Carbohydrate changes during dormancy release in floral, vegetative buds and bark tissues of pear cultivar 'Wonhwang' cuttings following dormancy breaking agents treatment

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Abstract— *The aim of this study was to elucidate the effect of hydrogen cyanamide (HC) and thiourea on dormancy release and carbohydrates metabolism in floral, vegetative buds and bark tissues of pear (Pyrus pyrifolia Nakai) cultivar 'Wonhwang'. Selected cutting were immersed in appropriate solutions of HC, thiourea and dH2O as a control while cultured in forcing condition for 5 weeks. Our results showed that both the treatments were more effective in dormancy release of both floral and vegetative buds as compared to control. However, the cuttings treated with HC started the floral and vegetative budbreak after 5 and 7 days of treatment followed by thiourea 7 and 9 days and control 13 and 13 days respectively. 50% floral budbreak was observed after 18, 22 and 30 days of treatment during forcing, on cutting treated with HC, thiourea and control while 50% vegetative budbreak was noted on cuttings treated with HC and thiourea after 22 and 26 days treatment during forcing. For control, 49.3% vegetative budbreak was observed after five week during forcing. Mean time to budbreak (MTB) for both floral and vegetative buds was less for HC followed by thiourea and control. HC and thiourea application caused an abrupt increase in starch hydrolysis and a transient accumulation of soluble sugars in buds and bark tissues during the first five and ten days after treatment. These variations, which happened shortly after HC and thiourea application, seemed to be linked with a process leading to endo-dormancy release. In fact, as budbreak started, we observed a rapid decline in sucrose, glucose and fructose concentrations in all treated tissue, while these concentrations remained high in untreated tissues and then decreased when starch concentration started to increase. Sorbitol concentration increased in treated and untreated floral buds up to 20 days in the same trend and then decreased. Our data suggest that the difference in the timing of soluble sugars accumulation/consumption process between HC, thiourea applications and control cuttings may account for the differences in the timing of growth resumption and budbreak growth.*

Keywords— Carbohydrates; Dormancy; Dormancy breaking agents; Pyrus pyrifolia; Stem cuttings.

I. INTRODUCTION

Temperate perennial woody plants annually pass through a period of active growth, growth cession, dormancy and growth resumption. Dormancy is defined as the temporary suspension of visible growth of any plant structure containing a meristem [1]. Dormancy helps to protect the plant tissues from harsh environmental conditions and secure their proper architecture. Bud dormancy in perennial fruit trees, including pear, is an adaptive developmental process for their survival under the adverse environmental conditions. Bud dormancy in perennial woody plants can be divided in three phases as para-dormancy (regulated by physiological factors within the plant but outside the dormant structure), endo-dormancy (regulated by physiological factors within the dormant structure) and eco-dormancy (regulated by environmental factors) [1]. Bud dormancy in temperate plants is well studied at the physiological level [2,3]. In winter, the short photoperiod and low temperature cause shoot extension growth to cease and the initiation of apical to protect the apical meristem [4,5]. The buds must receive an amount of chilling to resume growth which is genetically controlled and varies among genotypes [6].

Abnormal pattern of budbreak is mainly attributed to lack of chilling in temperate fruit trees in mild winter regions [7]. Due to warm winter temperature dormancy release become one of major limited factors for pear production in warm regions [8]. The pear industries in those regions may be adversely affected because of irregular blossoming and delayed flowering

behavior. The amount of chilling hours of exposure to temperatures <7.2°C is required to break dormancy and induce floral and vegetative bud break. Artificial means to break dormancy is needed for maintaining economic production of pear crop in particular regions lack of natural chilling and also for protected pear cultivation in some regions of China [9]. Artificial chilling exposure and bud breaking agents with different concentration interact in breaking dormancy of fruit trees. Different scientists from the beginning of the previous century until recent times attempted to break the endodormancy and stimulate the budbreak of deciduous fruit trees planted in warm regions using the dormancy breaking agents such as thiourea, cyanamide and potassium nitrate [10]. Many researchers have been conducted the research to artificially interrupt dormancy in areas lacking sufficient chilling units with synthetic chemicals [11,12,13,14]. A number of chemicals have been used to break the dormancy, including hydrogen cyanamide (HC) [15] and thiourea [12]. Hydrogen cyanamide increases the percentage of budbreak and improves regularity in budbreak of fruit trees under low chilling conditions [16]. Synthetic application of 2% thiourea provided 360 hour of chilling (CH) which caused early budbreak in pear [17]. The effectiveness of these chemicals mostly depends upon the application dates, quantity and cultivars. Investigators determined and calculate the chilling requirements or accumulations of deciduous fruit species based on different ways. The common method used on a large scale is to force cuttings or seedlings into growth chamber, laboratory or greenhouse under room temperature (20-25°C or more) for two to three weeks or more then recording the percentage of budbreak [18].

Significant changes are found in carbohydrate contents in floral and vegetative buds in fruit trees submitted to chilling deprivation as compared to natural chilling conditions [19]. Carbohydrates are the main source of energy for the metabolic changes that occurred during the dormancy release stage. Carbohydrate accessibility is most probably of major relevance to the control of bud growth and development during dormancy induction and release [20]. During earlier summer, starch is accumulated in reserve tissues and then converted to soluble sugars during dormant season. There are two enzymes concerned in sucrose metabolism as sucrose synthase and invertase. In plants, various kinds of invertases are found such as alkaline and acid invertase. It was observed that invertases play an important role in sink initiation, growth and cell expansion while sucrose synthase is linked with the metabolism of carbohydrates storage, fruit maturation, and polysaccharides synthesis [21]. Likewise, soluble sugars are also documented as important signaling molecules involved in many processes of plant life-cycle, including dormancy [22,23,24]. However, budbreak pattern appear to be more correlated with the capacity of bud to use soluble sugars than with sugar abundance in dormant tissues [25,19].

