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Biochemical changes in kiwifruit buds during dormancy under controlled and natural chilling

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ABSTRACT

Chilling accumulation is associated with many metabolic changes that lead to release of endodormancy of buds. The aim of the present research was to assess the impact of both natural and controlled chilling on proline, total phenol and radical scavenging (RSA) , polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL) activities in the buds of kiwifruit cultivars during dormancy. This study was undertaken during 2015-16 in the North of Iran. The results indicated that Hayward and Tomuri had different enzyme activities during and after endodormancy. Hayward cultivar showed higher PAL, RSA, total phenol and proline contents and lower PPO than Tomuri cultivar. Under the natural chilling method, PAL activity, RSA and total phenol contents increased significantly at the first chilling. The effects of controlled chilling treatments on the pattern of biochemical changes in buds were similar to those of natural chilling during the endodormant period. However, the activities and contents of the mentioned biochemical compounds were lower under controlled chilling than natural chilling. Higher phenylpropanoid pathway activity as shown by higher PAL/PPO enzymes activities giving rise to higher phenols accumulation and higher DPPH scavenging capacity can be used as valuable biochemical indicator (marker) for timing of bud endodormancy releasing in kiwifruit under natural chilling.

Key words: *Actinidia deliciosa*, antioxidant, enzyme, phenol, proline.

INTRODUCTION

Bud dormancy is a process that causes the woody plants to survive in an environmentally unstable conditions during winter. There are three types of dormancy: paradormancy where the growth prohibition rises from other section of the plant; ecodormancy which happens because of restriction in environmental agents; and endodormancy (rest) in which the inhibition stays in the resting structure itself. Accumulation of the minimum chilling temperatures is essential for endodormancy release, maximum bud break and flowering (Mcpherson *et al.,* 8). The biochemical changes in different parts of fruit trees during the dormant season have been studied by numerous researchers. During dormancy, the changes in biochemical variability may be related to the end of dormancy and start of growth, as described by many scholars (Ben Mohamed *et al.,* 2). During endodormancy period, accumulation of chilling units is the cause of these changes which is essential for the development of certain phonological stages. Examination of oxidase and peroxidase activities in the varieties of pistachio buds showed seasonal variations of them in the November-March interval (Pakish *et al.,* 9). In terms of the phenol, buds have similar differences in the various stages of dormancy within the varieties of the same species.

In flowering buds of apricot, total phenol showed a gradual increase in endodormancy, and disappeared during flowering (Laslo and Vicas, 7). In certain cultivars of pistachios (*Pistacia vera* L.), the content of polyphenols showed large changes during the period from November to March under natural chilling (Pakish *et al.,* 9). Proline content in the buds of Superior Seedless® grape increased following 300 hours of controlled chilling and decreased with further chilling (Ben Mohamed *et al.,* 2). Furthermore, in higher plants, proline accumulation has been reported as a response to abiotic stresses such as low temperature in sugarcane and hydrogen cyanamide in kiwifruit (Walton *et al.,* 15). In two Hayward and Tomuri cultivars and two female and male Golden genotypes of kiwifruit buds, total phenols and antioxidant capacity of buds significantly increased at the beginning of endodormancy and subsequently decreased at the end of the endodormancy (Abedi Gheshlaghi *et al.,* 1).

Previous studies revealed that, under natural chilling, biochemical changes can be used as a biomarker to demonstrate of dormancy phases of buds in pistachio (Pakish *et al.,* 9) and kiwifruit (Ben Mohamed *et al,.* 2; Abedi Gheshlaghi *et al.,* 1). The

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effects of controlled chilling treatments on the pattern of biochemical changes in buds may be similar to natural chilling treatments during the dormant period, but there are limited data on the effect of controlled chilling on biochemical changes. The aim of the present research was to assess the impact of both natural and controlled chilling on proline and total phenol contents, radical scavenging activity (RSA), activities of polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL) enzymes in order to determine of dormancy stage in the buds of kiwifruit.

