

DETERMINATION OF CARBOHYDRATE ACCUMULATION AND PLANT GROWTH DYNAMICS IN SNOWDROP (*Galanthus elwesii* Hook.) RESPONSE TO MOISTURE LEVELS IN DIFFERENT SUBSTRATES

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ABSTRACT

In terms of botany, geophytes are known by their own survival strategy due to have a swollen storage organs. Snowdrops (*Galanthus*, 20 spp.; Amaryllidaceae) are important type of wild-sourced ornamental bulb genus in all geophyte species. Also, have a great deal of potential for use on landscape designs. Whereas, not much study has been done regarding the growth dynamics of snowdrops at harsh environmental conditions. This experiment was conducted to evaluate the effects of abiotic stress conditions on the performance of snowdrop (*Galanthus elwesii* Hook.) in soilless culture. Substrates and moisture were the variables. Peat + perlite and cocopeat were used as a substrate in pots. Moisture levels were applied; ML₁, ML₂ (well-watered and moderately tolerant treatments), ML₃ (moderate stress) and ML₄ (severe stress). Moisture had a statistically significant effect ($P < 0.05$) on circumference size, height and weight of the snowdrop bulbs. Plant height and carbohydrate accumulation were also affected by moisture levels in different substrates. The correlation between total carbohydrate ($r = 0.95$) and starch ($r = 0.98$) were positively determined. The reduced sugar, total sugar, starch and total carbohydrate values were increased by the severe stress treatment (ML₄).

Key words: geophytes, *Galanthus elwesii* Hook., substrate, plant growth, carbohydrate accumulation

INTRODUCTION

Galanthus species are perennial and herbaceous plants. When active growth is complete, the above-ground parts of the plants dry out and bulbs remain dormant during the drought period in summer [Aksu et al. 2002, Zencirkıran 2002, Avcı 2005]. Snowdrop (*Galanthus elwesii* Hook.) is an important species within the *Galanthus* genus. It is native to a large area from Europe to the middle east, stretching across Greece, Bulgaria, the Aegean islands, the Balkans, Turkey and Iran to Lebanon, is well-known and widespread in the northwest, west and southwest part and also in the Anatolia region of Turkey [Aksu et al. 2002]. The Snowdrop is known to be highly resistant

to cold, so it is used as an ornamental plant for landscape designs in winter gardens. It is regarded as a medicinal plant as it contains some important alkaloids (galanthamine, lycorine, elwesine, flexine) which are extensively used in pharmacological products [Latvala et al. 1995].

The snowdrop can be propagated either from bulbs or seeds, but it will take so long to be mature and to reach the stage of flowering. In recent years *Galanthus* species are threatened by habitat destruction, illegal collecting and also climate change. Therefore, if current rates of exploitation of wild stocks are not preserved, it may face extinction in the next decade and

may have its genetic base severely reduced. For this reason, endemic geophytes such as *Galanthus* species have to be cultivated. On the other hand, variable environmental conditions such as the growth medium and the amount of water can directly affect the growth of bulbous plants such as snowdrops. Hence, soil type and water requirements for all kinds of bulbous plant have to be determined to improve water management and to evaluate their response to the abiotic stress.

Water stress is one of the main abiotic stress factors, affecting plant growth, and may have both qualitative and quantitative effects on plant constituents. Carbohydrates, play an important role in adaptations of plants to changing environmental conditions, keep water and nutrients to provide energy, found in plant organs and also storage organs of geophytes [Rees 1992, Miller 1992, Fediuk et al. 2020, Zohouri et al. 2020]. Storage organs contain different carbohydrate forms such as starch and soluble sugars. *Galanthus nivalis* known, has the highest amount of starch and sugar concentration amongst geophytes. According to Hendry [1987], snowdrop store maximum concentrations of starch and fructan in the winter months. Recently, some studies pointed out that there was a strong correlation between the water content of plant tissues and variations in carbohydrate accumulation [Orthen 2001, Orthen and Whermayer 2004, Yıldırım et al. 2009].

