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ASPECTS RELATED TO THE BIOCHEMISTRY OF RESISTANCE TO COLD-INDUCED STRESS IN 4 APRICOT CULTIVARS IN THE CLIMATIC CONDITIONS OF 2018 IN NW ROMANIA

Laslo Vasile*, Oneț Aurelia*, Vicaș Simona*, Teușdea Alin*, Oneț Cristian*, Moș Georgiana*

*University of Oradea, Faculty of Environmental Protection, 26 Gen. Magheru St., 410048, Oradea, Romania, e-mail: vasilelaslo@yahoo.com

Abstract

The accumulation of chill portions started in October and in early January this stage was completed (58CP), the need for cold of the cultivations studied being satisfied. The maximum amount of peroxidase in all cultivars was recorded in February when the temperatures were the lowest (0.41 U / mg at the Siren and 0.15 U / mg at CR2 / 63). Phenolic compounds are more present in the ecodormal phase of late cultivars. High values of soluble proteins were recorded in the coldest months for the determined values varying according to the ripening age of the variety (1.23 mgABS / mg at Comandor and 0.45 mgABS / mg in Rareș).

Key words: apricot, cold, climat conditions, temperature, enzyme

INTRODUCTION

Satisfying the cooling requirements of fruit trees is a determining factor in achieving a quantitative and qualitative production. Responding to lower temperatures and shorter days in autumn, buds of fruit trees are in a state of latency, a state that gives them increased resistance to low temperatures. Depending on the period of the year when latency is installed, reference is made to ectodormality or forced rest, endometrium or rest, and ecodormality or secondary rest. The first form of rest is caused by unfavorable environmental factors in the autumn, which causes rest to enter before the characteristic period. Endodormality is caused by internal factors and it has a genetic determinism (Samish, 1954). The norm is determined by the action of unfavorable factors after vegetation such as cold or drought stress, which induce critical signals that prevent the growth of buds (Lang, 1987, 1996; Horvath et al., 2003).

Deep rest is a feature of wood species in the temperate climate. It is not a mechanism that manifests abruptly, but a progressive one that evolves to a deep resting state (Erez et al., 1979; Lang et al., 1987), involving the expression of some genes that stabilize the cell membranes against the effects of low temperatures (Faurobert, Gomez, 2006).

The onset of resting state takes place before negative temperatures occur, and the endodontic intensification takes place with the setting of negative temperatures (Darbyshire et al., 2013). Recent studies have

highlighted the fact that interaction, photoperiod-temperature plays an important role in controlling the resting state of Prunus species (Ola, 2008).

There is limited information on the resting state of the apricot (Prunusarmeniaca L.). In order to appreciate the depth of the resting state of the buds in the absence of visual morphological changes of buds and due to the lack of endogenous markers, the depth of the resting state is being assessed by the accumulation of cold units (Arora et al., 2002; Laslo et al., 2015).

There are still great gaps in the knowledge of the physiological and biochemical mechanisms underlying the induction of the resting state of the flower buds, the support and the end of this state (Arora et al., 2002).

Currently, evaluation of the resting moment in different apricot cultivars is done using both physical and physiological parameters (weight of floral buds and phenological stages) (Guerriero et al., 2002; Ruiz et al., 2007); histology of flowering buds and their weight after forcing (Viti et al., 2003).

A series of changes in the budding biochemistry seem to indicate the transition from endodormal to ecodormal (Szecskó et al., 2002; Pakkish et al., 2009). Crabbe, 1994 suggests that some biochemical markers could indicate relative levels of rest in organs, tissues or even cells.

In superior plants, antioxidant metabolism undergoes changes according to the annual cycle. Protection against free radicals is achieved by the antioxidant action of some enzymes (catalase, peroxidase, superoxide dismutase etc.) High increases in antioxidant activity have been reported in apricot cultivars with low requirements of cold at the end of the endodormal period. The results suggest that minimal thresholds for antioxidant activity may be decisive in eliminating free radicals formed during winter (Bartolini et al., 2006).

Alongside the destructive effect that free radical oxygen produces in plants, it seems that they also play a critical role in transduction signals. Due to these considerations, it is important to maintain a balance between the free radicals formed and the removed / annihilated. It is considered that the plants resistant to oxidative stress, generated by free oxygen radicals, are resistant to frost (KohIba, 2002; Laslo et al., 2018).

Studies on the activity of sulfhydryl (SH) compounds, reduced glutathione (GSH) and catalase activity during the flowering cycle of flower buds have been made in apricot cultivars that have a different production capacity. The activity of catalase, SH and GSH changes according to a 3-step model from November to February. In the first two phases, there is a gradual increase in the activity of catalase and reduced glutathione, in parallel with a decrease in the total concentration of the sulfhydryl compounds. In Phase 3 (the beginning of February), at the same time as SH

and GSH increase, catalase activity tends to decrease (Viti, Bartolini, 1998). Many studies report that in buds the content in phenols is different, varying both in the time of rest and variety. Thus, at the beginning of resting, the phenomenon of phenols is increased at the beginning of rest, followed by a reduction and a complete elimination when flowering (Szalay et al., 2005). A close relationship between protein content and accumulation of cold units has been determined by Hisayo Yamane et al. 2006 in Japanese apricot (PrunusmumeSiebold&Zucc.) In another study, Tamura et al. (1998) suggested that the 19 kDa protein may be a suitable marker for measuring the extent to which the Japanese pear develops its resting state (PyruspyrifoliaNakai). The study we initiated aims to establish a correlation between the need for the cold required to go through the dormancy phase and the presence of biochemical markers that signal the passage of some stages.