MATERIALS AND METHODS

Kiwifruit vines were located at the National Citrus and Subtropical Research Center of Iran (75.36°N 33.51°E) and trained on a T-bar support system with a planting distance of 4×6 m. Cuttings were taken from the middle section of one-year-old canes of 10-year-old own-rooted vines. This research was carried out using two methods of chilling: controlled and natural environmental conditions. In natural chilling, bud samples of Tomuri and Hayward cultivars (*Actinidia deliciosa*) were collected from the end of October 2015 until the end of January 2016 as weekly. Bud samples were collected when they received 0, 218, 346, 480, 585, 692, 773,881 and 966 chilling hours (0-7.2 °C) in the vineyard. For controlled chilling, bud samples of the same cultivars were collected when negated chilling reached the lowest value and daily mean temperatures were recorded below 16 °C on sampling time (Richardson *et al.,* 11), and were then chilled with the same natural chilling treatments hours at 4±0.5 °C in the dark (Wall *et al.,* 14).

Bud samples were collected from the middle parts of annual canes after the chilling accumulation of each treatment. The bud samples were frozen in liquid nitrogen and stored at -80 °C for further analysis to assess biochemical changes. The activities of PAL, PPO, RSA, total phenol and proline contents were determined in buds during dormancy.

Chilling requirements of kiwifruit cultivars were calculated using single-node cuttings test (Dennis, 3). After the chilling accumulation of each treatment, 15 single cuttings in three replications of each cultivar, with the base immersed in tap water, were forced in a growth chamber with 16 h of light for 40 days at 25 ºC (Wall *et al.,* 14). The bottom of the cuttings was submerged in water during forcing and removed every 5 days to barricade xylem vessel blockage. For estimation of CR of buds in Hayward and Tomuri cultivars, the lowest mean time to budbreak (MTB) until 50% bud burst was considered as an endodormancy release in two cultivars.

Through the Folin-Ciocalteu procedure, the total phenol content of the buds was extracted and evaluated. Radical scavenging activity was determined using spectrophotometric. The bud samples, weighing 100 mg, were ground in liquid nitrogen and homogenized with 2 mL of 1.0 M borate buffer containing 1.0% of polyvinyl pyrrolidone. The extracts were centrifuged at 13,000 rpm for 15 minutes in cooling centrifuged at 4 °C. The supernatants stored at -80 °C. The activity of PAL was assayed using to the method explained by Yu *et al.* (17). Two-hundred mg of kiwifruit buds was ground in liquid nitrogen and homogenized with 2 mL of potassium phosphate buffer (50 mM, pH=7.0) containing 0.05% EDTA and 1% polyvinyl pyrrolidone (PVP) (W/V) at 4 °C. The extract was centrifuged at 14000 rpm for 15 min in cooling centrifuged at 4 °C. The supernatant kept for further assessments at -80 ° C. Polyphenol oxidase activity was estimated using spectrophotometric. The proline content in buds was determined by triturating the frozen buds. Subsequently, 100 mg of triturated buds was mixed with 2 mL of 3% sulfosalicylic acid. The extract was centrifuged at 4 °C and 15000 rpm for 15 min. Based on proline's reaction with ninhydrin, proline colorimety was performed.

The experiment was designed as two-way factorial with three replications. The first factor was the two cultivars, the second factor was two types of buds (from natural and controlled chilling conditions) during 2015-16. The SAS general linear model procedure was used to analyze all studies. Means were compared using Duncan’s multiple range test at the $P \leq 0.05$ level.

RESULTS & DISCUSSION

The onset time for chilling accumulation was considered to be when a constant chilling accumulation occurred and temperatures producing a negative impact were infrequent (Richardson *et al.* 1974). In this research, this date was October 27, 2015 (Table 1). According the results of this study, maximum profundity of endodormancy was in late November (218 hours of chilling, Fig. 1). The chilling requirements of the cultivars were different in the two chilling methods (Fig. 1). In natural chilling, their CR were estimated to be higher than those in controlled chilling ($P \leq 0.05$).