The acts of artificial dormancy breaking agents, such as HC and thiourea have been associated with the onset of sub lethal stress which leads to budbreak [26,27,12]. Therefore, the present work was planned to study the effect of exogenous application of hydrogen cyanamide and thiourea on floral and vegetative budbreak. To better understand the starch and soluble sugars concentrations would be effected by HC and thiourea application to endo-dormant cuttings of *Pyrus pyrifolia* Nakai cv. 'Wonhwang.

II. MATERIALS AND METHODS

2.1 Plant materials

Adult trees of pear *Pyrus pyrifolia* Nakai cultivar 'Wonhwang' were used in this trail. They were located in experimental pear orchard of Zhejiang University, Hangzhou City of Zhejiang Province, China, latitude 36°13' N, longitude 120°12' E and elevation 41.7 m. These cultivars were grafted on *Pyrus calleryana* Decne rootstocks, planted in rows. Liu, 2013 [28] estimated the cultivars chilling requirement for budbreak to be about 992 CU (using Utah model).

2.2 Experimental design and treatment

One year old cuttings (≈80 cm long) were randomly collected from adult trees during leaf fall (1st November 2012), and before any natural chilling accumulation had occurred. These cuttings were cut into seven buds segments (≈60 cm long) by removing the apical and basal buds and then divided into two sets each of floral and vegetative buds for low temperature treatments. Each set of cuttings were covered with paper, kept in plastic bags and then were exposed to continuous low temperature (5°C) in cold room to stimulate the accumulation of 650 chilling hours, the equivalent of 2/3 of the cultivar chilling requirement. Different chemicals are used for breaking the dormancy in different time but the adequate time for treatment is after 2/3 of the chilling hours of the cultivars [29]. Each hour in cold treatment is the equivalent of 1 positive chill unit as stated by [30]. After chilling hour's treatment, cuttings were pulled out from the cold room and each set of cuttings were further divided in to three groups; one group of each set was sprayed to run off with the aqueous solution of Dormex® containing 3% (v/v) HC. The second group of each set was sprayed with thiourea solution containing 3% 9v/v) thiourea and the third group of each set was sprayed with distilled water to serve as a control. After treatment, cuttings were

placed with their basal tip in water containing 500 ml vials and forced in a phytotron day/night 25± 1/18±1°C, with a 12-h photoperiod of white light (320 µmol m^{-2} s^{-1}) and 75% humidity for 6 weeks to induce the budbreak. During forcing, the basal ends of the cuttings were cut and water in the viols was changed after every one day regularly to avoid xylem vessel clogging by algal growth up to 35 days. Floral, vegetative buds and bark tissues were sampled after 0, 5, 10, 15, 20, 25 and 30 days of forcing. The samples were immediately frozen in liquid nitrogen and stored at -80°C until used.

Three replicates of 9 cuttings from each group were prepared from HC, thiourea treated and control cuttings and were used to measure the dormancy status of floral and vegetative buds. Data were collected three times a week. Buds that reached to balloon or green tip stage were recorded open and budbreak percentages were determined at 10, 14, 18, 22, 26, 30 and 35 days of forcing. According to the dormancy status classifications in pear of [31] with some modifications, when 50% of buds on the branch cuttings were in the green tip stage then we considered the buds to have broken the endodormancy. However, if less than 50% buds had broken on the branches then those to be considered in endodormancy phase. Results were expressed as the percentage of budbreak.

Mean time to budbreak (MTB) of both floral and vegetative buds was calculated, after the treatments of the cuttings. The results were expressed as mean time to budbreak (MTB) in days (arithmetic means of each three group of nine excised cuttings).

2.3 Determination of starch and sugars concentrations in floral, vegetative buds and bark tissue

Sugars and starch were extracted as the method described in Huang et al., 2009 [32], with slight modifications. Approximately, 1 g of the frozen sample (floral buds, vegetative buds and bark) was homogenized in 10 mL of 80% ethanol for 10 min at 80°C. The extract was centrifuged at 14,000 rpm for 10 min. The extraction was repeated three times and the supernatants were collected and pooled. To remove the phenolic compound, 5% (w/v) polyvinylpolypyrorolidone (PVPP) was added to the combined extracts and they were left over night. The pellets were saved and stored at -40°C for further starch analysis. The combined extracts were centrifuged at 3000 rpm for 15 min, and the supernatant was evaporated to dryness under vacuum below 40°C until the ethanol was removed and then adjusted to the volume of 1 mL with distilled water for analyses of fructose, glucose, sucrose, sorbitol using HPLC (LC-20T, Shimadzn, Japan). System Gold Software (LC-20T, Japan) was used to run the HPLC and to process the results. Concentration and composition of sugars were determined by using Komatsu et al., 1999 [33] method with some modification. A 20 µL aliquot solution was injected into the 5.0 µM NH_2 (4.6 mm × 250 mm) column (Dalian Sipore Co. Ltd. China) and the eluted peaks were detected with a refractive index detector RID-10A (LC-20AT, Japan). Acetonitrile:water (80:20) was used as the mobile phase with a flow rate of 1.0 mL min^{-1}. Sugars were quantified from a standard peak using control sugars (Sangon Biotech, China).

Starch content was determined using the perchloric acid method according to Rose et al., 1991 [34]. The residue in tubes, left after sugar extraction, was further extracted three times with 5 ml of 35% (v/v) perchloric acid with continuous shaking at low speed for 15 min. The extracts were pooled and centrifuged for 5 min at 10,000 × g. The supernatants were collected in graduated tubes and brought to 20 ml with distilled water. For colorimetric determination, a 1-ml aliquot of extract was mixed with 5 ml of anthrone reagent (0.175% w/v in 75% cold sulfuric acid) in tube and mixed shortly. The mixture was placed into boiling water for 12 min and then kept on ice. The mixture's absorbance was read at 620 nm using Appendrop-Biospetrometer. Glucose standards from 0 to 100 g ml^{-1} were used for calibration.