MATERIAL AND METHOD

The study was conducted to determine the content of phenolic compounds, proteins, peroxidases and their correlation with the cold units necessary for the passage of the phenological stages of endodorming and ecodormance in 4 apricot cultivars cultivated in the NW Romania. We start from the premise that in this it is possible to perform comparative studies between different years and different geographical areas, it is possible to estimate the beginning and the duration of a physiological stage based on a quantitative parameter. Using the number of days is an imprecise way of expressing the initiation or duration of a phenological process due to the specific conditions of each year.

The biological material used

Floral buds were sampled at 12-14 days from 15-year-old apricots from S.C. Prototera S.R.L. (Prunuscerasifera), cultivated at a distance of 4x3 m and belonging to the cultivars: Rareş, CR 2/63, Sirena, Comandor.

Rareş. Low sturdiness tree, resistant to frost. The fruit has a medium-to-large spherical shape, weighing 60-70 g, the color of the epidermis is light orange with dashes of raspberry red, with a pale-yellow light orange pulp, juicy, the pulp does not stick to the kernel, very pleasant taste. The maturing period being June 5-10.

Sirena. The tree is of medium sturdiness with a globular crown, with solid skeletal branches, well garnished with short, medium and medium fruiting bands. The fruit is medium to large, on average 65 g, globular to ovoid, slightly asymmetrical. Orange peel with red spots on the sunny side. The pulp is orange with a firm, succulent structure, with a pleasant taste and fine aroma. The kernel represents 6.1% of the weight of the fruit and has a sweet core. The ripening time is between 10-15 August.

C.R. 2-63 (Cream Ridge 2-63). Variety of American origin Early ripening age, 88-112 days from flowering; early flowering, very abundant; high vigor trees, planting distance 5/5 or 5/6 m; predominant bearing on short branches; high productive potential 12-15 t / ha. Large fruit 60-80 grams; the peel is a orange with red carmine on the sunny side; orange pulp, juicy, aromatic, the pulp does not stick to the kernel; large kernel with a bitter core.

Comandor. Medium vigour growth tree, globular crown, with bouquets and mixed branches. It bears fruit from the fourth year since planting, it has good resistance to frost and spring frosts as well as drought. The fruit is large, 75 g, spherical, slightly elongated, with a very attractive appearance. The peel is elastic, yellow and orange, with dashes of red. The pulp is light orange, it is consistent and juicy with a balanced, sweet and aromatic taste. The kernel is nonadherent, relatively large, representing 6.8% of the weight of the fruit.

Preparation of the vegetal extract

Twenty floral buds were detached from the annual branches, weighed, cold milled in the presence of phosphate buffer Ph 7.2, passed through Ependorf tubes and centrifuged at 10,000 rpm at 40^0 C for 20 minutes. The harvest of buds has been conducted during Phases B, F and G after Flekinger, J (1945). The ratio of buds weight to phosphate buffer solution was 1:20 (w / v).

Biochemical determinations

a. Determination of peroxidase. Enzymatic activity of the peroxidase was measured using a spectrophotometer, the method being the oxidation of p-phenylenediamine by the peroxidase enzymes present in a plant extract and obtaining a violet colored solution. There is a direct proportion between the color intensity of the solution, the measured value with the spectrophotometer at 483 nm wavelength (UV minis UV-VIS Spectrophotometer, Shimadzu) and the peroxidase activity.

b. Determination of total soluble protein. The protein concentration in the bud extract was determined as described by Bradford (1976), using bovine serum albumin (BSA) as standard. Soon, buds were harvested for protein extraction, from which an extract was obtained using 6.7 · 10-3M phosphate buffer (pH 6.1). The extract was centrifuged for 20 minutes at 10,000 rpm and 40^0 C. The supernatant was used for spectrophotometric determination of the protein at 595 nm wavelength (Shimadzu UV-Vis mini-1240).

c. Determination of polyphenolic compounds. The total phenol content was determined by the Folin-Ciocalteu method. This method combines 100 µl of buds extract, 1700 µl of distilled water and 200 µl of Folin-Ciocalteu reagent; then mix thoroughly using a Vortex. The mixture

was allowed to react for 3 minutes and then we added 1 ml of 15% Na 2 CO 3 (Arnous et al., 2001) solution and mix thoroughly. Samples were incubated at room temperature in the dark for 2 hours. Absorbance was measured at 750 nm using a UVminis-UV spectrophotometer (UV-VIS), Shimadzu). The standard curve was linear, between 0.1-0.5 mg / ml gallic acid. The results were expressed in gallon equivalents (GAE, mg / g fresh weight). Appropriate dilution was required if the measured absorbance value was above the linear range of the standard curve.

d. Temperature Determination. It was made from hour to hour using the HANNA 143 HI logger between November 2017 and May 2018

Calculation of cold needs was done using dynamic (Fishman et al., 1987). I also received information on temperatures on

RESULTS AND DISCUSSION

Dynamics of temperatures during the studied period 2018 was atypical in terms of recorded temperatures (Fig. 1). December was a relatively warm month with 23450^0 C accumulated, of which 1099^0 C temperatures above 6.5^0 C (considered the apricot heat threshold). Degrees of negative temperatures were 1770^0 C.