The radical scavenging activity (RSA) indicated a significant difference ($P \leq 0.05$) between both cultivars and it was higher in Hayward buds than Tomuri buds (Table 2). Chilling condition, controlled and normal chilling, had significant effects on variation of RSA in kiwifruits buds ($P \leq 0.05$). The buds of kiwifruit had higher RSA under normal chilling than controlled chilling (Table 3).

Table 1. Air temperature, relative humidity (RH), Sunny hours, precipitation, mean daily transpiration (mmd-1) and freezing days during 2015-16.

Month	Temperature (°C)					Precipitation (mm)	Relative humidity (%)	Evaporation (mm/d)	Sunny hours	Freezing days
	Min. Abs.	Max. Abs.	Min.	Max.	Mean					
October	12.2	28.4	16.48	22.84	19.66	755.6	83.08	64.3	106.5	0
November	4.6	22.4	10.7	16.4	13.55	329.9	82.23	42.5	104.1	0
December	3.6	17	8.03	12.85	10.44	273.6	85.06	28.5	63.2	0
January	2.4	21.8	6.31	13.19	9.75	195.5	78.33	30.7	116.6	0
February	3.8	21.8	7.44	11.82	9.64	193	84.98	27.8	51.5	0
Mars 2016	1.8	16.2	5.83	11.11	8.47	98.3	81.74	39.7	97.1	0

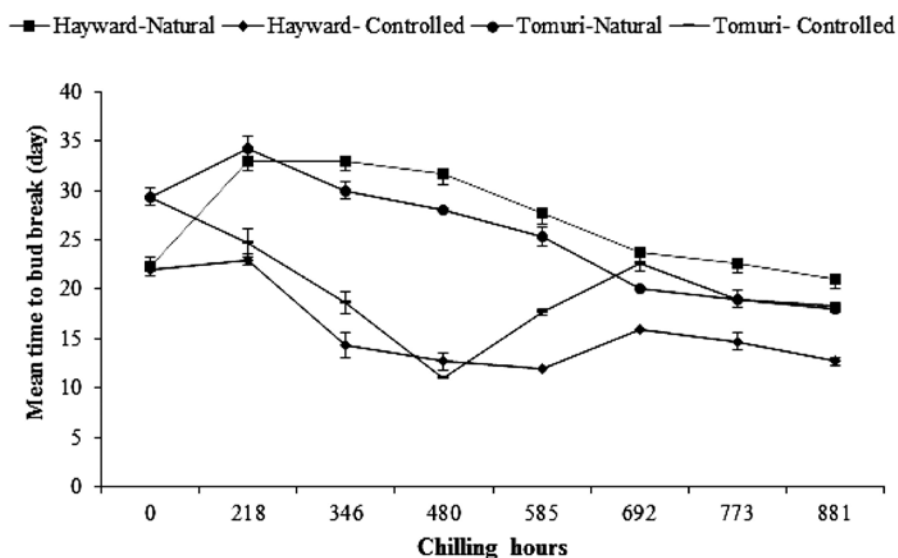

Fig. 1. The effect of chilling hours on mean time to bud break (day) in the buds of Hayward and Tomuri kiwifruits under natural and controlled chilling methods in 2015-16.

Table 2. Phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO) activity, radical scavenging activity (RSA), total phenol and proline contents of Hayward and Tomuri kiwifruits buds under natural and controlled chilling.

Cultivar	RSA %	PAL mg/gFW/60 min	PPO U/g FW min	Total phenol mg/gFW	Proline μM/gFW
Hayward	32.81 a	2.16 a	0.389 a	113.91 a	1.02 a
Tomuri	27.73 b	1.93 b	0.265 b	90.38 b	0.98 b
Coefficient of Variation (%)	5.85	7.09	11.11	6.16	6.60

Different letters within a column indicate significant differences by Duncan test at $P \leq 0.05$.