2.4 Statistical Analysis

Statistical analyses of the data were performed by analysis of variance (ANOVA) technique using Data Processing System statistical software version (DPS, v. 7.05, Zhejiang University, Hangzhou, China). The effect of HC and thiourea treatment on carbohydrate concentrations was analyzed with least significant difference (LSD) test to compare control to treated samples for each sugar, for sampling date. In all figures, the error bars represent standard errors of the means which were calculated from the replicates. Differences were considered significant $P \leq 0.05$ level.

III. RESULTS

3.1 Effects of dormancy breaking agents on floral and vegetative budbreak

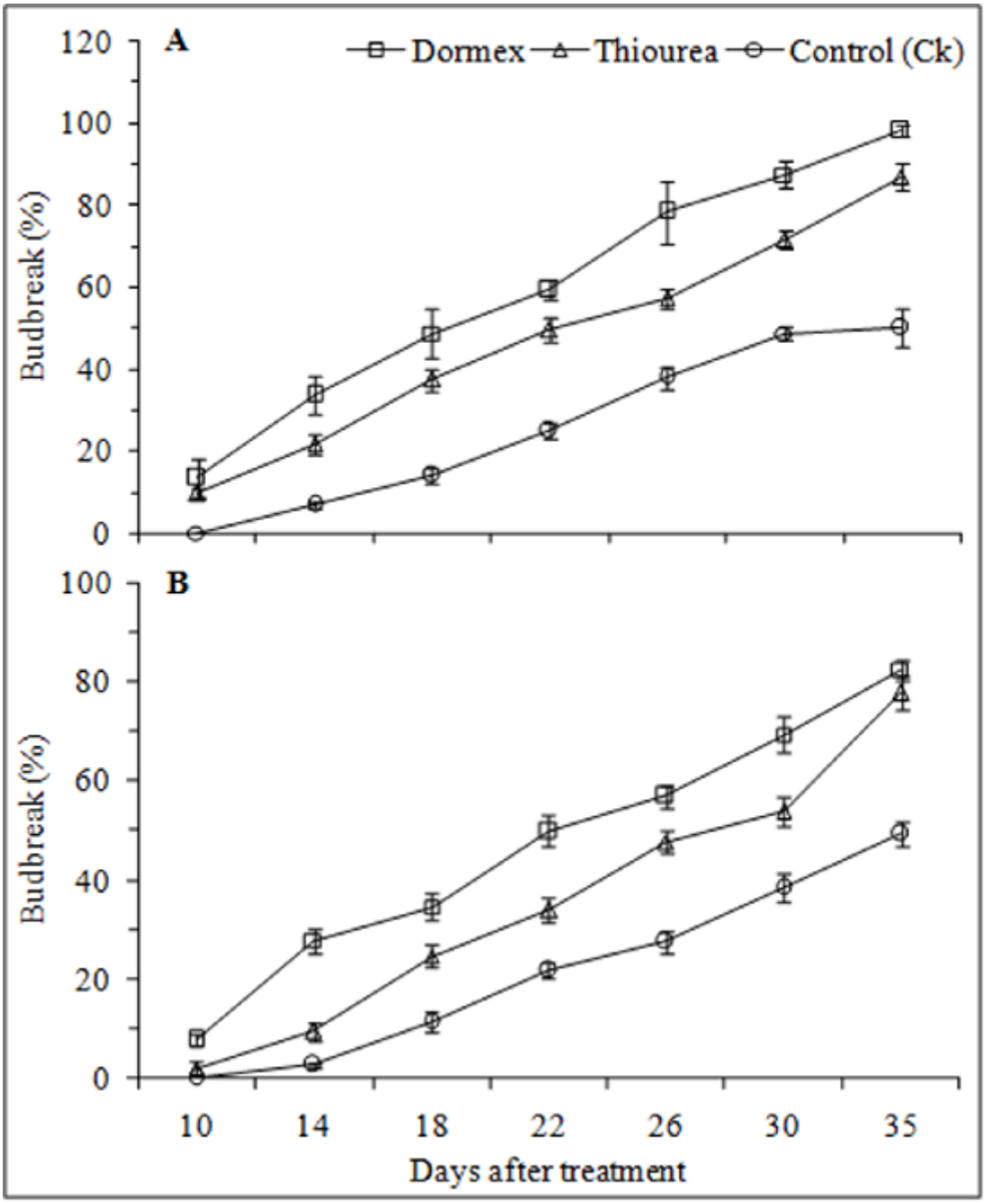

FIG. 1. EFFECT OF THE 3% (V/V) HC, 3% (V/V) THIOUREA AND DH2O (CONTROL) APPLICATION ON A CUMULATIVE FLORAL (A) AND VEGETATIVE (B) BUDBREAK OF EXCISED CUTTINGS OF PEAR CV. 'WONHWANG', FORCED AT DAY/NIGHT 25± 1/18±1°C, WITH A 12-H PHOTOPERIOD OF WHITE LIGHT (320 µMOL M-2 S-1) AND 75% HUMIDITY FOR 5 WEEKS. BARS REPRESENT ± SE OF THREE REPLICATES.

Figure 1A indicate that the application of HC and thiourea hastened the floral budbreak as compared to control. Under forcing conditions, floral budbreak with HC and thiourea treated buds started about 5 and 7 days respectively after treatment, whereas for untreated buds (control) it began about 13 days after treatment (Figure 1A). Furthermore, during the whole treatment period, floral budbreak percentage was constantly higher for HC and thiourea treated as compared to control buds. HC treatment was more effective than thiourea for budbreak while thiourea was more effective than control. Under forcing condition, 50% floral budbreak was observed during 19, 22 and 32 days after HC, thiourea and control treatment. HC and thiourea caused significant differences in the rate and the percentage of floral budbreak. After five weeks of forcing, 98.3, 86.9 and 50.1 % of floral budbreak was observed for HC, thiourea and control treatment respectively (Figure 1A).

