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Full Length Research Paper

Garlic physiological characteristics from harvest to sprouting in response to low temperature

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Garlic is a perennial vegetable crop which belongs to liliaceae family and propagated by bulbs. Garlic sprouting depends on air temperature and its storage period. This study was conducted to investigate physiological trait variations from sprouting to harvest of garlic cv. White Gorgan stored in low temperature (4°C). Toward this end, variations of chlorophyll, carotenoid, carbohydrates, as well as the activity of Amylase and Invertase enzymes were measured before and after chilling treatment for 15 and 30 days. Obtained results showed a significant difference among measured traits ($p < 0.01$). Chlorophyll a, chlorophyll b, chlorophyll ab, carotenoid, glucose, and activity of Amylase and Invertase enzymes were increased in response to increment of chilling period which reached their highest values after 30 days. Sucrose and starch had the highest contents in the beginning of experiment and reached to their lowest value after chilling treatment ($p < 0.01$). Results of correlation analysis indicates negative correlation of all chlorophyll types with total sugar, sucrose and starch, while all chlorophyll types had positive correlation with glucose, α-amylase, β-amylase and Invertase. Increment of chilling treatment is followed by α-amylase and β-amylase increment as well as decreased starch content of Bulblets. Starch content showed a negative correlation with α-amylase and β-amylase. Also sucrose content was negatively correlated with invertase ($p < 0.01$). In conclusion, storage of garlic cv. White Gorgan in 4°C for 30 days had the best sprouting result.

Key words: Garlic, sprouting, chilling, carbohydrate, enzyme.

INTRODUCTION

Garlic (*Allium sativum* L.) belongs to liliaceae family and it is an important bulb vegetable due to its medicinal and nutritional values (Nosraty, 2004). Garlic bulblet changes during sprouting are important for garlic storability and the understanding of sprouting physiology. During garlic sprouting, its nutritional and commercial values decreases and interesting physiological changes occurs. Garlic sprouting depends on temperature and storage period (Vazquez-Barrios et al., 2006). Vazquez-Barrios et al. (2006) and Contwell et al (2003) reported that garlic dormancy ends by storage in low temperatures. According to Rosa et al. (2009) low temperature affects enzymes involve in regulation of sucrose/starch ratio in plants. In fact, sucrose is the free sugar in plant which changes in low temperatures (Gupta and Kaur, 2005). In such condition, sucrose is catabolized to simple sugars (Athes et al., 1998). Generally, soluble sugars increase in temperate zone plants during low temperatures has been reported and it is regarded as a mechanism for cold stress tolerance (Hill and Luck, 1991). In another study the effect of different storage temperatures (4, 10 and 20°C) on glucose, sucrose and fructose content of onion were investigated (Benkeblia and Varoquaux, 2003). Also, Takagi (1990) studied the respiration rate of garlic in different temperatures. So many studies have revealed correlation between sugars content of bulbs and storage temperature (Gupta and Kaur, 2005; Iraqi et al., 2005; Kingston-Smith et al., 1999; Salama et al., 1990). On the other hand, Zwicker and Schoen (1993) showed chlorophyll

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changes in wheat by low temperatures. Chlorophyll synthesis is affected by genetic, light, oxygen, mineral elements and temperature (Mousavizadeh et al., 2010). Starch content of plants varies during low temperatures and since starch is the most important source of plant reserved carbohydrate, it must turn to simpler sugars for energy production (Lin et al., 2009). Starch hydrolysis can occur in different temperature ranges which depend on plant physiological condition (Lee, 2007). The storage of garlic bulbs in cold environment eliminates dormancy and stimulates sprouting. As investigation of biochemical variation correlated to carbohydrate metabolism is of importance, in this study the changes of chlorophyll, carbohydrate contents, amylase and invertase enzymes during stratification in 4°C in garlic cv. White Gorgan were measured.

MATERIALS AND METHODS

White garlic (*Allium sativum*) cv. Gorgan bulbs were collected from local fields in Gorgan when they were fully ripe and their dormant period just began.