However, for *Galanthus* species there is not very much specific information available, especially with regard to growth strategy and also relations between carbohydrate reserves and drought tolerance.

The objective of the present study was to evaluate the response of snowdrop to different growth conditions with a reduced amount of water and to determine the carbohydrate accumulation of bulbs exposed to variable moisture levels in different substrates.

MATERIALS AND METHODS

Plant material and experimental design

The present study was conducted in the greenhouse, Department of Horticulture, Faculty of Agriculture, Canakkale Onsekiz Mart University in Turkey. Bulbs of snowdrop (*Galanthus elwesii* Hook.) with an average circumference of 4–6 cm, were transplanted into pots on January 4, 2017. The pot dimensions were 12 cm in diameter and 10 cm in depth, resulting in a 113.04 cm² surface area and a volume of 1080 m³. Each pot had four drainage holes of 0.5 cm diameter at the bottom. Snowdrop plants were growth according to the substrate techniques used in soilless culture method. Two different substrate were used in the trial. Some physicochemical characteristics of the substrates (Peat+perlite (v/v = 50%), cocopeat) were given in Table 1. Each pot contained 1^{-L} substrate. For each irrigation treatment, there were 5 pots and in one substrate there were 20 pots for all the irrigation treatments. Evapotranspiration of the plant was measured by weighing the pots. The water quantities were then regulated by the weight of the pots. All pots were weighed daily to determine daily evapotranspiration (ET). To achieve this, the water balance method was used between the two irrigations [Yıldırım et al. 2009]. Therefore, there was almost no leaching of water in all treatments.

$$ET = [(W_{i-1} - W_i) + I - D] / A \quad i = 1, 2, 3, \dots, n$$

According to the equation; ET is the evapotranspiration (mm), W_{i-1} and W_i are the mass (kg) of the pot at day $i-1$ and i , respectively. I is the amount of irrigation water (kg), D is the quantity of the drainage water available (kg), and A is the pot surface area (m²).

Moisture levels (ML) were provided by the irrigation treatments included four gradient irrigation levels

Table 1. Physicochemical characteristics of the substrates used in the experiment

Substrates	pH	EC (mS/cm)	BD (g/cm ³)	Porosity (%)	WHC (%)	C/N (%)	CEC (Cmol/kg)
Peat	6.2	1.4	0.16	91	75.6	64.85	93.5
Cocopeat	6.8	2.7	0.17	56	90.1	50.22	141.8
Perlite	7.4	1.8	0.11	72	97.3	0.0	0.0

EC – electrical conductivity, BD – bulk density, WHC – water holding capacity, CEC – cation exchange capacity

from well-watered to severe drought. Irrigation was scheduled according to pre-set intervals, so that, in the full moisture treatment 100% (ML₁) depleted soil moisture was refilled up to the field capacity at 4-day intervals. In the deficit treatments, water was applied in the range of 75% (ML₂), 50% (ML₃) and 25% (ML₄) of the full moisture level.

All irrigation treatments were started on January 4 and the substrates were refilled up to field capacity until January 14 to establish root development. Then, different irrigation treatments commenced in both of the substrates. In all treatments the pots were weighed. Modified Hoagland's solution was used for the plant nutrition in soilless culture method [Jasoni et. al 2002]. During the experiment period, temperature and relative humidity (RH) at the greenhouse were measured at the same level of canopy for the plants by using a HOBO U12 instrument and a measurement range is from -20°C to 40°C for temperature, 5% to 95% for humidity (Fig. 1). The electrical conductivity of the irrigation water (ECw) was measured by an EC59 meter (Martini Institute); it was 0.25 ds m⁻¹.

Measurements of growth parameters. Measurements were taken for each irrigation according to the plant growth. Bulb fresh mass (g) per plant was determined by using digital precision scale (0.01 g). Plant height (mm) was determined by measurement of the part from the surface of substrate to the top of the plant shoot. The equation ($C = \pi \times R$) was used for the calculation of the circumference size (C), after diameter (R) of the bulbs were measured by digital display caliper (± 0.1 mm). Bulb height (mm) was also determined by digital display caliper (± 0.1 mm).