Application of HC and thiourea after 650 CH treatments significantly advanced the vegetative budbreak compared with control during forcing condition (Figure 1B). Application of HC and thiourea were more effective than control on percentage of vegetative buds breaking dormancy. HC was the most effective treatment on advancing percentage (82.3%) vegetative budbreak. Under forcing conditions, vegetative budbreak of HC, thiourea treated buds started about 7 and 9 days after treatment, while for untreated buds it began about 13 days. Moreover, throughout the forcing condition, budbreak percentage was consistently higher for HC and thiourea treated compared to control buds. HC and thiourea caused significant differences in vegetative budbreak percentage because after five weeks in forcing conditions 82.3, 77.6 and 49.3% budbreak was observed for HC, thiourea and control treatment respectively. During forcing condition, 50% budbreak was observed after 22 and 27 days for HC and thiourea treated buds, while for control 49.3% budbreak was observed after 35 days (Figure 1B). The application of HC and thiourea to endodormant floral and vegetative buds of pear cultivar 'Wonhwang' hastened budbreak (Figure 1A, B). Significant differences were observed in both floral and vegetative buds after HC and thiourea treatment because after 19 and 22 days, 50% budbreak was noted respectively. However, 50 and 49.3 % floral and vegetative budbreak was observed for control.

3.2 Mean Time to Budbreak (MTB)

When floral and vegetative buds were treated with HC, thiourea and dH_2O as a control after the 2/3 artificial CH treatments, the mean time to budbreak (MTB) was noted and showed in (Figure 2). Our results demonstrated that HC and thiourea treatments decreased the MTB value in both floral and vegetative buds. In control cuttings floral and vegetative budbreak occurred on the same time as after 13 days after treatment. The MTB value of vegetative buds was higher than that of floral buds for thiourea treated cuttings as compared to HC treated cuttings. Besides, the MTB value was stabilized in cuttings of vegetative buds treated with HC and cuttings of floral buds treated with thiourea (Figure 2).

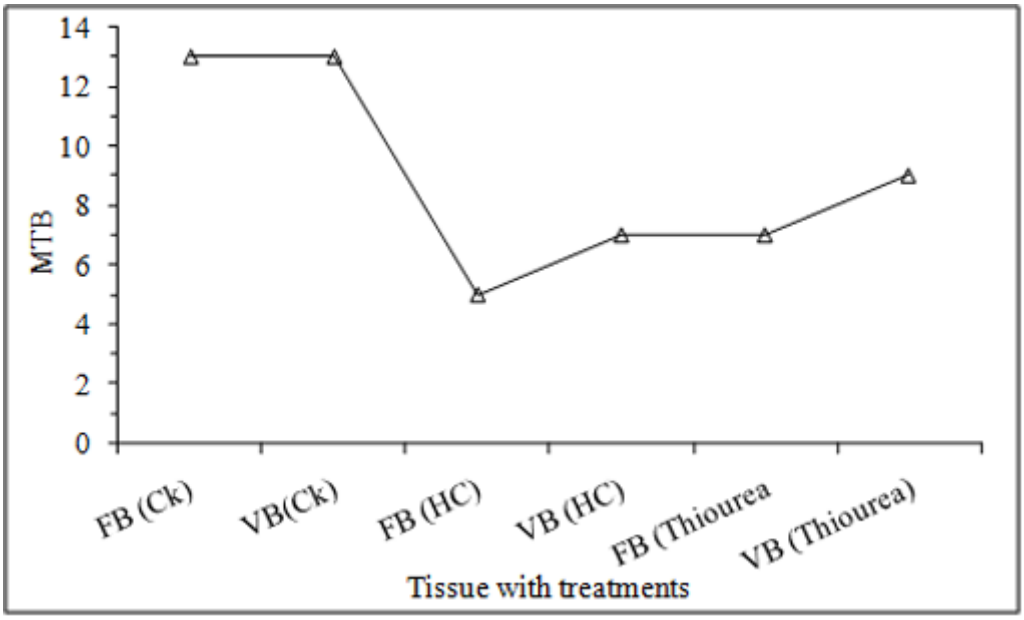

FIG. 2. MEAN TIME TO BUDBREAK (MTB) OF FLORAL AND VEGETATIVE BUDS AFTER HC, THIOUREA AND DH2O (CONTROL) APPLICATION DURING FORCING CONDITION. ERROR BARS ARE PRESENTED AS THE MEAN ± SE; N =3.

3.3 Carbohydrates concentration in floral, vegetative buds and bark tissues after treatments

The effect of HC and thiourea application on starch and sugars concentrations in dormant floral, vegetative buds and bark tissues of pear cultivar 'Wonhwang' are mentioned and summarized in (Figure 3, 4 and 5). Starch concentration was high in dormant floral buds and declined during forcing. This concentration showed a significant and rapid decrease (103% and 90%) in floral buds and reached to the lowest level after 5 and 15 days of forcing after the application HC and thiourea respectively, followed by a slight increase from the rest of the forcing period. However, starch concentration in untreated floral buds was decreased gradually and reached to the lowest point after 20 days of forcing and then increased. Furthermore, there were no significant changes in starch concentration between treated and untreated floral buds after 20 days of forcing. However, the pattern of changes in starch concentration were similar for both treated and untreated floral buds, but the rate of

decline was more rapid in HC and thiourea treated than control floral buds (Figure 3A). Furthermore, all sugars concentrations increased in floral buds after the application of HC, thiourea treatment and control during forcing period. But the sucrose, glucose and fructose concentrations increased quickly in floral buds after 5 days of treatment and reached to their highest level followed by thiourea treated floral buds (Figure 3B, C, D). After getting the highest level their concentrations were decreased while their quantity during the decreased was high in control floral buds then treated. Sorbitol concentration increased in same pattern in treated and untreated floral buds but its quantity was higher in HC treated floral buds then thiourea and untreated (Figure 3E).