Storage condition and sprout treatment

For cold storage period investigating, 2500 garlic of the same diameter were arranged in plastic trays enclosed in aluminum foils in order to avoid light and kept at 4°C, favorite temperature for garlic sprouting (Hartman, 2002). The experiments were carried out in a cold storage with 75% humidity for periods of 15 and 30 days.

Carotenoid and chlorophyll content

Carotenoid and chlorophyll contents were extracted by acetone, from 1 g of garlic. The amount of these pigments were measured by Arnon, 1956 method spectrophotometerily (S 2000 UV/Vis) at 480, 510, 645, 652, 663 nm and expressed as $mg.g^{-1}$ fresh weight.

Total soluble sugar

Total soluble sugar was determined according to the Antron method spectrophotometry at 630 nm (Sadasivam and Manickam, 1992).

Glucose and sucrose contents

Glucose and sucrose contents were calculated by dinitro salicylic acid (DNS) method, spectrophotometerically (Sadasivam and Manickam, 1992; Malhotra and Sarkar, 1979).

Starch measurement

Starch was determined according to the Antron method spectrophotometry at 630 nm (Sadasivam and Manickam, 1992).

α-amylase and β-amylase activities

The amount of α-amylase and β-amylase activities were measured by 3,5-dinitro salicylic acid color indicator and starch substrate of 1%, spectrophotometry at 540 nm (Bernfeld, 1955).

Invertase special activity

3,5-dinitro salicylic acid color indicator and starch substrate of 2.5% was used to measure invertase special activity according to Mahadevan and Sridhar method (1986), by using spectrophotometry at 540 nm.

Experimental design and statistical analysis

Experiments were conducted as a completely randomized design with three treatments (0, 15 and 30 days cold storage) and four replications of 80 garlic cloves per study unite. Data were analyzed by SAS software (2001) and mean comparison was conducted with calculation of LSD at $P < 0.05$.

RESULTS

Carotenoid and chlorophyll content

Results indicated a significant difference between recorded traits in garlic ($P < 0.01$). Results showed that chlorophyll a, b, ab and carotenoid in the beginning of experiment and before chilling treatment were 0.26, 0.28, 0.28 and 0.26 $mg.100\ mg^{-1}$ FW, respectively with an increasing trend during chilling period. As if, it reached twice of primary content after 30 days (Table 2, Figure 1). Existence of correlation between chlorophyll a, b and ab contents during hardening of temperate zone crops to cold as well as positive correlation between resistance of plant to cold and its chlorophyll content have been reported (Zwicker and Schoen, 1993). Chlorophyll increment in cold condition might be due to cell relative water changes. During chilling period, leaves water content decreases and chlorophyll content gradually increases (Netto, 2005), that is in accordance with the present study results. Our result showed that chlorophyll a, b, ab, were correlated with total sugar, sucrose and starch content, negatively ($P < 0.01$). On the other hand, positive correlation between storage period and glucose, α-amylase, β- amylase and Invertase were found (Table 3). This might be an indication of carbohydrate catabolism activation during chilling period. Carotenoid content was correlated negatively with total sugar, sucrose and starch content as well as with chlorophyll a, b, ab, glucose, α-amylase and Invertase (Table 3). In fact, chlorophyll was not formed until sugars were reduced. Mousavizadeh et al. (2010) reported a positive correlation between carotenoid and chlorophyll a and b synthesis. It seems that chlorophyll isn't formed until sugar reduction. Chlorophyll formation occurs during storage in the plants with reserved sugars (Ebrahimzadeh, 2000). Mashayekhi and Neumann (2006) reported a direct correlation between chlorophyll formation and secondary metabolites specially anthocyanins in carrot.

Table 1. Analysis of variance for measured traits of garlic based on mean squares.