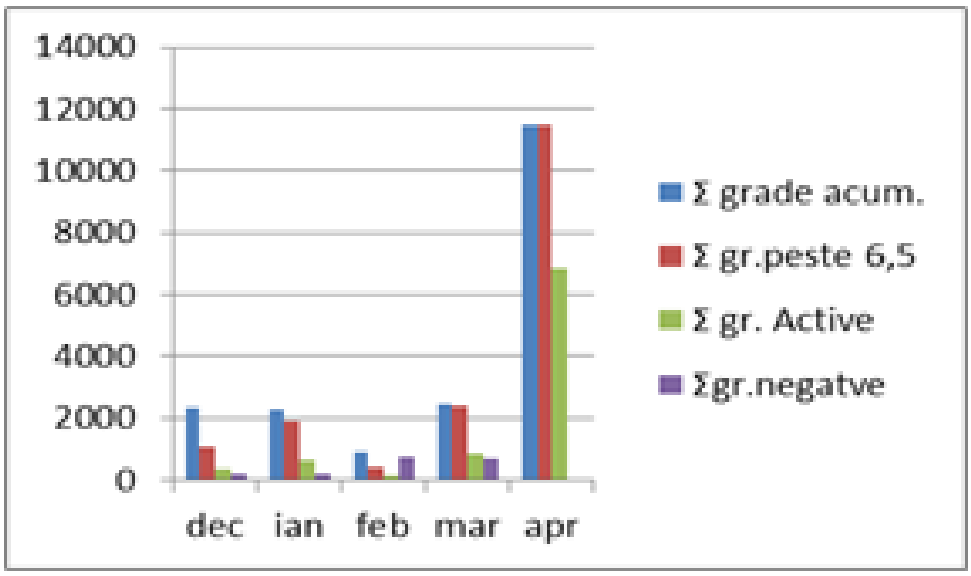

Fig. 1. Dynamics of temperatures between December 2017 and April 2018

The month of January was under the thermal regime of December. Although the number of accumulated grades was lower than in December 2291^0 C (which suggests a cooler weather) the sum of temperatures above 6.5^0 C was 1882^0 C, with the sum of the 635^0 C active temperatures. In terms of active temperatures, January was warmer than December. In the

study period February (Fig. 2) was the coldest with a total sum of only 881^0 C and a sum of negative temperatures of 726^0 C.

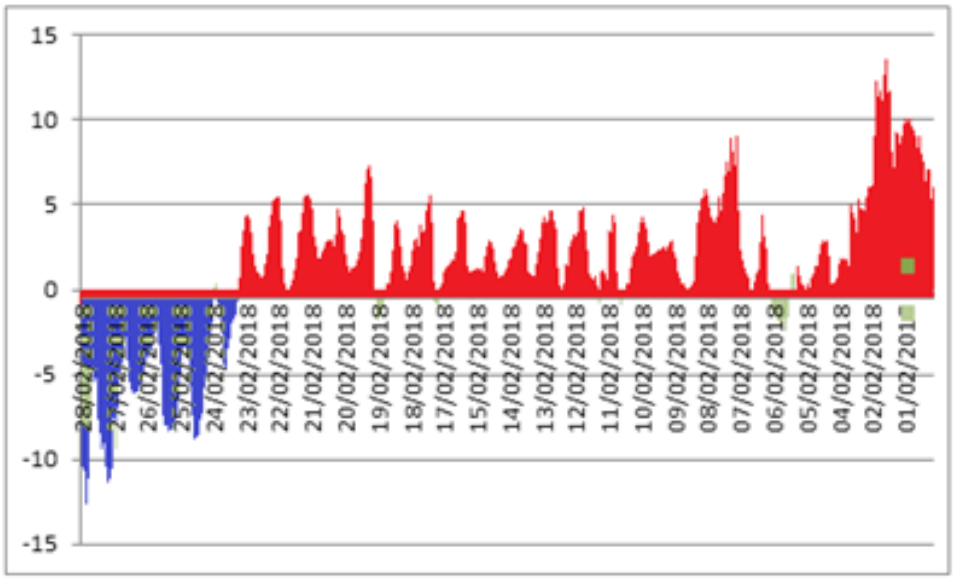

Fig. 2. Dynamics of Temperatures for February 2018

Determination of the accumulation of cold portions (CP) from the resting state to starting vegetation (Fishman et al., 1987 method)

The dynamic model, developed by Fishman et al., 1987a, 1987b, is based on the assumption that the accumulation of cold results from a two-stage process. In the first step, low temperatures produce an intermediate cooling compound, which can be destroyed by heat. The intermediate product can be transformed into a permanent "cooling portion" by a process that is most effective at moderate temperatures. These cooling parts are accumulated during winter. The equations on which this model is based are more complex than the other models (Chilling Hours, Chill Units according to the Utah Model), but have been described by several authors (Luedeling et al., 2009).