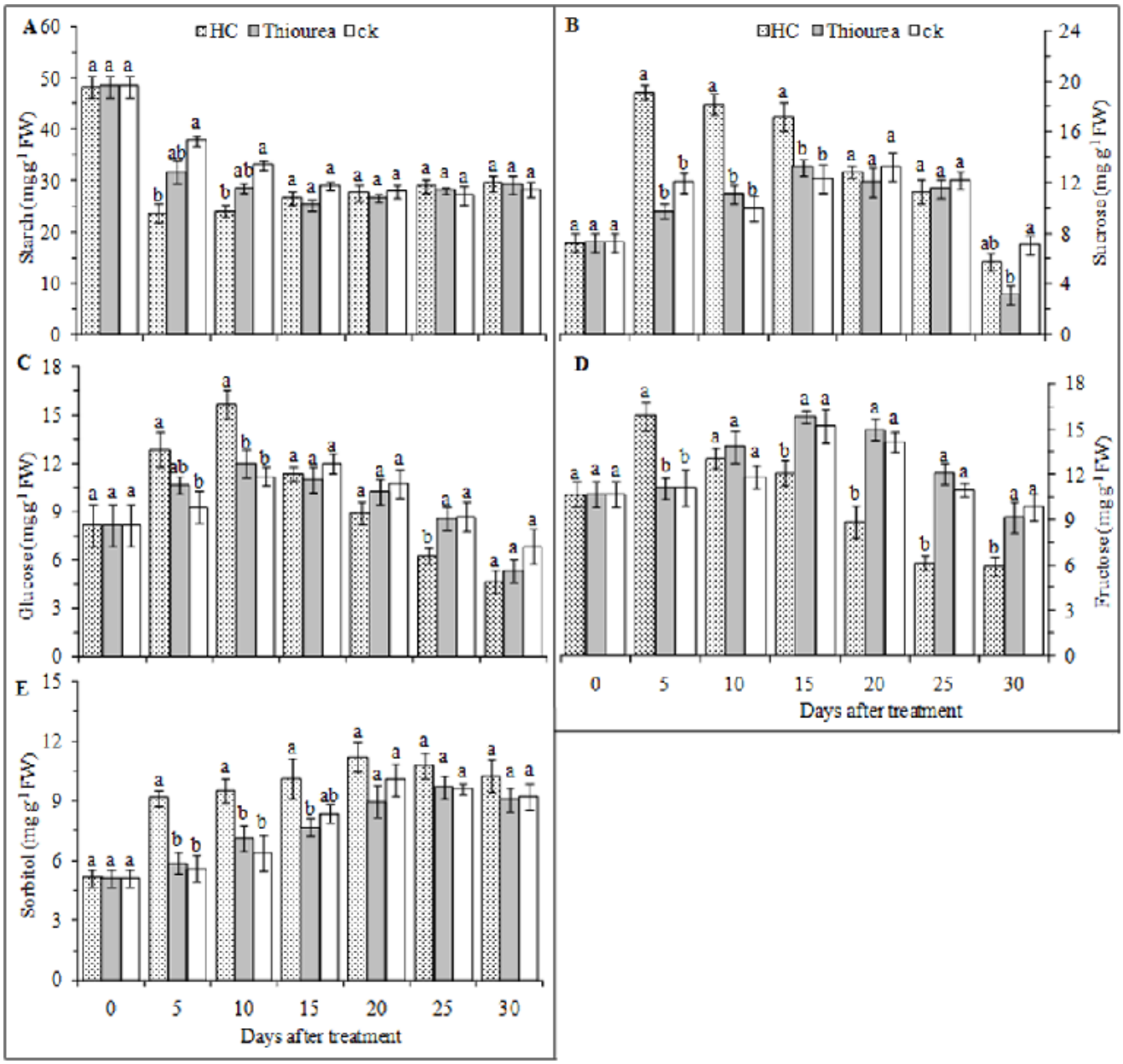

FIG. 3. CHANGES IN STARCH (A), SUCROSE (B), GLUCOSE (C), FRUCTOSE (D) AND SORBITOL (E) CONCENTRATIONS IN DORMANT FLORAL BUDS OF PEAR CV. 'WONHWANG' AFTER HC, THIOUREA AND DH2O (CONTROL) APPLICATION DURING FORCING CONDITION. BAR REPRESENT ± SE OF FOUR REPLICATES. VALUES FOLLOWED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT ACCORDING TO LEAST SIGNIFICANT DIFFERENCE (LSD) TEST AT $P \leq 0.05$.

Our results revealed that the amount of starch in vegetative buds was at its maximum level just after treatment (Figure 4A). We observed a rapid decline in starch concentration (56%) in vegetative buds after 5 days for HC treated buds followed by thiourea (28%) and control (12%). Starch concentration decreased up to 20 days after treatment for all treated vegetative

buds, thereafter, its concentration increased (Figure. 4A). Starch concentration was in high quantity during the increase in untreated vegetative buds as compared to treated buds. Initially, there was a rapid increase in sugar concentration concomitant with a rapid decrease in starch concentration of HC treated vegetative buds (Figure 4B). Data in Figure 4B-E, show that, the contents of sucrose, glucose, fructose and sorbitol increased gradually from the start of the treatment and reached to their highest levels after 5, 10 and 15 days of treatment in floral buds treated with HC, thiourea and control respectively. Our results demonstrated that the amount of sugars was high in HC treated vegetative buds followed by thiourea and control during the increase while their amount was high in control followed by thiourea and HC treated vegetative buds during the decrease.