Determination of carbohydrate accumulation. To determine the carbohydrate content of the snowdrop bulbs at the beginning of generative phase, the spectrophotometric method was used. Soluble sugars (glucose + fructose + sucrose) as reduced and total sugar concentrations were determined by the dinitrophenol method [McCormick and Green 1999] and starch analyses by the athrone test [Tiwari 2015]. Readings from the spectrophotometer (UV-1800 Shimadzu, Japan) were taken at 600 nm for reduced sugar and 620 nm for both total sugar and starch. Total carbohydrate was determined from the equation of total carbohydrate (%) = total sugar (%) + starch (%), as defined by Yeşiloğlu et al. [1992].

Statistical analyses. Experimental data were analysed using randomized block design with five replications for each treatment. Analysis of variance was performed by Two-Way ANOVA and using Statistical Analysis System [SAS 9.1, SAS Institute, Inc. Cary NC, USA] based on the following statistical model in order to evaluate the effect of moisture level and substrates.

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + r_{ik} + \varepsilon_{ijkl}$$

where Y_{ijkl} : observed value, μ : grand mean, α_i : effect i. substrate (I = 1, 2), β_j : effect of j. moisture level (j = 1, 2, 3, 4), $(\alpha\beta)_{ij}$: effect of substrate \times moisture level interaction, r_{ik} : l. replication effect (l = 1, 2, 3), ε_{ijkl} : random error term.

Least significant difference (LSD) test at $p < 0.05$ was used for mean comparison. Data for interaction between the different substrates and moisture levels were analysed by using R package program [R Core Team 2019]. To show mean differences as graphically, analysis of means (ANOM) technique was used.

RESULTS AND DISCUSSIONS

Snowdrop is known to be cold resistant up to -15°C [Atay 1996]. Therefore, climatic parameters during the trial did not have any adverse effect on the plant growth parameters (Fig. 1). However, fluctuations in relative humidity affected the amount of evapotranspiration, while the temperature values were at an almost constant level throughout the trial (Fig. 1). The amount of evapotranspiration varies in different plants and it can also change even in the same plants as their morphological and physiological structure varies [Selvi 2012]. Therefore evapotranspiration must be determined for all plants. In this experiment, the moisture levels varied according to the irrigation treatments and water consumption of plants for different substrates (Figs 2 and 3).

The moisture levels (ML) applied to the peat + perlite medium were as follows: 85.86 mm for the ML₁, 76.19 mm for the ML₂, 64.63 mm for the ML₃, and 51.66 mm for the ML₄ (Fig. 4). But for the coco-peat substrate, in the same treatment, lower amounts of water was observed as follows; 73.95 mm for the ML₁, 65.15 mm for the ML₂, 54.42 mm for the ML₃, and 40.84 mm for the ML₄ (Fig. 4). Therefore, some

