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Viburnum tinus L. as a new Mediterranean element for central Europe urban landscapes

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Abstract : *Growth phenology of woody plants refers to seasonal biological life cycles driven by environmental factors, and is considered to be a sensitive and significant indicator of climate change. The proposed study attempted to quantify changes in Viburnum tinus L. phenology between the years 2011 and 2014. Phenological studies were carried out during three years, from April 2011 to March 2014, in the Botanical garden of the Slovak University of Horticulture in Nitra, by using ground observations. Ground observations of Viburnum tinus L. growth processes from April 2011 to April 2014 in 15 day intervals were performed both visually growth and by measuring of the leaf chlorophyll content. Two types of planting were managed in the study area; plants planted direct in the ground and were outside during wither time only the part which near the soil was covered by leaves and plants planted in pots which removed to greenhouse during winter time from end of November till end of March. Leaves in this Viburnum tinus begin to bud break and full leafing from the beginning of the April and continued up to the end of May in plants planted in pots, while bud break and full leafing in plants planted direct in the soil begin from end of April and continues up to second week of June. The results show that during study period, the growing season duration has lengthened in plants planted in pots in comparison with plants planted direct in the soil. The chlorophyll content in leaves was measured after taking the leaves from plants in 27.01.2014 when the temperature during the night was (-7°C) and at 9 am was (-3°C) while inside greenhouse the temperature was (8°C). The results showed that there is a significant difference between the plants in each planting types in content of chlorophyll and carotenoids.*

Keywords: *Viburnum tinus, Mediterranean plants, climate condition, winter hardiness, growth phenophases pigments content*

I. Introduction

Viburnum tinus L. follow to *Caprifoliaceae* family is an evergreen shrub that grows naturally in Mediterranean ecosystems. It is frequently used as ornamental plant [1]. *Viburnum tinus* is a large evergreen shrub or small tree. In late spring it produces clusters of small white flower which are followed by blue-black berries. A good structural plant for a medium to large garden.

Every landscape is changing in the course of the historic development and this is expressed mainly by its character and the areal share 3 of secondary landscape structure components, Landscape as a territorial system of physical and biological elements is extremely rich in its diversity and represents not only natural but also anthropogenic cultural heritage [2];[3];[4]. According to [5] the ability of woody plants to cold harden is promoted in early autumn in leaves where sugars and other protective substances accumulate, proteins are rearranged, the cells become less turgid and the central vacuole is divided into many small vacuoles. Concomitantly, growth cessation also occurs. The combination of a decline in temperature and a decrease of photoperiod allow reaching the highest freezing tolerance during winter. Vegetative growth tends to continue in summer in Australian and South African Mediterranean sites[6]. Where as in the Mediterranean basin vegetative growth during the beginning of summer only happens when roots go deep enough [7].

Introduction of an ever-increasing number of ornamental trees and shrubs, exclusively, however, from European gardens, and may be considered as extending from the middle of the l8th to the middle of the l9th century. Almost all introductions of woody plants were introduced by horticulturalists, botanists, doctors, foresters, agro foresters or gardeners [8]. One example of this is the introduction of coniferous woody plants to rural settlements situated in southern regions of Slovakia surrounded by bottomland vegetation and agricultural landscape e.g. *Thuja occidentalis, Platycladus orientalis, Picea abies, Picea pungens, Pinus nigra, Pinus sylvestris* and other species [9];[10].

Plants can be differentiated according to their freezing resistance and hardening capacity. Freezing sensitive plants are injured as the consequence of ice formation at rather high sub-zero temperatures (-2 – (-10) °C). Some freezing sensitive plants can enhance their freezing resistance by few degrees, via cellular accumulating of antifreeze substances. Others cannot be frost hardened at all and are injured at very mild