SOV	df	Ch a (mgg^{-1}FW)	Ch b (mgg^{-1}FW)	Total Ch (mgg^{-1}FW)	Carotenoid (mgg^{-1}FW)	Total sugar (mg 100 mg^{-1} FW)	Glucose (mg 100mg^{-1}FW)	Sucrose (mg 100 mg^{-1} FW)	Starch(mg 100 mg^{-1} FW)	α-mylase (mgmin^{-1})	β-amylase (mgmin-1)	Invertase (mg 100mg^{-1}FW)
Treatment	9	0.187**	0.225**	0.216**	0.196**	0.702**	0/00064**	0.734**	1.982**	0.00027**	0.00016**	3.95**
Error	2	0.00216	0.0023	0.0021	0.005	0.012	0.0000002	0.0122	0.052	0.00000009	0.0000002	8.91
CV%		9.3	8.6	8.5	18.1	5.8	1.3	5.9	5.4	1.9	3.5	7.1

As secondary metabolites increased during differentiation there is more chlorophyll formation in somatic embryogenesis of carrot. In the other hand, Mousavizadeh et al. (2010) reported a positive correlation between carotenoid and chlorophyll a and b synthesis in cucumber that was in accordance with Mashayekhi and Neumann (2006) result and the present studies result.

Sugar, starch, sucrose and glucose

According to Table 2, total sugar content of garlic before chilling treatment was at the highest value (2.363 mg 100 mg^{-1}FW), while this amount decreased to about a half (1.553 mg 100 mg^{-1}FW) after 30 days of chilling treatment. Such decreasing trend was observed for sucrose and starch content too. Thus the highest amount of sucrose (2.333 mg 100 mg^{-1}FW) and starch (4.869 mg 100 mg^{-1}FW) recorded before chilling and the lowest amount of sucrose after chilling treatment, respectively. Indeed chilling accounts for sucrose and starch decreasing in garlic (Figure 2). Takagi (1990) stated that garlic respiration rate in 5, 10 and 15°C was more than 0°C. Other researchers have been reported that storage of garlic in 5°C causes total soluble solid decrease of 33% and prompt sprouting (Vazquez-Barrios et al., 2006). Rosa et al. (2009) showed that starch content of *Chenopodium quinoa* exposed to low temperature (2.5°C) followed a decreasing trend 2nd day, whereas sucrose content increased until 4th day and then decreased until 9th day of the experiment. On the other hand, Benkeblia and Varoquaux (2003) declared that sucrose content of onion in 4°C decreased significantly after 4th to 6th week. Our results showed a significant and positive correlation between starch and sucrose contents during chilling treatment (Table 3). However, no positive correlation observed between sucrose synthase and starch content in response to low temperature for *C. quinoa* plantlets (Rosa et al., 2009). Based on our results, chilling treatment caused increase in glucose content of garlic cloves so that the highest glucose value (0.052 mg 100 mg^{-1}FW) recorded in 30th day after chilling in comparison with the beginning value (0.03 mg 100 mg^{-1}FW). Naturally, starch and sucrose catabolism to glucose is a time consuming procedure, Therefore, there was no significant difference observed before and after chilling treatment for 15 days (Table 2, Figure 2). A delay stage was observed in glucose increase of garlic. Salama et al (1990) reported that temperature was the most important factor affecting sugar content in onion. Moreover, the increase of soluble sugars of temperate crops in low temperature has been reported (Hill and Luck, 1991). On the other hand, sugars not only act as energy transport in plants but act as gene expression regulators (Gupta and Kaur, 2005; Iraqi et al., 2005). Gene expression initiation accomplish with phosphorylation level by hexokinase, achieved from sucrose hydrolysis. During sucrose catabolism by invertase, two hexoses is produced which might have been a signal for hexokinase activation (Kingston-Smith et al., 1999). Therefore, glucose decrement can be attributed to its conjugation to other molecules or its metabolism during energy production.

α-amylase, β-amylase and Invertase

In this study, α-amylase, β-amylase and invertase enzymes showed significant variations during garlic cold storage at 1% level of confidence (Table 1). Accordingly, α-amylase (0.006 mgmin^{-1}), β-amylase (0.006 mg/min^{-1}) and invertase (0.0009 mg/100 mg^{-1}FW) contained their lowest amount before chilling treatment. These enzymes activity increased as chilling treatment days advances (Figure 3). β-amylase activity reached to three folds (0.018 mg/min^{-1}) of initial value after 15 days and α-amylase and Invertase achieved their highest value after 30 days of chilling treatment (Table 2). Results of correlation coefficient showed a negative and significant correlation

Table 2. Mean comparison of measured traits of garli.