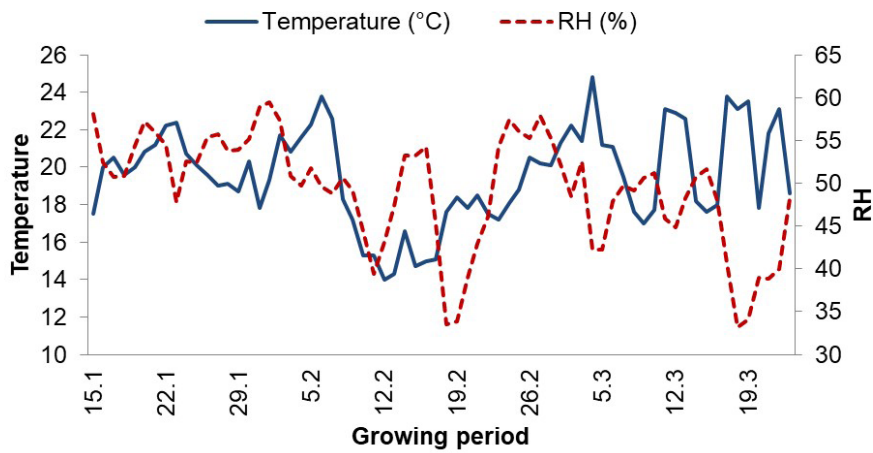


Fig. 1. Variations in climatic parameters during the experiment

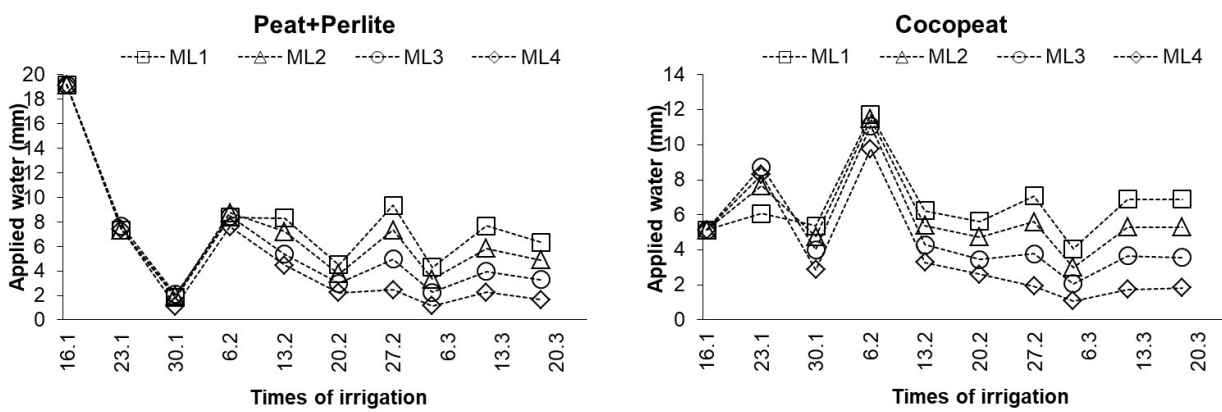


Fig. 2. Variations in applied water (mm) at different moisture levels in different substrates through the whole growing period of 8 week

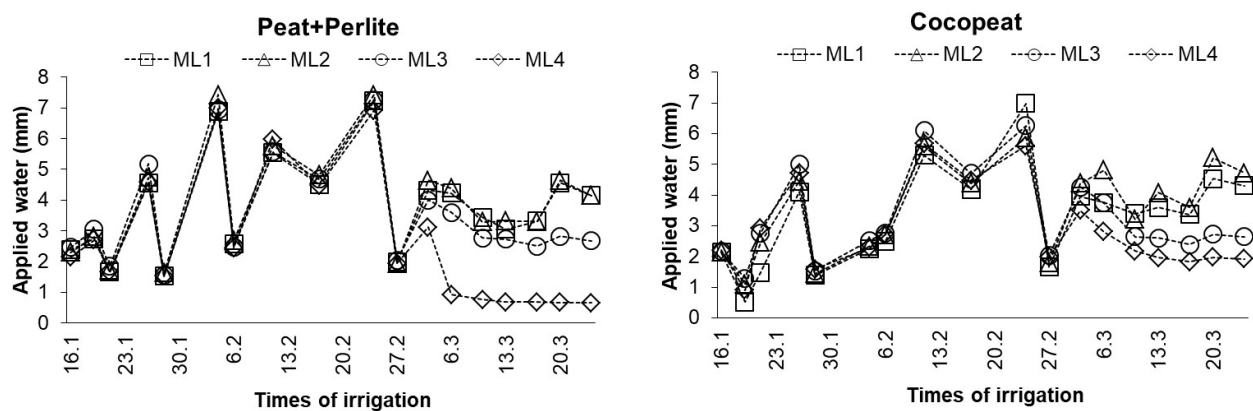


Fig. 3. Variations in evapotranspiration (mm) at different moisture levels in different substrates through the whole growing period of 8 week