From Fig. 3 it can be noticed that the accumulation of chill portions started after the fall of the leaves as early as October, so at the end of December already 52 PS were accumulated, ie the need for cold of the earlier cultivars was already satisfied from the second half December.

The process continues in the following months, 18 CP in January, 17 CP in February, 16 in March and 3 CP in April, totally 106 CP. The active phenophases started on 09.03.2018 with the Rareş variety. The late variety analysed (Commander) budding infusion phenophase started 12 days later (Table 1).

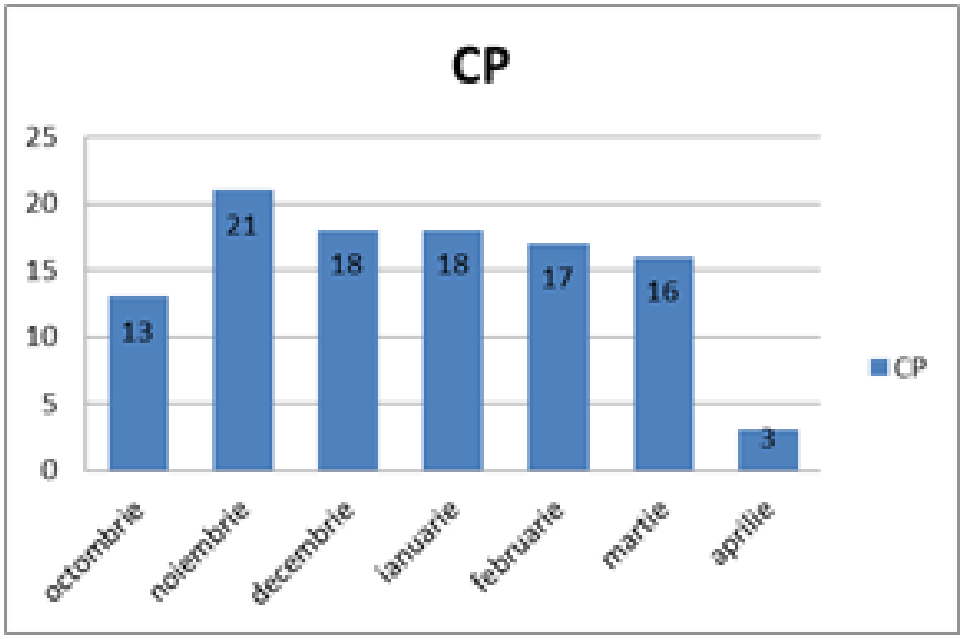

Fig. 3. Monthly accumulation of cold portions (CP) between October 2017 and April 2018

Table 1

Date of triggering and duration of phenophases in the studied apricot tree cultivation

Nr. crt.	Type	Flower bud distension	Pink bud	Beginning of blooming	Ending of blooming
1	Rareș	09.03	22.03	02.04	10.04
2	CR2/63	11.03	25.03	06.04	10.04
3	Sirena	15.03	03.04	09.04	12.04
4	Comandor	21.03	04.04	12.04	17.04

Table 2

The accumulation of chill portions (CP) until the phenological stages are triggered

Type	Flower bud distension	Pink bud	Beginning of blooming	Ending of blooming
Rareș	93	98	103	106
CR2/63	94,84	96,84	104	106
Sirena	94,84	100	105	106
Comandor	94,94	100	105	106

Endodormance and ecodormance of studied cultivars

In the last days of December, the need for cold to trigger the swelling of the buds in the Rareş variety was already accumulated (36CP). Upon entering the phenophase the puffiness of the buds accrued 93CP (Fig. 4). The climatic conditions of 2017 and the beginning of 2018 lasted practically 75 days, accumulating the over 57 CP over the necessary. Ecodormance was imposed by temperatures below the biological threshold (6.50 ° C).