freezing temperatures. In fully freezing tolerant plants freezing tolerance is extended to all parts of the plant, and they can, without injuries, survive the lowest temperatures recorded on earth [11]. Cold acclimation can be defined as a process involving physiological and biochemical changes whereby plants become increasingly tolerant to subzero temperatures. Parallel to cold acclimation temperature woody plants form terminal buds and develop dormancy [12]. Cold-adapted plants tend to be slow growing, have the C3 mode of photosynthesis and store sugars in underground tissues. Plants well adapted to cool environments have evolved an efficient respiration system, which allows them to rapidly mobilise stored reserves during the short growing season. Phenology is the study of the seasonal occurrence of developmental or life cycle events, such as bud break, flowering, or autumn leaf drop. The timing of these events is known to be sensitive to short- and longterm variability in climate and is thus a robust indicator of the effects of climate change, especially observed rising temperatures [13];[14]. Chlorophyll *a* and Carotenoids are the most important characters because they are present in most plants species and play the key role in the photosynthesis process of phytoplankton. Among the most common spontaneous or induced mutations in higher plants are those that cause alternations in plant pigmentation, particularly the chlorophyll pigments [15]. It is known that acclimatization of plants to low temperatures requires a biochemical and physical restructuring of cell membranes in order to increase their fluidity [16].

The aims of this study to delimiting the length of growing season of *Viburnum tinus* growing in different growth conditions and to determine the effect of low temperature on pigment contents in the leaves. Determine the hardiness against the winter coldness, finding out whether the phenological rhythms of the introductions agree with the annual climatic rhythm of the Slovak Republic.

II. Materials and methods

The study was carried out in 2011 – 2014 year at Botanical garden of Slovak University of Agriculture in Nitra, Slovak Republic. *Viburnum tinus* L. was planted in two types of planting and in three replications for each planting type. First plants planted in the ground and stayed outside during winter condition, the second was planted in pots and protected during winter time, and the plants remove in to greenhouse from end of November until end of March. The plants selected from Florence nursery in Italy all the plants were in the same size and in the same age. The plants irrigated by drip irrigation system and during the experiment time the plants controlled against the pest, the occurrences of diseases and pests on study woody plants processed according to method of [17]. Also the plants supplied with NPK twice in a year.

Dates for leaf bud swelling event in the spring were used to define the beginning of growing season (BGS) and for the end of growing season (EGS) the timing of blossom fall event in spring was used. The length of the growing season (LGS) was determined from the number of days between BGS and EGS, Juliana dates was used for determining each phenophases, this expansion we express with abbreviation in month (J1-31-January 1-31, F- February, M- March, A- April, My- May, Jn- June, Jl- July, Ag- August, S- September, O-October, N- November, D- December) and by number adjusting to the date (M_{11} is 11^{th} of March).

Winter hardiness hardeness and bio- growth expression was evaluated on a 7-point scale of hardiness and 5-point scale for Bio-phenological and reproductive characteristics according to [18]:

i) Frost resistance

I, plants do not freeze;
II, 50% of the annual shoots' length freezes;
III, 50–100% of the annual shoots' length freezes;
IV, older shoots freeze;
V, the aboveground part of the plant freezes to the snow cover height;
VI, the entire aboveground part of the plant freezes;
VII, plants are winter-killed

ii) Bio-phenological and reproductive characteristics

VIII, Wood species vegetate, but do not bloom
IX, They bloom, but do not fruit
X, They fruit, but give ungerminant seed
XI, They fruit and give germinant seed
XII, Plants permanently regenerate in a natural way

The following phenophases were registered as in (Table1)

Table1. Studied growth phenophases in *Viburnum tinus* L.

1. Beginning of leaf bud swelling	8. Total flower budding
2. Total leaf bud swelling	9. Beginning of flowering
3. Beginning bud breaking	10. Total flowering
4. Total bud breaking	11. Beginning of blossom fall
5. Beginning of leafing	12. Total blossom fall
6. Total leafing	13. Beginning of fruit bearing
7. Beginning of flower budding	14. Total fruit bearing

The plants height was measured every year in the end of growing season by using a Biltmore stick. Measurement of shrubs height carried out from land level to mean of top branches. Terminal shoot growth (year increment) of 15-20 from each side of plants selected branches was measured from each individual plant.
The sample of leaves was taken when the temperature was (-3°C) at 9 am and during night was (-7°C) outside, the young leaves was taken from plants and the pigments was determined. Assimilation pigments contents were measured in control leaves as follows: The segments of the youngest mature leaves of *Viburnum tinus* L. were homogenized with using sea sand, MgCO3 and 100% acetone and then extracted with 80% acetone. Extracts were centrifuged 2 minutes at 2500 rpm. Absorbance (A) of the solution was measured by UV-VIS spectrophotometer (Jenway, UK), at 470 nm, 647 nm, and 663 nm, with correction for scattering at 750 nm; the measurements were done in three repetitions [19]. The concentrations of chlorophyll a (Chl *a*), chlorophyll b (Chl *b*) and carotenoids (Car) in $mg*l^{-1}$ was determined by using the equations of [20]:

Chl $a = 12.25*(A_{663} - A_{750})-2.79*(A_{647} - A_{750})*D$
Chl $b = 21.50*(A_{647} - A_{750})-5.10*(A_{663} - A_{750})*D$
Chl $a+b = 7.15*(A_{663} - A_{750}) +18.71*(A_{647} - A_{750})*D$
Car $= [(1{,}000*(A_{470} - A_{750}) -1.82*(\text{Chl } a) - 85.02*(\text{Chl } b))/198]*D$

The concentrations of the pigments were calculated in mg dm^{-3}; A_n was the absorbance at given wavelengths (n) after correction for scattering at 750 nm; D was the optical thickness of cuvette; results were also recalculated in mg. m^{-2} using the volume of solution and the area of leaf segments: [mg. m^{-2}] = V/1000*1/A, when V is volume of 80% acetone and A is area of leaf segments. Air temperature and rainfall were received from dates of metrological station of Botanical garden during study period (Table2). Samples of the soil were taken in 11.12.2012, from the direct planted plants and plant planted in pots was collected from a depth of 50 mm and 300 mm. (Table3, 4) Nutrients and trace elements in soil samples In the evaluation of elements of the samples taken from the experimental area was determined acceptable nutrient content according to Mehlich III. The values of the elements of soil analysis for the experimental area were compared within the assessment criteria analysis results of soil Mehlich III method. and according to Lindsay and Norvel under Annex no. 5 to Decree No. 338/2005 Coll. Ministry of Agriculture of the Slovak Republic on 6 July 2005 on the procedure for the collection of soil samples [21].
An experiment was designed as Factorial Randomized Complete Design (RCD) in three replications, the data were analyzed with the general linear model procedures in Statistical Analysis System (SAS), and Duncan test at level 0.05 was used for the means separation.

Table2. Average temperature (°C) and rainfall (mm) in Nitra (2011, 2012, 2013, 2014)

Temperature (°C)					**Rainfalls (mm)**				
Month	**2011**	**2012**	**2013**	**2014**	**Month**	**2011**	**2012**	**2013**	**2014**
January	-0.90	1.36	-0.8	2.42	January	25	61.1	71.2	1.19
February	-0.60	-2.49	1.5	3.86	February	6	23.5	75.6	1.1
March	5.90	7.41	3.1	14.94	March	27	2.8	113.9	32.6
April	12.70	11.23	12.1	18.17	April	13	36.1	20.4	43.5
May	15.80	17.29	15.6	20.45	May	48	19.6	77.8	49
June	19.80	20.86	19.3	-	June	91	70.1	46.7	-
July	19.70	22.77	22.8	-	July	122	61.4	2.1	-
August	20.90	21.47	21.9	-	August	152	7.3	73.9	-
September	17.70	17.02	14.7	-	September	92	32.7	60.0	-
October	9.90	10.46	12.1	-	October	37	76.1	30.5	-
November	3.00	7.45	6.8	-	November	1	34.6	71.3	-
December	2.20	-0.91	2.3	-	December	42	44.4	11.0	-
Year Average Temperature	10.51	11.16	11.0	-	Year Sum of Rainfalls	656	469.7	654.4	-

Table3. Nutrients and trace elements in soil samples (Department of Agro chemistry and plant nutrient of FAaFR, SAU Nitra, 2013 according to Mehlich III

Planting type	Depth mm	pH	Nan mg.kg-^{1}	The nutrient content mg.kg^{-1} **(Mechl.III)**			
				P	K	Ca	Mg
Ground planted plants	0-50	6,86	13,5	162,5	700	4710	952
Ground planted plants	0-300	6,88	8,6	111,25	537,5	4665	981,5
Pot planted plant	0-50	5,87	27,6	465	900	6700	1369
Pot planted plant	0-300	6,41	25,35	350	712,5	6635	1219

Table4. Evaluation of analysis of soil for the experiment (nutrient content limits in mg.kg^{-1}) according to Mehlich III