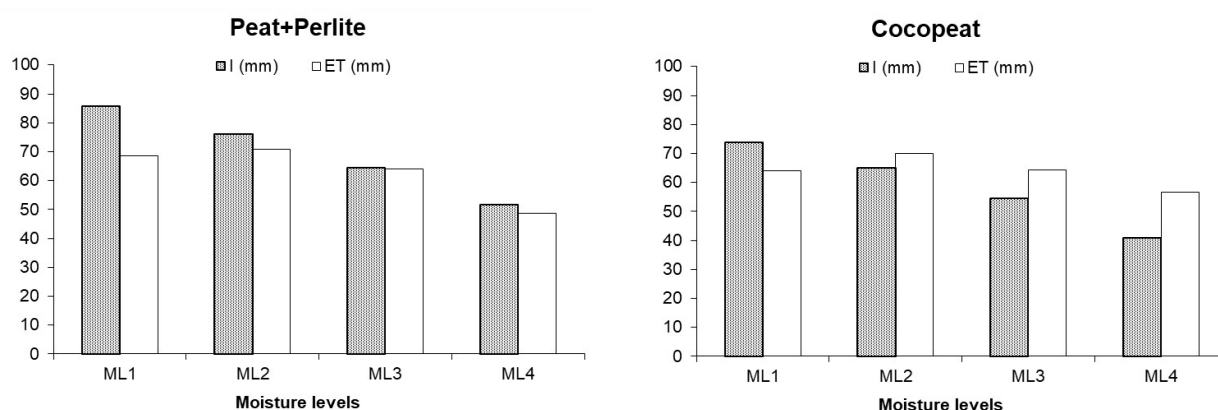


Fig. 4. Total amount of applied water (I) and evapotranspiration (ET) dependent on the substrates and moisture levels

differences were observed in terms of water consumption and the amount of water applied. These differences are thought to have arisen from the particle size of the substrates and the different water holding capacities. The mixed peat + perlite medium also had a higher porosity rate (81.5%) which led to more water retention than the cocopeat medium. According to De Hertogh and Tilley [1991], whom carried out a study on species of *hippeastrum*, concluded that the particle sizes of the substrates had an important effect on water holding capacity and drainage features.

The irrigation amounts and evapotranspiration values in relation to the different substrates and moisture levels were given in Figure 2 and Figure 3. The amount of water applied, was decreased especially in those periods when the plants were seen out of the substrate surface between the dates 16.01.2017 and 23.01.2017. This can be explained by the poorly advanced root development since at these time, the snowdrop's roots were just beginning to develop. For this reason, the water consumption of the snowdrops was low during this period. On the other hand, in the second week after planting, an increase in the amount of irrigation water applied was observed. This situation can be explained by the fact that, the plants began enzymatic activity and photosynthesis after shoot meristems started to produce leaves, and developmental processes are involved in leaves. Evapotranspiration was similar for all substrates and moisture levels for a period of 8 weeks, but significant changes were observed after

this time. The amount of evapotranspiration decreased in response to the water stress treatments (Fig. 3).

Plant growth parameters

The interaction between the substrates and moisture levels for plant growth parameters were statistically significant, shown in Figure 5. Moisture levels had a statistically significant effect ($p < 0.05$) on the plant height of snowdrop in both of the substrates, but there was no statistically significant difference between the mean of substrates for the plant height (Tab. 2). The amount of water in the ML₁ treatment resulted in a significant effect on the plant height, while there was no difference between ML₂, ML₃ and ML₄ treatments. On the other hand, the reduction in the amount of water applied caused a decrease in plant height. Therefore, it is very likely that the increase water quantity has the effect of boosting the carbohydrate assimilation and photosynthesis. This result agrees with the findings for flower stem length and plant height in the studies on gladiolus [Bastuğ et al. 2006, Porto et al. 2014].

