to early plant senescence and a greater accumulation of carbohydrates such as glucose and fructose in the bulbs. Likewise, Kaur et al. (2021) reported that the storage temperature negatively influenced physiological and biochemical parameters such as plant weight, plant height, total phenols, total flavonoids, ascorbic acid and total soluble sugars.

Our findings show that the highest bulb yields were recorded by 'Gravina' in both research years (Table 2). This result could be due to the absence of bolting in this cultivar (Khokhar et al., 2007). The yield varied significantly in the two crop cycles, showing a 23 % increase in the second year accompanied by a significant improvement in all morpho-productive parameters: mean weight, height and diameter of the bulbs, mean weight of the bulblets. Also, the dry biomass production, both total and underground (bulbs), as well as the harvest index, had a similar trend. These differences may be related to higher rainfall in

the second year (+20 %), which likely enhanced bulb growth and yield (Khokhar, 2023).

5. Conclusions

The results of this study highlighted clear differences in the response of the two Italian garlic cultivars to pre-planting cold storage. Overall, 'Gravina' was more productive than 'Rosso di Sulmona', particularly in the second year. The highest bulb yields were obtained with chilling treatments for 4–6 weeks in the first year and for 2–4 weeks in the second year. 'Gravina' responded more positively to longer cold storage, whereas 'Rosso di Sulmona' benefited from short-term exposure (2 weeks). These findings confirm that genotype-specific behaviour must be considered when defining storage protocols, since bolting and non-bolting cultivars follow different developmental pathways. This has practical implications for garlic cultivation in Mediterranean environments, where optimizing pre-planting practices may contribute to higher yields and improved market quality. To better identify the physiological mechanisms underlying these responses, future investigations should be extended including additional cultivars and a wider range of storage conditions. Lastly, attention should also be given to quality-related traits, such as phenolic content, anthocyanins and flavonoids, which are increasingly relevant for both nutritional value and consumer preference.

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CRediT authorship contribution statement

Vincenzo Candido: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Investigation, Formal analysis, Data curation, Conceptualization. **Michele Denora:** Writing – review & editing, Formal analysis. **Donato Castronuovo:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Loriana Cardone:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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