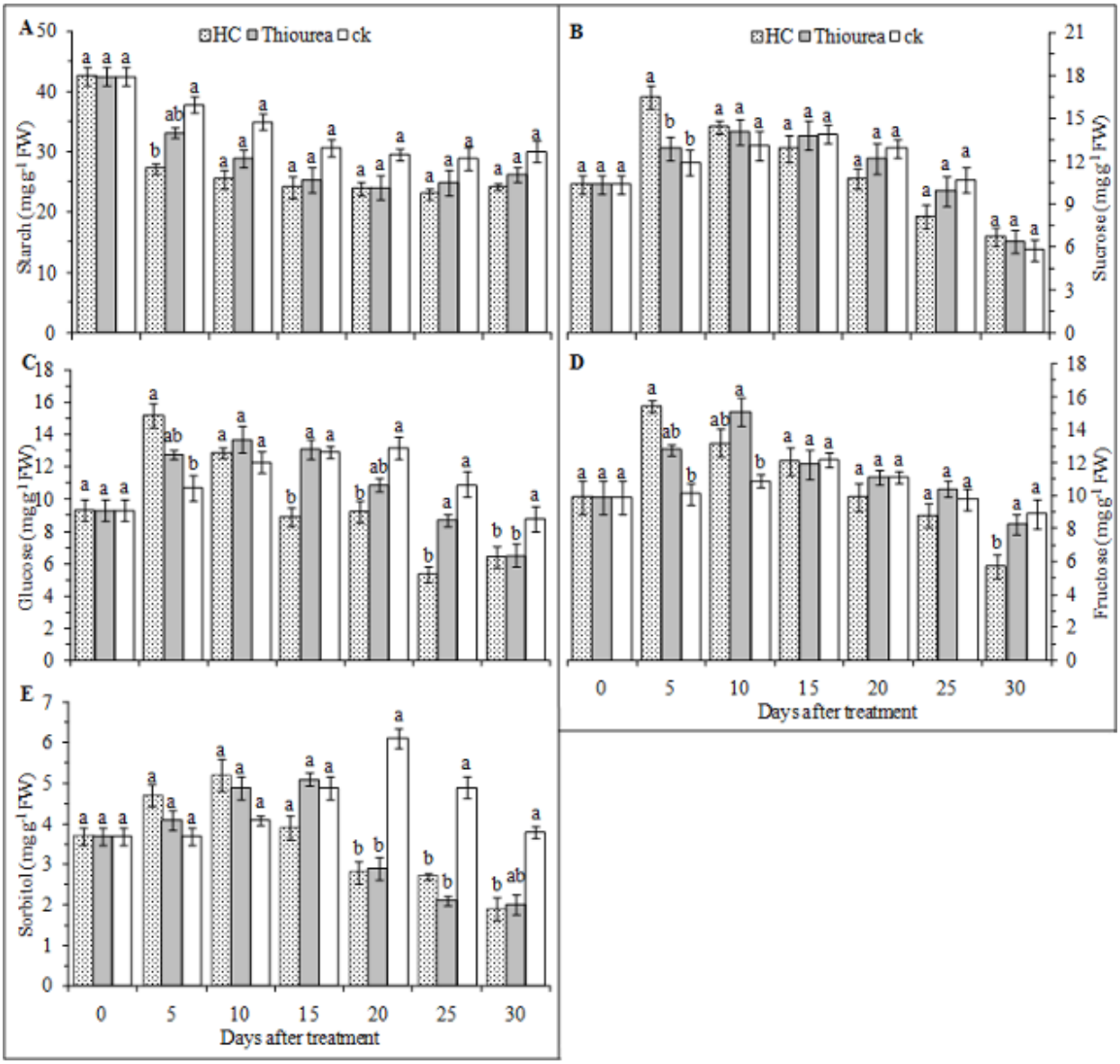

FIG. 4. CHANGES IN STARCH (A), SUCROSE (B), GLUCOSE (C), FRUCTOSE (D) AND SORBITOL (E) CONCENTRATIONS IN DORMANT VEGETATIVE BUDS OF PEAR CV. 'WONHWANG' AFTER HC, THIOUREA AND DH2O (CONTROL) APPLICATION DURING FORCING CONDITION. BAR REPRESENT ± SE OF FOUR REPLICATES. VALUES FOLLOWED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT ACCORDING TO LEAST SIGNIFICANT DIFFERENCE (LSD) TEST AT $P \leq 0.05$.

Starch concentration was higher in bark tissue then in floral and vegetative buds but it decreased in a similar trend (Figure 5A). Indeed, starch degradation was more rapid in HC and thiourea treated bark then control. However, up to 15 days after treatment starch concentration decreased in treated and untreated bark tissue while thereafter, no significant differences was observed in starch concentration. Following HC treatment, sucrose concentration in bark tissue increased quickly and reached to maximum level (14.7 mg g^{-1} FW) on day 10 of forcing then started to decrease immediately after (Figure 5B).