SOV	Ch a (mgg^{-1}FW)	Ch b (mgg^{-1}FW)	Total Ch (mgg^{-1}FW)	Carotenoid (mgg^{-1}F W)	Total sugar (mg 100 mg^{-1}FW)	Glucose (mg 100 mg^{-1}FW)	Sucrose (mg 100 mg^{-1} FW)	Starch(mg 100 mg^{-1} FW)	α-mylase (mgmin^{-1})	β-amylase (mgmin-1)	Invertase (mg 100 mg^{-1} FW)
Chilling start	0.26^{c}	0.286^{c}	0.281^{c}	0.268^{b}	2.363^{a}	0.03^{b}	2.333^{a}	4.869^{a}	0.006^{c}	0.006^{b}	0.0009^{c}
15th day	0.546^{b}	0.644^{b}	0.629^{b}	0.296^{b}	1.773^{b}	0.03^{b}	1.742^{b}	4.335^{b}	0.019^{b}	0.018^{a}	0.0015^{b}
30th day	0.685^{a}	0.735^{a}	0.723^{a}	0.666^{a}	1.553^{c}	0.052^{a}	1.500^{c}	3.473^{c}	0.021^{a}	0.017^{a}	0.0016^{a}
LSD 5%	0.0074	0.076	0.074	0.119	0.176	0.0008	0.176	0.365	0.0005	0.0008	0.0002

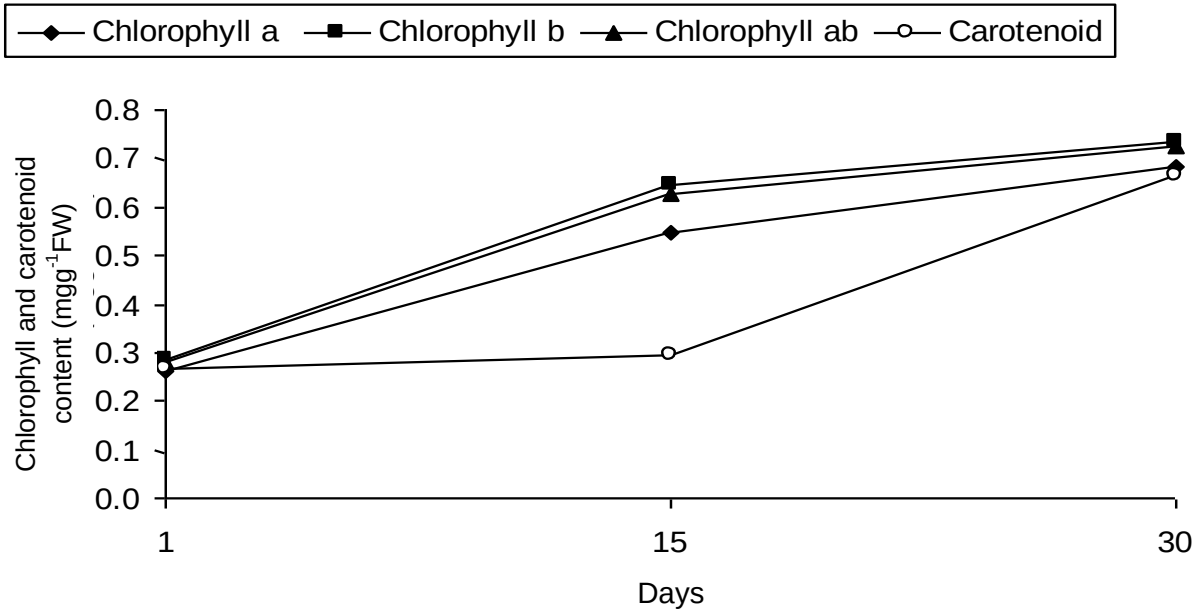

Figure 1. Chlorophyll a, b, ab and Carotenoid content changes during chilling period.

between starch content and α-amylase and β-amylase activities (Table 3). Rosa et al. (2009) reported that activity of enzymes involving sugar hydrolysis increased in 5.5°C.