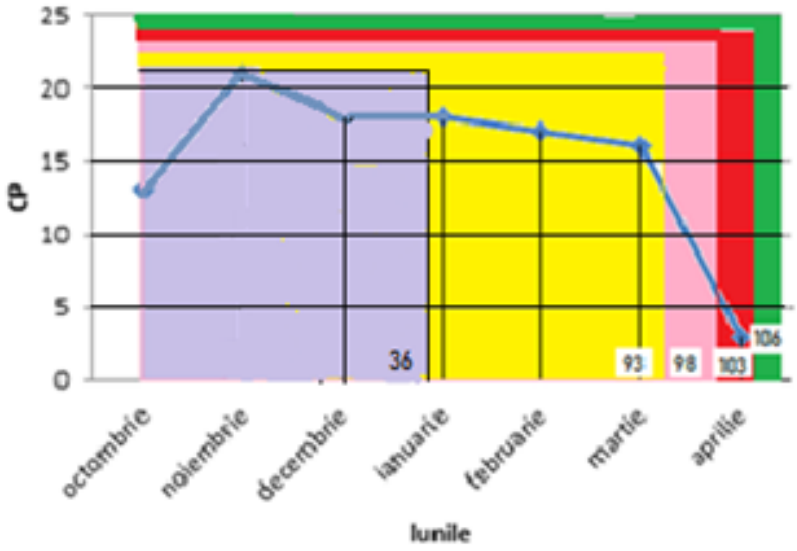

Fig. 4. Additional accumulation of CP in the Rareş variety

In the CR2 / 63 variety the end of the endodormancy occurred at the end of December and the bud sputum phenophase started on 11.03.2018. The long period of ecodormancy was due to the temperatures below the biological threshold in January and the low temperatures in February and March.

The end of endodormancy in the Sirena variety was recorded in the last days of December, and budding puff phenophase began on March 15th, 2018.

The high temperatures recorded in April caused a reduction in the gap between the latest and the earliest cultivars analysed. Among the budding puff phenophase the gap was 11 days, followed by pink button phenoxy with a 12 day gap then the beginning of blooming with 10 days and shedding the petals for 7 days.

In the climatic conditions of the last 3 months of 2017 and the first 3 months of the 2018 stage of endodormancy was practically completed in all four cultivars at the beginning of January (Fig. 8).

The subsequent evolution of temperatures to the limit of the biological threshold in February, continuing with very low temperatures in the beginning of March, followed by a few days with very high temperatures and then again low temperatures (21-23 March 2018), caused the massive affliction of buds in cultivation of Rareș and CR2 / 63. In these cultivars the degree of damage was over 90%.

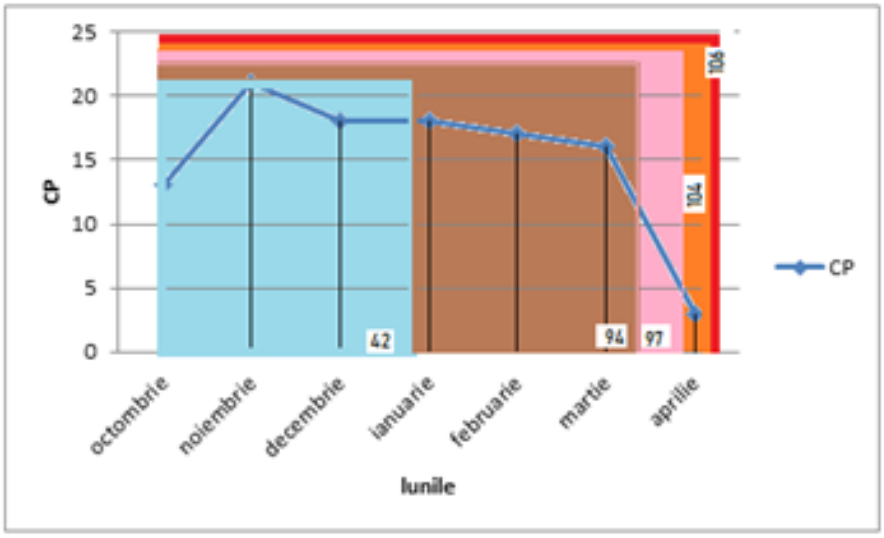

Fig. 5. Additional CP build-up in CR2 / 63 variety

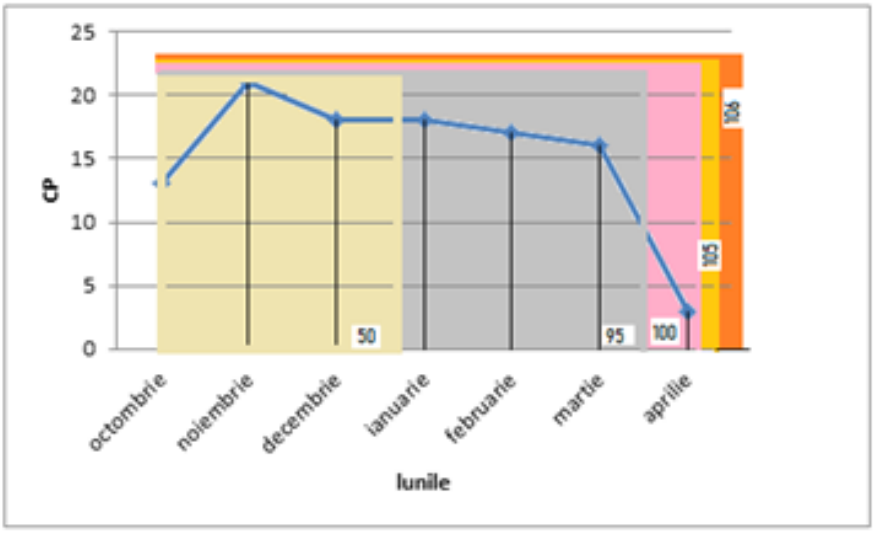

Fig. 6. Additional accumulation of CP in the Sirena variety

Peroxidase activity as a stress marker due to low temperatures

Peroxidases are involved in many physiological processes in plants, involving responses to biotic and abiotic stress. They are involved in

biosynthesis of the lignin polymer responsible for plant stiffness and hydrophobicity of cell walls. Peroxidases are also involved in the polymerization of lignin precursors. Another important physiological role they have is to be involved in the elimination of reactive oxygen species (ROS), which are partially reduced atmospheric oxygen forms, very reactive and capable of causing cellular oxidative lesions. Peroxidases may be a source of hydrogen peroxide (H_2O_2), but are also capable of eliminating it (Vicuna, 2005).