Planting type	Depth mm	pH	Nan mg.kg-^{1}	The nutrient content mg.kg^{-1} **(Mechl.III)**			
				P	K	Ca	Mg
Plants in the ground	0-50	Neutral	Suitable	Very high	Very high	Very high	Very high
Plants in the ground	0-300	Neutral	Low	Very high	Very high	Very high	Very high
Plants in pot	0-50	Slightly acid	Good	Very high	Very high	Very high	Very high
Plants in pot	0-300	Neutral	Good	Very high	Very high	Very high	Very high

III. Reslts and discussion

Data in (Table5) showed that *Viburnum tinus* during 2011 and 2012 50% of annual shoots freeze while during 2013 and 2014 all parts of plants not freeze. The biochemical, physiological and morphological changes associated with low temperature tolerance clearly affect active growth and development and, as a result, a plant must be programmed to recognize and respond to temperatures that are favourable for growth and to the environmental cues that signal seasonal changes [22]. [23] Stated that the growth rates fluctuate throughout the year according to weather conditions However, he stressed that the growth rate depends not only on climate conditions in the year of assessment, but also on the conditions of previous years, especially in months where apical buds form. The ability of woody perennials to survive winter is depending on their entry into dormancy state as well as the development of their cold acclimation achieved by a continuous exposure from -5 to -15°C. The wide distribution of this species and its adaptation to a variety of environments, its short generation time and its small stature make it ideal for eco physiological and genetic studies, and it has been used for nearly 40 years in a biotic stress and dormancy research [24]. According to the (Table5) plants planted in the ground blossomed but there was no fruit during 2012 and 2013 while in 2014 they started fruiting. *Viburnum tinus* which was planted in pots blossomed all studied years but the seed was ungerminant. Genetic variations in growth and development of woody plants differ among species, populations within species and individual plants [25].

Table5. Winter hardiness rating, Bio-phenological and reproductive characteristics according to (BENČAT, (1967)

Origin	**Winter hardiness rating**				**Phenological and reproductive characteristics**							
					Plants in the ground				**Plants in pots**			
	2011	2012	2013	2014	2011	2012	2013	2014	2011	2012	2013	2014
Mediterranean	II	II	I	I	IX	IX	IX	X	X	X	X	X

According to (Figure1) the height of *Viburnum tinus* which was planted in pots was highest comparing with *Viburnum* tinus which was planted in the ground. Plants in pots showed the highest value of plant height in 2012 (83.3 cm) while plants planted in the ground decreased the height of plants (76.3 cm). (Figure2). Inverse relationship between 2013 and 2011, 2012 was shown in this study, the highest height for plants was in plants planted in the ground (96.7 cm) while the height of plants which were planted in pots had less height (91.7 cm). (Figure3). Probably for plants in pots the plants growing and competition for space and nutrients increasing with the age and plants in the ground adapted for the new condition also air temperature was changed for warmer comparing with 2011 and 2012. Adaptations develop over time and generations as a response to the ever changing environment [26]. There are however, several factors that can limit these adaptations: availability of water, light, predation and temperature.