The highest value of RSA was observed in Hayward cultivar after 218 hours of natural chilling accumulation (Table 4). The lowest amount in RSA was observed in Tomuri cultivar following 480 hours of controlled chilling during endodormancy release (Table 4).

The activity of PAL enzyme without considering chilling conditions has been presented in Table 2. Hayward buds showed significantly higher PAL activity than Tomuri ones. The variations of PAL activity indicated a significant difference in the kiwifruit buds varieties in various chilling hours under the two different methods ($P \leq 0.05$). PAL enzyme activity of kiwifruits buds was significantly less in controlled chilling than normal chilling (Table 3).

Table 3. Effect of natural and controlled chilling treatments on changes of phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO) activates, radical scavenging activity (RSA %), proline and total phenol contents in the buds of Hayward and Tomuri kiwifruits.

Chilling hours	RSA %	PAL mg/gFW/60 min	PPO U/g FW min	Total phenol mg/gFW	Proline μM/gFW
0	27.33 ef	1.24 h	0.298 de	73.89 gh	0.85 f
N 218	40.76 a	2.07 e	0.459 b	154.78 a	1.10 df
N 346	40.03 a	2.88 b	0.337 d	144.16 b	1.47 a
N 480	36.52 b	3.04 a	0.312 de	132.06 c	1.07 de
N 585	31.24 d	2.92 ab	0.297de	118.42d	1.14d
N 692	34.29 c	2.37 d	0.327 de	143.11 b	1.04 e
N 773	21.25 i	2.56 c	0.411 c	90.23 e	1.22 c
N 881	35.80 cb	2.77 b	0.279 e	142.25 b	1.51 a
C 218	29.76 de	1.34 gh	0.298 de	86.47 ef	1.04 e
C 346	28.30 ef	1.44 g	0.191 f	86.59 ef	1.28 cb
C 480	24.02 h	1.28 gh	0.114 g	64.52 i	1.33 b
C 585	25.31 gh	1.8 f	0.172 f	81.22 fg	0.39 h
C 692	26.99 ef	1.22 h	0.408 c	78.15 g	0.78 f
C 773	24.78 h	1.90 ef	0.520 a	68.99 ih	0.51 g
C 881	27.67 ef	1.79 f	0.485 ab	67.31 ih	0.29 i
Coefficient of Variation (%)	5.85	7.09	11.11	6.16	6.60

N, C Natural and Controlled chilling respectively.
Different letters within a column indicate significant differences by Duncan test at $P \leq 0.05$.

The lowest activity of PAL activity was in Tomuri cultivar in the beginning of chilling accumulation and the highest activity of this enzyme was in Hayward buds after 346 hours of natural chilling (Table 4).

Based on Table 2, PPO activity was significantly higher in Tomuri buds than Hayward ones ($P \leq 0.05$). After the endodormancy release of the buds in the two cultivars, PPO enzyme activity was enhanced further with continuing controlled chilling than natural chilling (Table 3). The interaction effect of cultivar type and chilling conditions was significant on PPO activity (Table 4). PPO activity was the highest in Tomuri at 881 hours under controlled chilling (Table 4). However, the lowest enzyme concentrations was observed in Hayward at 585 hours controlled chilling in the edndodormancy stage (Table 4).

The difference in the assessment of chilling can be the result of fluctuations in tree physiology, rainfall, and temperature conditions of vines in natural chilling. The chilling requirement of Hayward and Tomuri vegetative buds was estimated to be 692 and 692 hours in the natural chilling method, and 552 and 480 hours in the controlled chilling method, respectively (Fig. 1). Guerriero *et al.* (4) reported CR of kiwifruit buds to be more under natural chilling than Controlled chilling, corresponding with the results of this study.