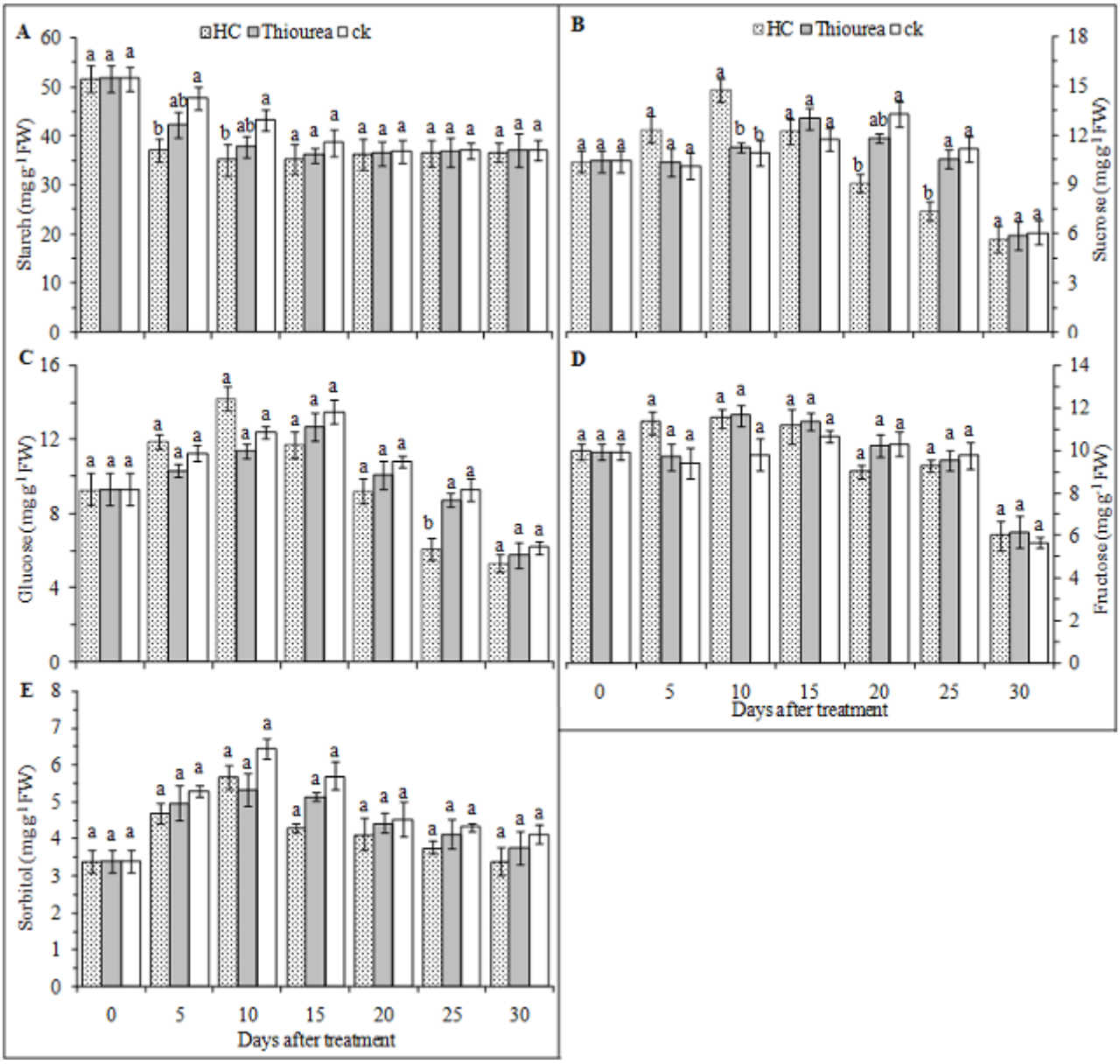

FIG. 5. CHANGES IN STARCH (A), SUCROSE (B), GLUCOSE (C), FRUCTOSE (D) AND SORBITOL (E) CONCENTRATIONS IN BARK TISSUE OF PEAR CV. 'WONHWANG' AFTER HC, THIOUREA AND DH2O (CONTROL) APPLICATION DURING FORCING CONDITION. BAR REPRESENT ± SE OF FOUR REPLICATES. VALUES FOLLOWED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT ACCORDING TO LEAST SIGNIFICANT DIFFERENCE (LSD) TEST AT $P \leq 0.05$.

However, in thiourea treated and control bark tissue, there was a delay and less pronounced sucrose accumulation and reached to maximum point (13 mg g^{-1} FW) and (13.3 mg g^{-1} FW) after 15 and 20 days of treatment before declining thereafter respectively. By the end of the forcing period, sucrose concentration was almost equivalent in all treated and untreated bark tissues (Figure 5B). Glucose concentration have increased temporarily up to (14.2 mg g^{-1} FW), (12.7 mg g^{-1} FW) and (13.5 mg g^{-1} FW) by HC, thiourea and control bark tissue about 10 and 15 day of forcing respectively; thereafter, it decreased in all type of treated bark tissues but more rapidly in HC treated bark tissue. Glucose concentration progressed in the same trend for all treated and control after 5 days of treatment during forcing but the magnitude was different (Figure 5C). During 5 days of forcing, fructose concentration was increased in bark tissue treated with HC while decreased in thiourea treated and untreated bark tissues. However, after 10 days of treatment fructose concentration decreased in all treated and untreated bark tissues and no significant differences were observed (Figure 5D). Unlike sucrose, glucose and fructose, sorbitol concentrations were not significantly different between treated and untreated bark tissues during forcing

period (Figure 5E). Sorbitol concentrations increased after 5 days of treatment in all treated and untreated bark tissues and reached its highest level after 10 days in treatment and then decreased until the end of the forcing period. Furthermore, the magnitude of sorbitol concentration was high in untreated bark tissues then in treated bark tissues in the whole forcing period (Figure 5E).

It was obvious that soluble sugars concentrations, mostly sucrose and glucose, endured an accretion phase followed by a decline or utilization phase when budbreak started for all treated and untreated cuttings. However, the amount of accumulation was greater in HC treated cuttings followed by thiourea treated and control while the rate of decline was greater in control cuttings followed by thiourea and HC treated cuttings.