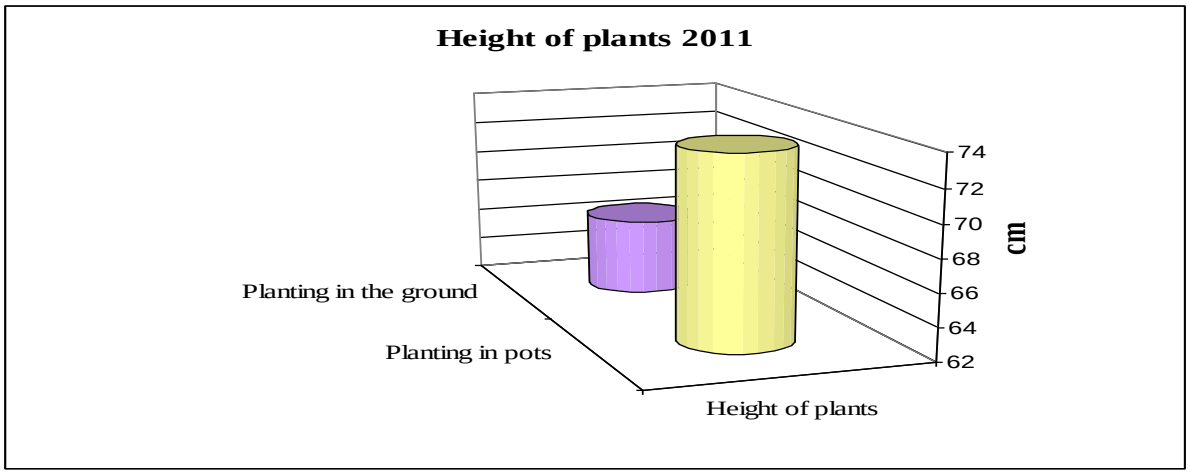

Figure1. Height of *Viburnum tinus* in 2011

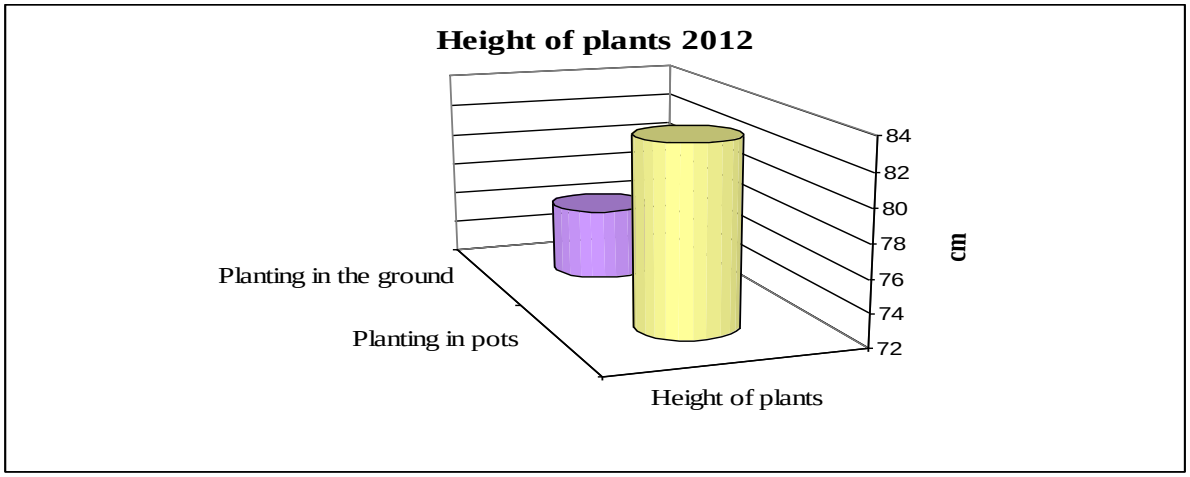

Figure2. Height of *Viburnum tinus* in 2012

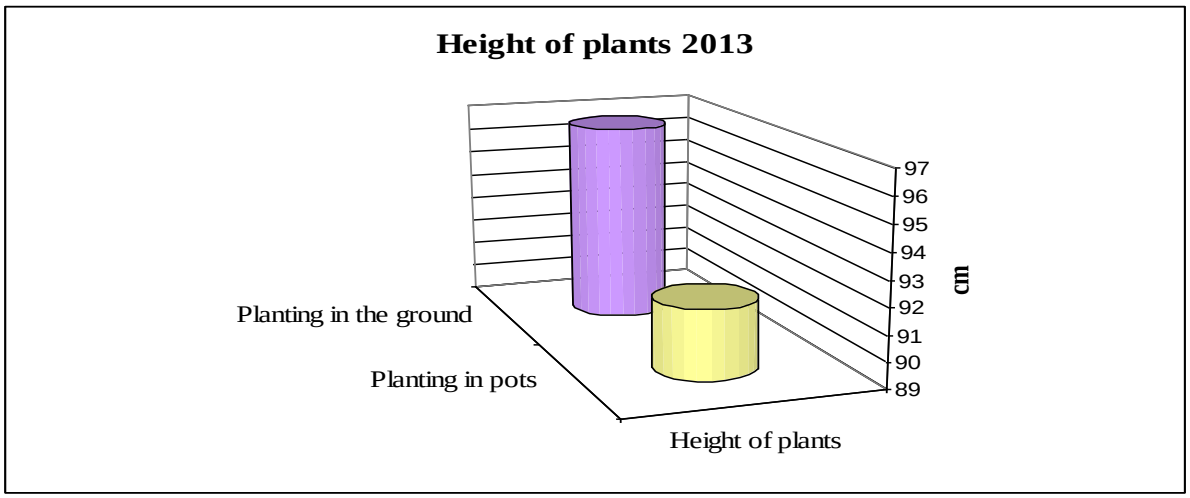

Figure3. Height of *Viburnum tinus* in 2013

Year increment in *Viburnum tinus* which was planted in the ground recorded 13.17 cm during 2011 while the increment was increased to 21.33 cm during 2012 and in 2013 the increment increased to 27.50 cm the differences between year increment was significant, while year increment in plants which were planted in pots recorded 11.08 cm during 2011 the amount increased to 15.33 cm in 2012 then in 2013 the increment increased to 17.68 cm but the differences was not significant (Table6). Perhaps due to the limited space in which plants were planted, generally height and diameter growth increments decrease with increasing age. Environmental