For controlled chilling study, all cutting were collected once on October 27th, whereas the buds for natural chilling were collected on at variable time as indicated earlier. Therefore, cuttings of natural chilling are mostly controlled by the vine system. At leaf fall, more than 80% of the kiwifruit vines' starch accumulated in its root system, and a quarter of these accumulation are utilized throughout winter and spring. Results indicate that bud dormancy may be adjusted from other parts of the plant, for example in the root system. As recommended by several researchers (Dennis, 3; Wall *et al.,* 14), we used single-node cuttings to study biochemical and physiological processes leading to bud endodormancy release because they show only the effect of endodormancy and not apical dominance.

After the cessation of growth, endodormancy is developed gradually (Dennis, 3). In addition, in autumn the intensity of endodormancy increases and then moderately fades away through the removal of the physiological impediments of growth by the chilling process. In this study, MTB in sampling time was higher than that on the endodormancy release date for Hayward and Tomuri cultivars. Therefore, lateral buds of kiwifruit may enter endodormancy before the end of summer or the beginning of autumn as reported by Dennis (3).

Table 4. Effect of natural and controlled chilling treatments on changes of phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO) activates, radical scavenging activity (RSA %), proline and total phenol contents in the buds of Hayward and Tomuri kiwifruits.

Cultivars	Chilling hours	RSA %	PAL mg/gFW/60 min	PPO U/g FW min	Total phenol mg/gFW	Proline μM/gFW
Hayward	0	29.26 efg	1.48 klm	0.302 jk	83.64 klm	0.81 i
	N 218	43.35 a	1.86 i	0.333 ghij	168.1 ab	1.35 c
	N 346	44.11 a	3.19 a	0.311 ijk	161.0 bc	1.745 a
	N 480	37.83 b	2.87 bc	0.224 lm	140.2 de	0.97 gh
	N 585	32.80 cd	3.03 ab	0.209 m	129.7 ef	1.01 g
	N 692	38.00 b	2.53 ef	0.178 m	153.6 c	1.28 cde
	N 773	20.04 l	2.35 fg	0.447 de	87.10 ijkl	1.24 de
	N 881	44.11 a	2.89 bc	0.386 fg	175.8 a	1.27 cde
	C 218	31.35 de	1.67 jk	0.310 ijk	98.41 ghi	1.08 fg
	C 346	29.60 defg	2.13 gh	0.0942 n	96.97 hij	1.32 cd
	C 480	27.66 fgh	1.03 op	0.112 n	76.81 lmn	1.49 b
	C 585	28.57 efgh	1.68 jk	0.0721 n	97.93 ghi	0.53 j
	C 692	29.75 defg	1.27 mno	0.193 m	86.50 jkl	0.48 j
	C 773	27.16 ghi	2.10 ghi	0.486 d	75.57 lmno	0.46 j
	C 881	28.57 efgh	2.22 g	0.320 hijk	77.37 lmn	0.25 k
Tomuri	0	25.41 hij	0.99 p	0.296 jk	64.15 pqr	0.89 hi
	N 218	38.16 b	2.27 g	0.585 bc	141.5 d	0.85 i
	N 346	35.96 b	2.56 def	0.366 fghi	127.3 f	1.19 e
	N 480	35.21 bc	3.21 a	0.401 ef	123.9 f	1.16 ef
	N 585	29.69 defg	2.81 bcd	0.385 fg	107.1 gh	1.27 cde
	N 692	30.60 def	2.21 g	0.475 d	132.7 def	0.80 i
	N 773	22.45 jkl	2.77 bcde	0.375 fgh	93.36 ijk	1.21 de
	N 881	27.49 fghi	2.65 cde	0.173 m	108.7 g	1.76 a
	C 218	28.16 efgh	1.01 op	0.285 jk	74.53 mnop	1.016 g
	C 346	27.01 ghi	0.75 q	0.288 jk	76.21 lmn	1.24 cde
	C 480	20.38 l	1.54 kl	0.116 n	52.22 s	1.17 ef
	C 585	22.06 4mkl	1.92 hij	0.270 kl	64.52 opqr	0.25 k
	C 692	24.23 ijk	1.17 nop	0.624 ab	69.80 nopq	1.08 fg
	C 773	22.40 jkl	1.71 jk	0.554 c	62.41 qrs	0.57 j
	C 881	26.78 ghi	1.35 lmn	0.648 a	57.25 rs	0.34 k
Coefficient of Variation (%)			5.85	11.11	6.16	6.60

N, C Natural and Controlled chilling respectively.
Different letters within a column indicate significant differences by Duncan test at P ≤ 0.05.