IV. DISCUSSION

In this experiment, we investigated the effect of HC and thiourea application on floral, vegetative budbreak and carbohydrate reserves metabolism in floral, vegetative buds and bark tissues after the exposition of 2/3rd CH of cultivar by using excised cuttings in forcing condition of pear cultivar 'Wonhwang'. Lack of chilling is the main factor of abnormal pattern of budbreak, development and flower bud abortion in temperate fruit trees associated with mild winter [7,35,36,37,38]. To overcome these problems, different artificial dormancy breaking agents are used to influence the dormancy release and cover the chilling insufficiency. Dormex and thiourea has been used effectively in the replacement of lack of chilling, achieve satisfactory budbreak and improve cropping. HC and thiourea advanced floral and vegetative budbreak by about 8, 6, 6 and 4 days compared with untreated buds respectively. Because for HC treated cuttings, the starting of the floral and vegetative budbreak was around day 5 and 7, while in cutting treated with thiourea, the floral and vegetative budbreak was started on day 7 and 9 respectively; however, day 13 was recorded for untreated floral and vegetative budbreak (Figure 1A and B). These findings corroborate the results of [39,40]. They observed that the time of budbreak was advanced by 4 and 8 days by HC and thiourea application as compared to control in superior seedless grapevine and pear respectively. After five weeks of forcing, the percentage floral and vegetative budbreak of HC treated buds was high followed by thiourea treated and untreated buds. These results are in agreement with the previous findings of [41,42,43], who found that different chemical application increased the flower and vegetative budbreak percentage compared with control in apricot and plum cultivars. These findings agree with the suggestions by [44,45,46], which they demonstrated that the application of different chemicals had hastened and constrained budbreak when applied to deciduous fruit trees in that region where insufficient chilling was a problem. Furthermore, we observed that dormancy breaking agents increased the budbreak ratio and decreased the MTB value as compared with control treatment during forcing condition. The stimulating effect of dormancy breaking agents in buds endodormancy release appears to be due to their positive effect of metabolic activities in the dormant buds. Moreover, [47,29,48], reported that, dormancy breaking agents (dormex and thiourea) were most effective in advancing the budbreak with different percentage compared with the control.

In this piece of work, we studied the effect of HC and thiourea application on reserve carbohydrates metabolism in dormant floral, vegetative buds and bark tissues of pear cultivar 'Wonhwang'. Reserved carbohydrates are the main energy source for budbreak and other metabolic process in the underlying tissues of perennial plants.

The effect of low temperature on carbohydrates concentration during dormant period can be explained due to amylase activities which are induced by cold temperature; starch hydrolyses and accordingly sugar concentration as in peach and poplar [19,49]. The increase in both floral and vegetative budbreak percentage in response to HC and thiourea applications were escort with significant changes in carbohydrate metabolism in both buds and bark tissues. Buds are a strong sink uses stored carbohydrates for restarting the growth [50]. Different key enzymes are involved in carbohydrate metabolism in sink, whereas, its size is given by cell number [51]. It was observed that rapid starch degradation and sugars accumulation in the buds and bark tissues immediately after HC and thiourea application (Figure 3, 4 and 5). Starch breakdown was intimately connected with the raise in sucrose, glucose and fructose concentrations. Here we can presume that sucrose accretion is credited to starch hydrolysis providing the strong evidence that starch was the main source for sucrose synthesis. These results agreed with the previous findings of [52], they stated that the highest starch content in branches under insufficient chilling could reflect the lack of induction of these starchy enzymes during the dormancy period in wood tissue of walnut trees. It was stated earlier that soluble sugars accumulation associates well with bud endodormancy release [53]. The energy required for budbreak comes mainly from the mobilization of products stored in the perennial parts of the plants. Because of that score, starch is known to serve as an important carbohydrate reserve during an extended period which begins at leaf drop and continues until some week after budbreak [54]. We found in our experiment that there is an overall imbalance in carbohydrate reserves as a consequence of the metabolic consumption during dormant period and forcing condition after

treatment. Furthermore, we observed the fleeting soluble sugars (especially sucrose) accretion which seems to perform a key role possibly by lowering the osmotic potential that activate the events leading to bud endodormancy release as was recommended in dormant onion bulb [55]. Both floral and vegetative budbreak was observed earlier (7 to 10 days) in HC and thiourea treated cuttings then control during forcing period, hence we noticed a rapid consumption of soluble sugars in buds and bark tissues which indicting that metabolic activity of the buds has increased as was mentioned earlier in peach [19,56]. In fact, on days 5 and 10 after HC and thiourea treatment, there was more sucrose in the floral and vegetative buds than in the bark (Figures 5B, 6B and 7B) and starch concentration declined more rapid in buds suggesting a hydrolysis of starch to sucrose in buds. Similar results have previously been reported for sprouting sugarcane buds [57]. Generally, there are some enzymes as (acid invertases) which convert sucrose into hexoses to provide cells with carbon and energy for the synthesis of different compounds essential for budbreak. Altogether, and based on the above explained results, we suppose that the decrease in starch concentration as well as the accumulation of soluble sugars may be a part of the mechanism leading to endodormancy release following HC and thiourea application, while the rapid consumption of sugars and sink strength development, an signal of high metabolic activity, are fundamentals for budbreak and active growth. In contrast, the starch hydrolysis and sugars accumulation and utilization were less rapid and less pronounced in untreated tissues, reflecting the differences in bud development which was higher and more intense in HC and thiourea treated cuttings, but delayed and uneven in untreated cuttings. It is therefore possible that the differences in the timing and amount of soluble sugars' increase and use processes between HC, thiourea treated and control cuttings are accountable for the observed effects of HC and thiourea treatments on budbreak timing and percentage.

V. CONCLUSION

In conclusion, the application of HC and thiourea caused significant increase in floral and vegetative budbreak as compared to control cuttings. Buds endodormancy release occurred concomitantly with the accumulation of sucrose and glucose in floral and vegetative buds whereas the starch content decreased in all tissue but most significant in floral buds. Less MTB and more budbreak percentage was observed on cuttings treated with HC followed by thiourea and control for both floral and vegetative buds during forcing condition. HC and thiourea treatments also affected the starch and soluble sugar contents in floral, vegetative and bark tissues. Increasing soluble sugars and high starch reduction were associated with HC and thiourea treatment and the release of endo-dormancy, as it was wondered that HC and thiourea treatments response could be related either to the dormancy release or to the carbohydrates metabolism or to both processes. Furthermore, stable starch concentrations and rapid soluble sugars utilization show the rising in metabolic activity and budbreak percentage.

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