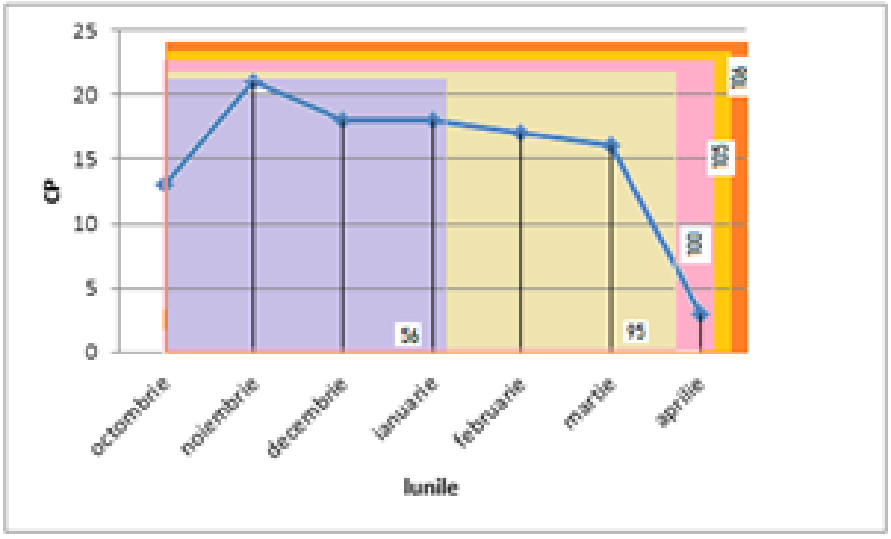

Fig. 7. Additional accumulation of CP in the Comandor variety

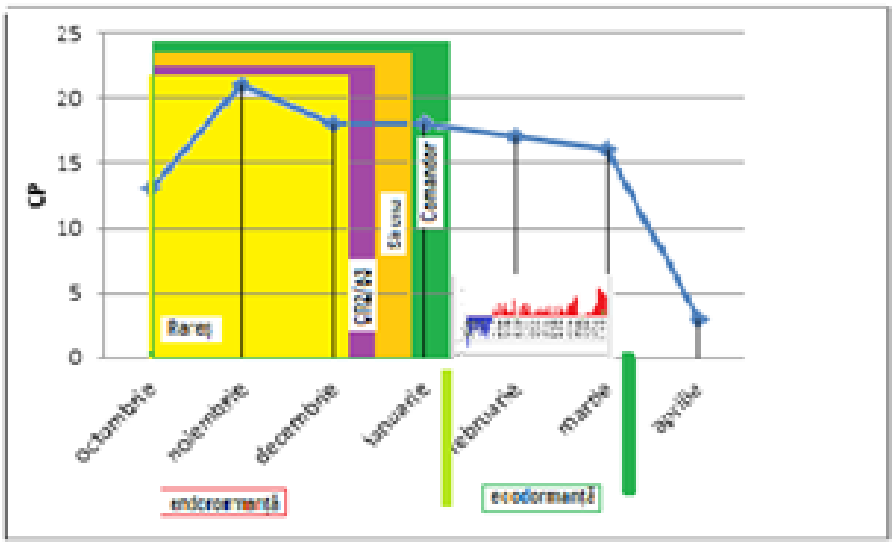

Fig. 8. Evidence of endodormancy and ecodormance in studied apricot cultivation

It can be seen from Fig. 9 and Tab. 3 that the cultivars are grouped in the sense that the earlier cultivars produce a smaller quantity of peroxidases. The maximum quantity of peroxidases in all cultivars was recorded in February when the temperatures were the lowest.