The circumference size of any bulb is an important parameter with a view to increasing its commercial value in bulbous ornamentals. Therefore, in the trial the interaction between the substrates and different moisture levels had a statistically significant effect ($p < 0.05$) on the bulb circumference size (Tab. 2). The bulb circumference size in both of the substrates decreased with the reduction in the amount of moisture level. The maximum mean of bulb circumference

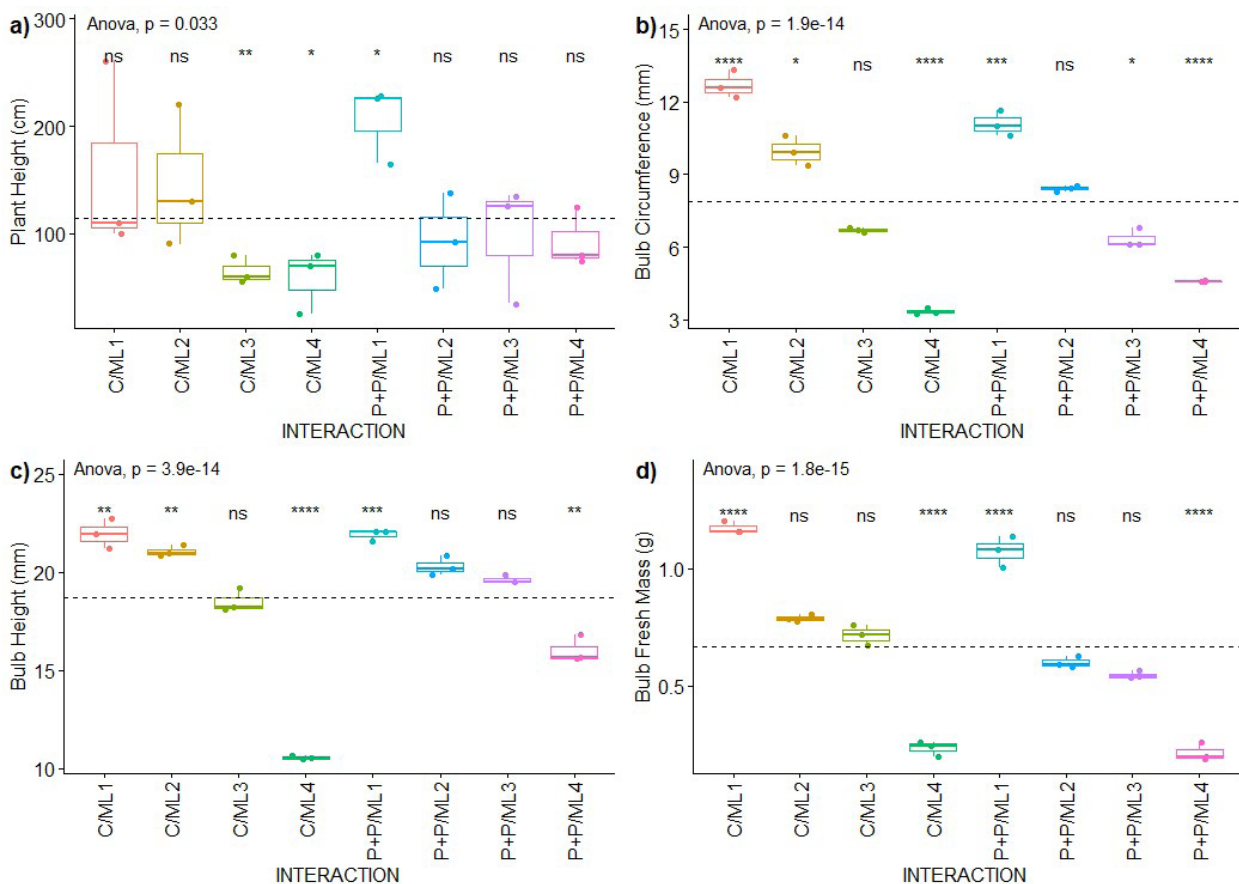


Fig. 5. The interactions of different moisture levels (ML) and substrates (C: cocopeat; P + P: peat + perlite) on plant growth parameters of the snowdrop. Dashed line shows grand mean and dots indicate measurement value for each traits