Amylase activity in *Ruditapes variegates* has been reported during storage temperature of 4 to 35°C by Lin et al. (2009). The obtained results by Athes et al. (1998) in sucrose content during chilling treatment was attributed to sucrose hydrolyser enzyme, invertase. In this study, sucrose correlated with invertase, negatively and significantly (Table 3). This correlation was the same as patterns

Table 3. Correlation coefficient among measured traits of garlic.

Traits	Ch a	Ch b	Ch ab	Carotenoid	Total sugar	Glucose	Sucrose	Starch	α-mylase	β-amylase	Invertase
Ch a	1										
Ch b	0.97**	1									
Ch ab	0.98**	0.99**	1								
Carotenoid	0.74**	0.66*	0.67*	1							
Total sugar	-0.94**	-0.94**	-0.94**	-066*	1						
Glucose	0.74**	0.65*	0.66*	0.93**	-0.70*	1					
Sucrose	-0.94**	-0.93**	-0.94**	-0.68*	0.99**	-0.71**	1				
Starch	-0.89**	-0.84**	-0.84**	-0.85**	-0.80**	0.83**	-0.88**	1			
□-mylase	0.95**	0.97**	0.97**	0.59*	-0.94**	0.60*	-0.94**	-0.79**	1		
□-amylase	0.91**	0.94**	0.94**	0.49^{ns}	-0.90**	0.48^{ns}	-0.90**	-0.71**	0.98**	1	
Invertase	0.92**	0.91**	0.92**	0.75**	-0.88**	0.78**	-0.88**	-0.85**	0.90**	0.85**	1

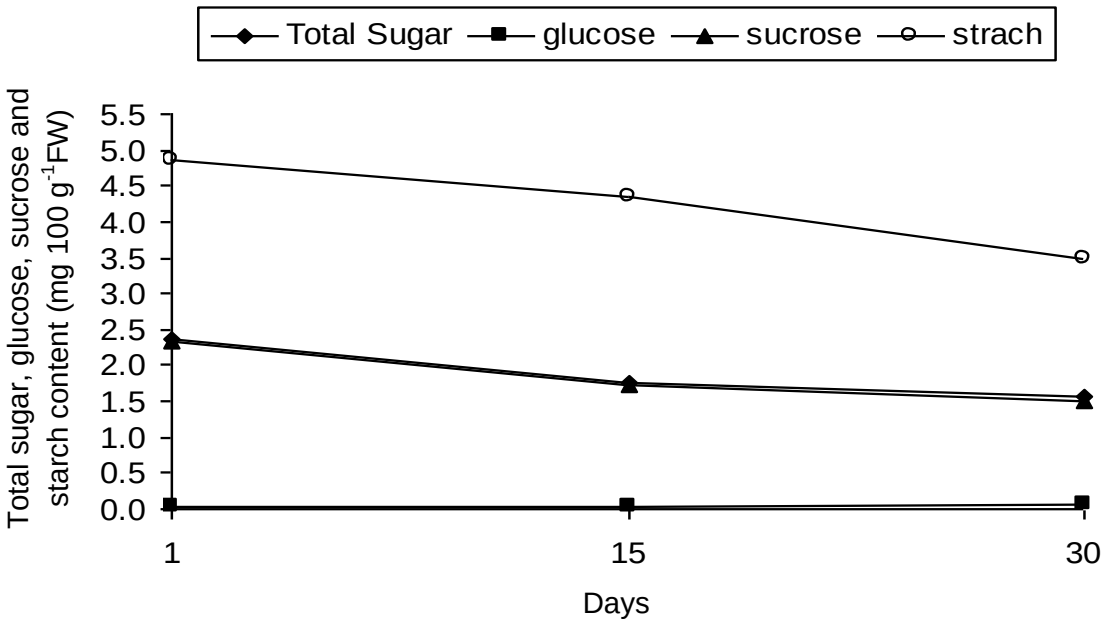

Figure 1. Total sugar, sucrose, glucose and starch contents changes during chilling per.

found by Rosa et al. (2009) in *C. quinoa* plantlets. In the present study, storage of garlic in 4°C caused sprouting which is similar to what was reported by Vazquez-Barrios et al. (2006) in garlic cv. Perla. They believed that storage of garlic in 5°C caused dormancy elimination. This period is in accordance with end of dormancy following by sprouting of garlic cv. Gorgan. Contwell et al. (2003) also reported that storage of garlic in temperatures of 5 to 18°C had promoted the sprouting. The same results were reported by Takagi (1990) about the sprouting at -2 to +9°C. Garlic sprouting decreases the quality and economical value. However, it is one of the suitable attributes for cultivation. Thus, evaluation of internal physiological traits during garlic sprouting can be used for investigation of dormant period (Vazquez-Barrios et al., 2006).