conditions both in the previous and current year would affect the annual height increment of a tree. The results agree with [27] who found that the terminal shoot growth showed significant differences in Minnis trees (39.4 cm) and campy trees (27.3 cm), it could be argued that genotypic differences could be the reason.

Table6. Mean and standard deviation for year increment during studied period (cm)

Planting type	2011		2012		2013	
	Mean	SD	Mean	SD	Mean	SD
In the ground	13.17[a]	18.72	21.33[b]	6.96	27.50[b]	5.72
In pots	11.08[a]	2.29	15.33[a]	3.34	17.68[a]	4.69

* Means with the same letters there is no significant differences

The longest growth season was observed in *Viburnum tinus* which were planted in pots from bud swelling until full fruiting while plants which were planted in the ground LGS was 218 days. The studied phenophases was observed wholly at the *Viburnum tinus* which planted in two types in Botanical garden in 2012-2014. Phenological visual had observed;

First mark of spring activity is beginning of bud swelling; in plants planted in the ground in 2014 was in F_{10} (41) days according to Julian day (JD) and it was earlier than 2012 (107 JD) and 2013 (93 JD). Perhaps this is due to temperature; the average temperature in 2014 was more than average temperature in 2012 and 2013. Plants planted in pots also showed differences in beginning of bud swelling in 2014 was earlier (60 JD) comparing with 2012 (75 JD) and 2013 (74 JD) and there was no different between 2012 and 2013. In plants planted in pots vegetative period was in middle of March and continued until end of May during 2012 and 2013, while in 2014 the vegetative period started (15) days earlier than 2012 and 2013. [28] Found that *Forsythia intermedia* showed significant difference in the onset of phenophases. Planting in the ground during 2012 and 2013 the vegetative period started in April while in 2014 started 35 days earlier, might be related to the environmental conditions in which they grow. The temperature increase affected beginning of phenophases and length of growing season; generally seasonal timing of spring phonological events such as leaf unfolding of trees depends highly on air temperature [29]. The study agrees with [30] they suggested that there is relationship between spring phenology and temperature before bud burst is highly species- specific.

Flowering period in plants planted in pots in 2012 started in middle of November (321 JD) and continued until beginning of April (94 JD) for next year, while flowering period in 2013 started from beginning of July (188 JD) until first of March (60 JD) in 2014, whereas plants planted in the ground in 2012 flowering period started from second week of July (196 JD) and the plants started in flowering without continued flowering period. In 2013 flowering period started in first of July (182 JD) until A_{20} in 2014. Fruit bearing in *Viburnum tinus* which were planted in pots started (87 JD) in 2013 and total fruit bearing was in (119 JD) while in 2014 fruit bearing was earlier (21 days) comparing with 2013 and total fruit bearing in 2014 was in (74 JD). Plants planted in the ground during 2012 and 2013 there was no fruiting and in 2014 the plants started fruiting in (107 JD) and total fruit bearing was in (125 JD). (Table7). The results agree with [31]. Growth rates fluctuate throughout the year according to weather conditions However, he stressed that the growth rate depends not only on climate conditions in the year of assessment, but also on the conditions of previous years, especially in months where apical buds form. Adaptable species, effective in stabilization of the environmental conditions, probably lower costs for their establishment and aftercare, broad base of their genetic sources in the landscape and large basis for selection of the most suitable phenotypes [32].