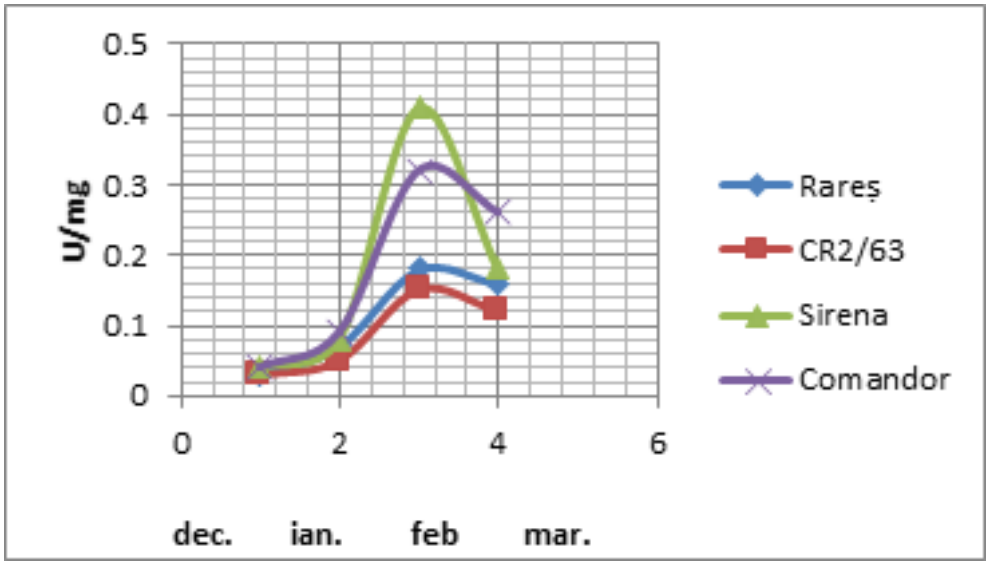

Fig. 9. The dynamics of peroxidases in the 4 apricot cultivars studied

Table 3

Peroxidase values (U / mg) in 4 apricot cultivars studied

Type	Dec.	Jan.	Feb.	Mar.
Rares	0,03	0,07	0,18	0,16
CR2/63	0,03	0,05	0,15	0,12
Sirena	0,04	0,08	0,41	0,18
Comandor	0,04	0,09	0,32	0,26

Exposure of plants to adverse environmental conditions increases the synthesis of reactive oxygen species (ROS), such as singlet oxygen, superoxide (O_2 • -), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH•). The process of detoxification of ROS in plants is essential for the protection of plant cells and organs against the toxic effect of these species (Mittler, 2002). Differences in subcellular location, biochemical properties of antioxidant enzymes and distinct gene expression responses, in addition to the presence of non-enzymatic mechanisms, lead to a versatile and flexible antioxidant system capable of controlling the optimal levels of ROS (Vranova et al., 2002).

ROS detoxification systems include enzymatic and non-enzymatic antioxidant components (Scandalios, 2005). Asbestos (AsA) and glutathione (GSH), non-enzymatic antioxidants are crucial to protect plants against

oxidative stress, playing a key role as antioxidant buffers (Mittler, 2002). Other non-enzymatic antioxidants involved include flavonoids, phenolic compounds, alkaloids, tocopherol and carotenoids (Gratão et al., 2005).

Enzymatic antioxidants include superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione peroxidase (GPX) and peroxiredoxin (PrxR). These enzymes are present in almost all sub-cellular compartments. Typically, an organ has more than an enzyme capable of escaping a single ROS (Scandalios, 2005). ROS capture enzymes in plants have been studied extensively and the results have shown that in environmental stress, APX activity is generally increased along with other enzymatic activities such as CAT, SOD and GSH reductase (Shigeoka et al., 2002). The peroxidase dynamics follows with particularities of the variety, the dynamics of the temperatures, the higher values of the peroxidases being registered when exposed to lower temperatures.

Dynamics of phenolic compounds

Modifications of secondary metabolism in buds of 4 apricot cultivars with different origins in vegetation and fruit ripening show a different resistance to stress induced by prolonged and steady low temperatures. Analyses have shown that the two late cultivars have a higher content of phenolic compounds, a higher free radical capture capacity and a higher reduction potential (Arnous et al., 2001).

Similar studies on vines have shown great differences in the function of secondary metabolism in response to the same stress factor (Król et al., 2015).

In all apricot cultivations studied phenolic compounds are more present in the ecodormance phase. Their higher values are noted in the later Comandor and Sirena cultivars with great differences in March especially (Fig. 10). The results obtained by us indicate that the thermal stress induces phenol accumulation in plants by activating their biosynthesis as well as inhibiting their oxidation. This could be considered a mechanism for acclimating the plant against thermal stress.

Protein content in buds of 4 apricot cultivars

Another mechanism by which plants increase cold resistance is accumulation of soluble proteins. Protein synthesis is a specific mechanism involved in increasing frost tolerance. Low temperatures can stimulate protein synthesis. Two possible functions of apoplastic proteins (AFP) are proposed (Antikainen et al., 1996).

The apoplast consists of the continuous cell wall of the adjacent cells as well as extracellular spaces, forming a tissue compartment comparable to the simplast. The apoplastic pathway facilitates the transportation of water and dissolved substances to a tissue or organ. This process is known as apoplastic transport (Antikainen et al., 1996).