Under controlled chilling, the mean time of budbreak (MTB) constantly decreased until the end of endodormancy. However, after endodormancy release, continuing chilling increased MTB from 2 days in Hayward to 11 days in Tomuri cultivar. Therefore, biochemical compound variations were not constant after endodormancy release. Under natural chilling, MTB constantly decreased until the end of endodormancy, and continuing chilling reduced MTB at the ecodormancy stage as well.

Commonly, it is said that low temperatures support buds to release from endodormancy (Ben Mohamed *et al.,* 2). Pérez and Rubio (10) demonstrated the conflicting result, maintaining that

the depth of endodormancy rather than the release of endodormancy is increased by low temperatures under controlled chilling. It may be proposed that, similar to grapes (Pérez and Rubio, 10), extreme chilling in kiwifruit buds might cause delay in their budburst and biochemical fluctuations.

The response of trees to chilling might be different under natural photoperiods, light intensity, and darkness. Pérez and Rubio (10) indicate that CR in buds is a reversible event that depend on the stage of dormancy and temperature, and a deeper endodormancy is more homogeneous with budburst response in grapevines. In this study, buds for controlled chilling condition were collected at the end of October 2015 when they were not on the depth of endodormancy. Therefore, the fulfillment of CR as well as biochemical compound changes may be correlated both with the degree of endodormancy depth and chilling accumulation in the darkness.

The pattern of RSA changes for each cultivar was almost identical in both methods. RSA simultaneously increased from the first chilling in the two cultivars under the two methods. Nevertheless, the peak period of the RSA of buds differed across the two cultivars (Table 4). RSA showed a significant reduction ($P \leq 0.05$), occurring simultaneously at the end of endodormancy in the buds. The total phenol of buds increased significantly ($P \leq 0.05$) with time and reached its maximum amount at the depth of endodormancy (Table 4) and remained at a peak during this period. After the endodormancy release, the amount of total phenol decreased significantly in the buds of two cultivars ($P \leq 0.05$). In two female and male Golden (*Actinidia chinensis*) genotypes of kiwifruit buds, antioxidant capacity and total phenol increased at the beginning of endodormancy and subsequently decreased at the end of the endodormancy, corresponding with the results of this study (Abedi Gheshlaghi *et al.,* 1).

Under the first controlled chilling treatment, increased activity of enzymes, RSA and total phenol content of the cultivars were much lower than those of the normal chilling. In addition, under the controlled chilling, subsequent changes of them to endodormancy release and even after it continued at a lower level than those of normal temperatures. This may be due to the impact of constant temperature or darkness in controlled chilling.

Total phenol content and radical scavenging activity were enhanced during in the progress of endodormancy and reached their topmost values in the depth of endodormancy (Table 3). The variation of these two variables was similar in the formation, survival, and release of endodormancy.

It has been widely accepted that chilling stress causes the formation of radicals and other reactive oxygen species (ROS) and the activation of antioxidant enzymes. The superoxide radical anion is changed into hydrogen peroxide (H2O2) by superoxide dismutase. Plants release H2O2 in response to environmental stress and low-temperature stress that accumulate in cells. Hydrogen peroxide, as the secondary informer of the enhancement in the activity of PAL enzyme, can direct the activity of this enzyme as a key enzyme in the phenylpropanoids' pathway, eventually, it leads to more total phenol and flavonoids accumulation (Wang *et al.,* 16).