Table7. Phenophases of *Viburnum tinus* according to Julian day calendar

Phenophases	Planting in pots			Planting in the ground		
	2012	2013	2014	2012	2013	2014
Beginning of leaf bud swelling	$M_{15\,(75)}$	$M_{15\,(74)}$	$M_{1\,(60)}$	$A_{17\,(107)}$	$A_{3\,(93)}$	$F_{10\,(41)}$
Total bud swelling	$M_{25\,(85)}$	$M_{25\,(84)}$	$M_{7\,(66)}$	$A_{28\,(118)}$	$A_{12\,(102)}$	$F_{15\,(46)}$
Beginning of bud breaking	$A_{1\,(92)}$	$A_{1\,(91)}$	$M_{13\,(72)}$	$A_{30\,(120)}$	$A_{14\,(104)}$	$F_{19\,(50)}$
Total bud breaking	$A_{18\,(109)}$	$A_{19\,(109)}$	$M_{25\,(84)}$	$M_{10\,(69)}$	$A_{22\,(112)}$	$F_{22\,(53)}$
Beginning of leafing	$My_{2\,(122)}$	$My_{2\,(122)}$	------	$M_{12\,(71)}$	$A_{29\,(119)}$	$F_{28\,(59)}$
Total leafing	$My_{30\,(150)}$	$My_{29\,(149)}$	-----	$Jn_{12\,(163)}$	$M_{29\,(88)}$	$M_{5\,(64)}$
Beginning of flower budding	$N_{16\,(321)}$	$Jl_{7\,(188)}$	------	$Jl_{15\,(196)}$	$Jl_{1\,(182)}$	------
Total flower budding	$D_{15\,(350)}$	$S_{13\,(256)}$	------	$O_{18\,(291)}$	$O_{18\,(291)}$	------
Beginning of flowering	------	$F_{12\,(43)}$	$J_{2\,(2)}$	$O_{22\,(295)}$	$O_{18\,(291)}$	-----
Total flowering	------	$M_{4\,(63)}$	$J_{18\,(18)}$	-----	-------	$M_{17\,(76)}$
Beginning of blossom fall	-----	$M_{28\,(87)}$	$F_{25\,(56)}$	------	--------	$A_{1\,(91)}$
Total blossom fall	------	$A_{4\,(94)}$	$M_{1\,(60)}$	-----	------	$A_{20\,(110)}$
Beginning of fruit bearing	------	$M_{28\,(87)}$	$M_{7\,(66)}$	------	-----	$A_{17\,(107)}$
Total fruit bearing	------	$A_{29\,(119)}$	$M_{15\,(74)}$	------	-----	$My_{5\,(125)}$

Data in (Table8) obtained that planting in the ground increased the value of chlorophyll *a*, chlorophyll *b*, chlorophyll *a+b*, chlorophyll *a/b* and caroteniods in the leaves comparing with plants planted in pots, chlorophyll *a* content in leaves was the highest (3.45 $mg.g^{-1}$), while the content of chlorophyll *b* was the lowest (1.01 $mg.g^{-1}$), while the content of carotenoids was (1.25 $mg.g^{-1}$) (Figure4). The results agree with [33]. The transfer of excitation energy from carotenoids to chlorophyll *a* is facilitated by the presence of chlorophyll *b* [34]. Carotenoids accumulation differed with time the amount of chlorophyll of leaves differ by the influences of many environmental factors [35]. Aslo the light planing important role in chlorophyll Configuration in plants ,prbably plants which planted inside had less light intensity. Some Chlorophyll *a* fluorescence ratios are frequently used to evaluate stress conditions [36]. The Chl a/b ratio increased while chlorophyll content decreased in response to planting type, the results agree with [37];[38]. The responses of all species were in the same direction, but differed in magnitude.Carotenoids have two important functions in plants. First, they can contribute to photosynthesis. They do this by transferring some of the light energy they absorb to chlorophylls, which then use this energy to drive photosynthesis. Second, they can protect plants which are over-exposed to sunlight. They do this by harmlessly dissipating excess light energy which they absorb as heat. In the absence of carotenoids, this excess light energy could destroy proteins, membranes, and other molecules. Biosynthesis of carotenoids in plants is a genetic characteristic, but environmental conditions also have an essential role [39].