DISCUSSION

Since low temperature during storage time affects garlic sprouting, some physiological changes occurred at 4°C for cv. White Gorgan. The obtained results indicate that low temperature causes sugar changes in garlic, qualitatively and quantitatively which finally promotes its sprouting. Of course garlic sprouting at low temperature is attributed to a complex of biochemical reactions which is carried out by interposition of different enzymes.

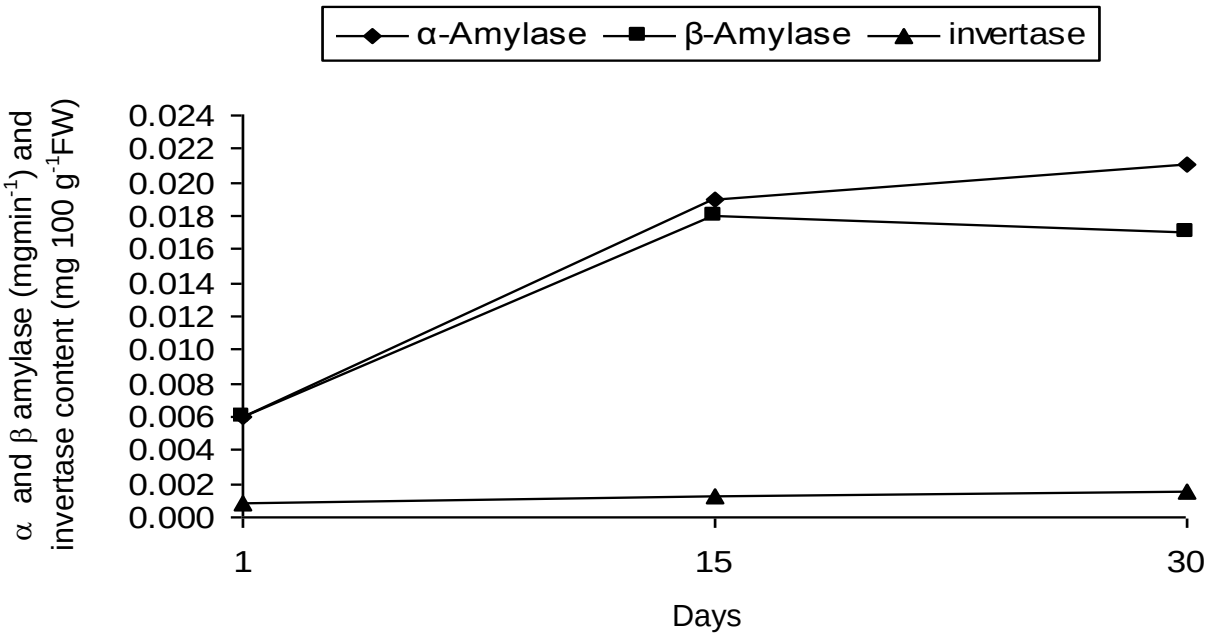

Figure 3. α-amylase, β-amylase and invertase changes during chilling period.

Similarly, increment of chilling treatment days increased chlorophyll, Carotenoid, amylase and Invertase as they achieved to their highest value in the 30th day of experiment. While in this period of time, sucrose and starch are consumed at a high content. A part of non-consumed carbohydrates causes glucose increscent of garlic tissue and sprouting. However, measurement of garlic glucose content can be used as an index for physiological stage of garlic sprouting. As glucose increment means sprouting initiation of garlic, each investigation on physiological changes during storage time till sprouting can be of high importance. On the other hand, since garlic bulblets can be regarded as a complete plant system containing bud, stem and stored part, it is regarded as a suitable tool for such investigations.

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