During the endodormancy of kiwifruit buds, due to oxidative stress, the activity of PAL enzyme and content of antioxidant substances such as phenols increased, resulting in a more radical scavenging activity in the buds within the endodormancy. Furthermore, it has been said that, based on the low temperature field to which the plants are exposed, the cold acclimatization of plants conducts to a considerable increase in PAL concentration (Stefanowska *et al.,* 12).

Under both chilling methods, after endodormancy release, PPO activity increased in the buds of Tomuri and Hayward varieties. The increase in PPO activity may be responsible in the elimination of certain growth-preventing phenols, and the phenolic compounds were found to be potent modifiers of enzyme activity, as both inhibitors and stimulators. With the first natural chilling treatment, there was a significantly rapid increase in PAL, PPO, RSA, and total phenol compared to controlled chilling. Their high activity can be a signal so that low temperature as the cold-stress causes more ROS production and, as a result, more ROS scavengers.

The results of this study in the field of PPO changes corresponded with its activity in pistachio flower buds (Pakish *et al.,* 9) during dormancy. Polyphenol oxidase activity increased in the early stage of endodormancy and decreased at late this period.

It seems that the role of antioxidant substances is the inhibition of reactive oxygen species and free radicals in cultivars during stress. Phenolic substances have valuable evidence for determining the differences between various cultivars of *Myrtus communis* and *Pistacia lentiscus* and play a key role in distinguishing genetic diversities in biochemical methods (Tattini *et al.,* 13). Therefore, it seems that the remarkable differences between Hayward and Tomuri cultivars in terms of the total phenol content are the result of their genetic differences.

Under favorable environmental conditions, phenolic compounds are synthesized in plant cells but their contents modify by environmental stresses in cells. During endodormancy period,

mid-autumn cold causes an increment of oxidative stress in plants. Plants contain a protective system, including an enzymatic antioxidant system such as catalase and peroxidase and non-enzymatic systems. Antioxidant activities of phenols, such as non-enzymatic antioxidant, are mostly because of their redox effects which let them to operate as singlet oxygen quenchers, hydrogen almoners and reducing agents (Huda-Faujan *et al.,* 5).

In higher plants, proline is accumulated in response to low temperature. Proline plays a role in osmotic regulation between the cytosol and the vacuole. Reactive oxygen species (ROS) formed under stress conditions such as chilling treatments in plant, and proline might protect subcellular structures via scavenging the ROS and, in the present study, proline in Hayward and Tomuri buds increased with chilling accumulation. Accumulation of proline in kiwifruit (Walton *et al.,* 15) and grapevine (Ben Mohamed *et al.,* 2) buds was reported, corresponding with the results of this study. More proline in the buds of the two cultivars under natural chilling method may be the result of extreme temperatures in this method.

It seems that proline cumulating within the early stage of chilling might be an accumulative reaction to low temperature that was different in cultivars. With further chilling after endodormancy release, the proline of Hayward and Tomuri did not decrease under natural chilling, but declined under controlled chilling. It is said that proline can be consumed as a source of nitrogen, carbon and energy (Ben Mohamed *et al.,* 2). For evaluating natural chilling method, the buds were sampled at consecutive stages, which might be affected by the physiology of vines. However, the buds of all controlled chilling treatments were collected once on October 27th, so that the decline of proline with continues chilling may be the result of proline oxidation to produce phosphorylated sugars, ATP, and NADPH, required for cell division, anabolic pathways and newly growing tissues after stress relief (Kishor *et al.,* 6).

Natural chilling treatments showed a better pattern of PAL, PPO, RSA, and total phenol in various stages of bud dormancy. Therefore, the changes in the biochemical activity of these compounds may indicate the phenological stages during dormancy. During and after bud dormancy, different treatments of controlled chilling did not show steady or constant patterns on biochemical variations. That may be affected by the time of bud sampling, darkness, or chilling temperatures, and needs further investigation.

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