Table8. Pigments content in leaves of *Viburnum tinus* according to planting type

Planting type	**Pigments in leaves**					
	Chlorophyll *a*	**Chlorophyll *b***	**Chlorophyll *a+b***	**Chlorophyll *a/b***	***Carotene***	**Chlorophyll *a-b/ carotene***
Planting in pots	1.33^{b}	0.53^{b}	3.15^{b}	2.45^{a}	0.52^{b}	4.0^{a}
Planting in the ground	3.45^{a}	1.01^{a}	4.35^{a}	2.67^{a}	1.25^{a}	3.57^{b}

* Means with the same letters in same column there is no significant differences

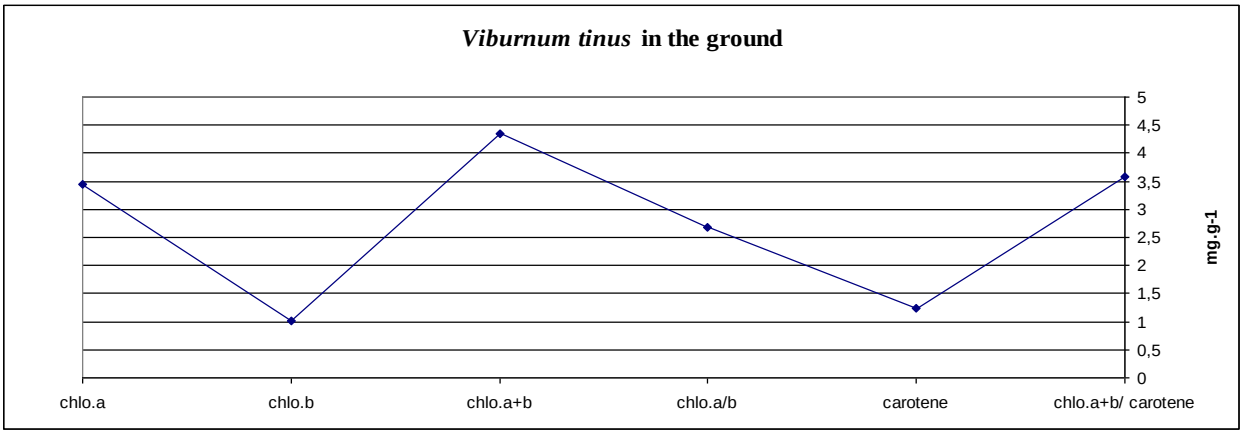

Figure4. Pigments in leaves of *Viburnum tinus* which is planted in the ground

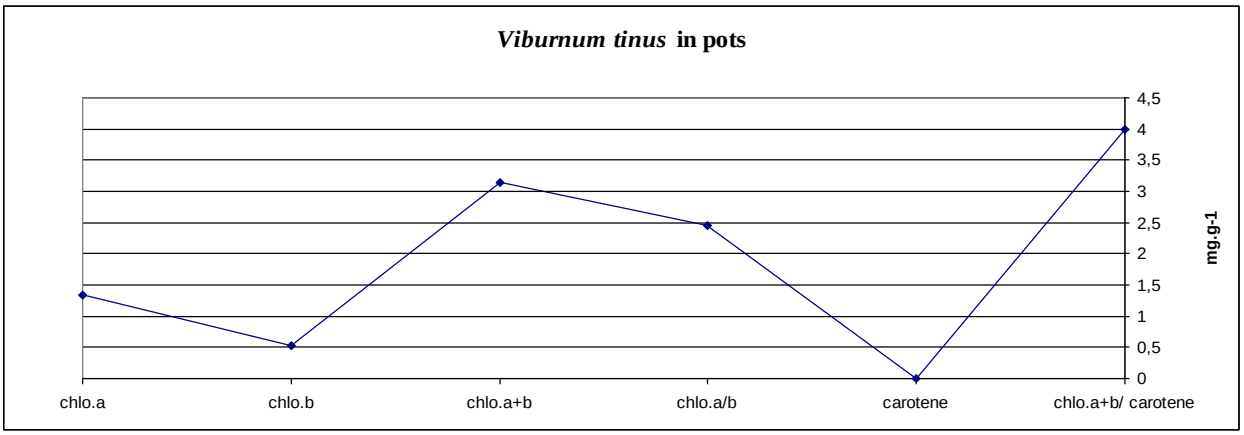

Figure5. Pigments in leaves of *Viburnum tinus* which is planted in pots

IV. Conclusion

The results show that during study period, the growing season duration has lengthened in plants planted in pots in comparison with plants planted direct in the soil, also there is a significant difference between the plants in each planting types in content of pigments, the highest content was in plants which were planted in the ground this might be the reason of increasing cold resistance during the winter time.

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Appendix

Winter time (plants direct in the ground)

Winter time (plants in pots and protected under greenhouse)

Spring time (in direct planted plants after surviving from winter coldness)