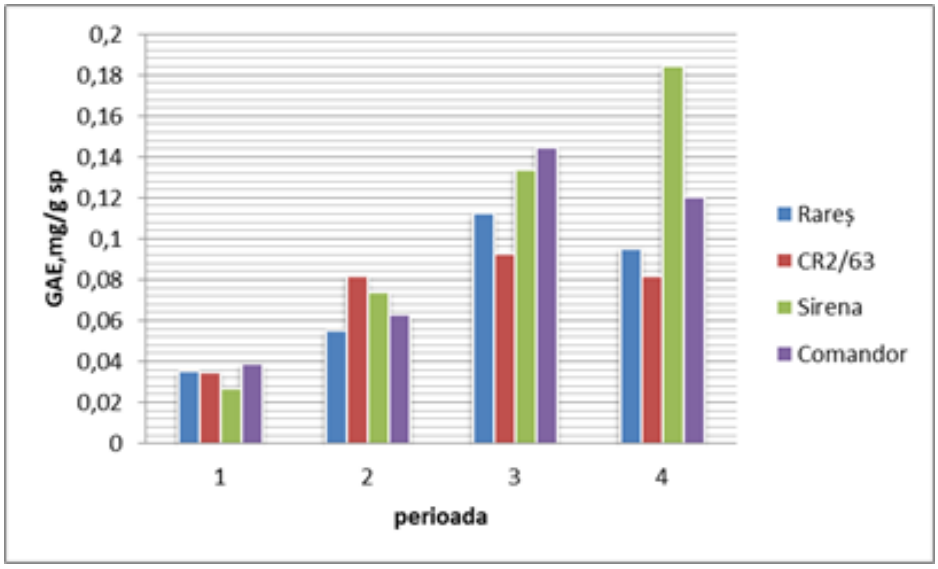

Fig. 10. The content in phenolic compounds of buds in 4 apricot cultivars

A first role of these proteins is to prevent the formation of large ice crystals. AFPs can also function as barriers that inhibit ice formation. To tolerate low temperatures, plants need to adapt to more cellular and metabolic functions. In the four cauliflower cultivars studied by us the highest values of soluble proteins we recorded in the coldest months respectively February and March. The determined values (Fig. 11) varies depending on the ripening age of the variety of the soluble protein values ranging for March from 1.23 mgABS / mg for Comandor and 0.45 mgABS / mg for Rareș.

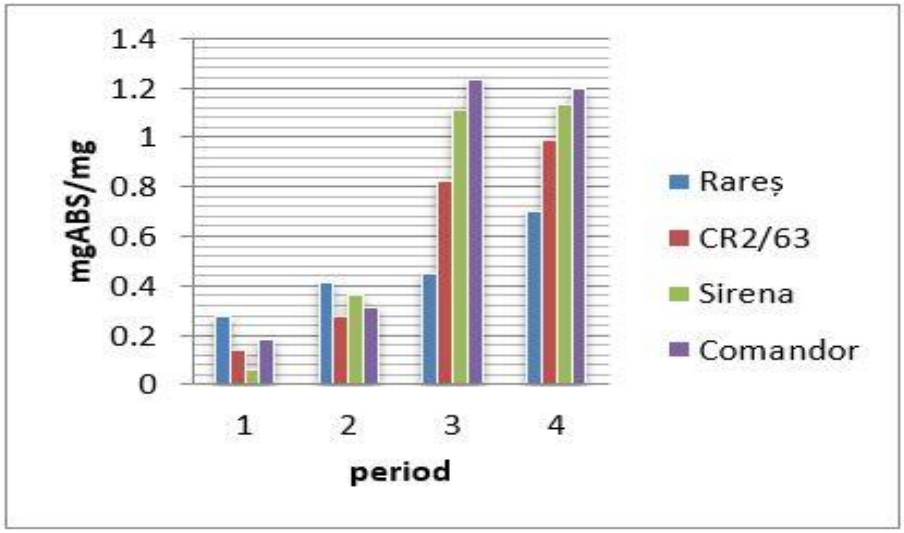

Fig. 11. Dynamics of protein accumulation in 4 apricot cultivars

CONCLUSIONS

The year 2018 was atypical in terms of recorded temperatures. December was a relatively warm month with 2354^0 C accumulated, of which 1099^0C temperatures above 6.5^0 C (considered the thermal threshold at the apricot). The negative temperature range was 177^0 C.

The accumulation of chill portions began after the fall of the leaves in October, so that at the end of December and the beginning of January, the need for cold of cultivated crops was accumulated (58CP).

The ecological condition imposed by the temperatures lasts from the beginning of February until the beginning of April.

The dynamics of peroxidases follows with particularities of the variety and the dynamics of the temperatures. Higher values of peroxidases were recorded at exposure to lower temperatures. Varieties are grouped in the sense that earlier cultivars produce lesser amount of peroxidases. The maximum amount of peroxidases in all cultivars was recorded in February when the temperatures were the lowest with values ranging from 0.41 U / mg to the Siren and 0.15 U / mg to CR2 / 63.

In all apricot cultivations studied phenolic compounds are more present in the ecodormant phase. Their higher values are noted in the later Comandor and Sirena cultivars with great differences in March especially. The results obtained by us indicate that the thermal stress induces phenol accumulation in plants by activating their biosynthesis as well as inhibiting their oxidation. This could be considered a mechanism for acclimating the plant against thermal stress.

The highest values of soluble proteins were recorded in the coldest months of February and March respectively. The determined values vary depending on the ripening age of the variety, the soluble protein values ranging from 1.23 mg ABS / mg in Comandor to 0,45 mgABS / mg in Rareş.

Acknowledgment

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