was obtained as 8.17 cm from the cocopeat substrate (Tab. 2), which was consistent with the work of other researchers, who reported that type of substrate significantly influenced size in bulbs of lily and corms of freesia [Yılmaz and Korkut 1998, Akçal 2014]. According to Akçal [2014], these differences between the substrates are related to their physical characteristics such as bulk density, porosity and water holding capacity.

The bulb height of snowdrop varied according to the amount of moisture levels treated. The highest mean value (21.92 mm) for the bulb height was observed in the well-watered (ML_1) treatment, while the lowest mean value (13.29 mm) was determined in the severe stress (ML_4) treatment (Tab. 2). Similarly, the fresh mass (FM) of bulb in snowdrop was significantly

affected ($p < 0.05$) by the moisture levels in different substrates (Tab. 2). Moreover, the interactions between the substrates and the moisture levels were found to be statistically significant for bulb FM (Tab. 2).

These results are in close agreement with the findings on the interactions between different water levels and a tuber weight in a species of *Ornithogalum* [Karagüzel et al. 2011]. This case gives important information about nutritional status and water use efficiency in tuberous and bulbous plants in effective root depths.

Variations in carbohydrate accumulation

The interaction between the substrates and moisture levels for the variations in carbohydrate accumulation were statistically significant, shown in Figure 6. The reduced sugar concentration (glucose + fructose)

Table 2. Effects of different moisture levels and substrates on plant height (mm), on bulb circumference size (cm), on bulb height (mm), on bulb fresh mass (g) of the snowdrop

Plant height (mm)					
Substrates	Moisture levels				Mean
	ML ₁	ML ₂	ML ₃	ML ₄	
Peat + Perlite	206.33 a	92.67 b	98.33 b	93.33 b	122.67 A
Cocopeat	156.67 ab	146.67 abc	65.00 c	58.33 c	106.67 A
Mean	181.5 A	119.67 AB	81.67 B	75.83 B	
LSD (0.05)	substrate: 45.553		moisture levels: 64.422		s × ml: 91.106
Bulb circumference size (cm)					
Substrates	Moisture levels				Mean
	ML ₁	ML ₂	ML ₃	ML ₄	
Peat + Perlite	11.08 b	8.43 d	6.33 e	4.57 f	7.60 B
Cocopeat	12.70 a	9.97 c	6.69 e	3.33 g	8.17 A
Mean	11.89 A	9.20 B	6.51 C	3.95 D	
LSD (0.05)	substrate: 0.31		moisture levels: 0.44		s × ml: 0.62
Bulb height (mm)					
Substrates	Moisture levels				Mean
	ML ₁	ML ₂	ML ₃	ML ₄	
Peat + Perlite	21.89 ab	20.30 cd	19.63 d	16.03 f	19.46 A
Cocopeat	21.96 a	21.05 bc	18.51 e	10.56 g	18.02 B
Mean	21.92 A	20.67 B	19.06 C	13.29 D	
LSD (0.05)	substrate: 0.44		moisture levels: 0.62		s × ml: 0.88
Bulb fresh mass (g)					
Substrates	Moisture levels				Mean
	ML ₁	ML ₂	ML ₃	ML ₄	
Peat + Perlite	1.07 b	0.60 e	0.55 e	0.22 f	0.61 B
Cocopeat	1.17 a	0.79 c	0.72 d	0.24 f	0.73 A
Mean	1.12 A	0.69 B	0.63 C	0.23 D	
LSD (0.05)	substrate: 0.03		moisture levels: 0.04		s × ml: 0.06

Capital letters show the significant differences between the means of substrates in the same column and show the significant differences between the means of moisture levels in the same row; small letters show the significant differences between moisture levels on the same column and same row, LSD, $p < 0.05$

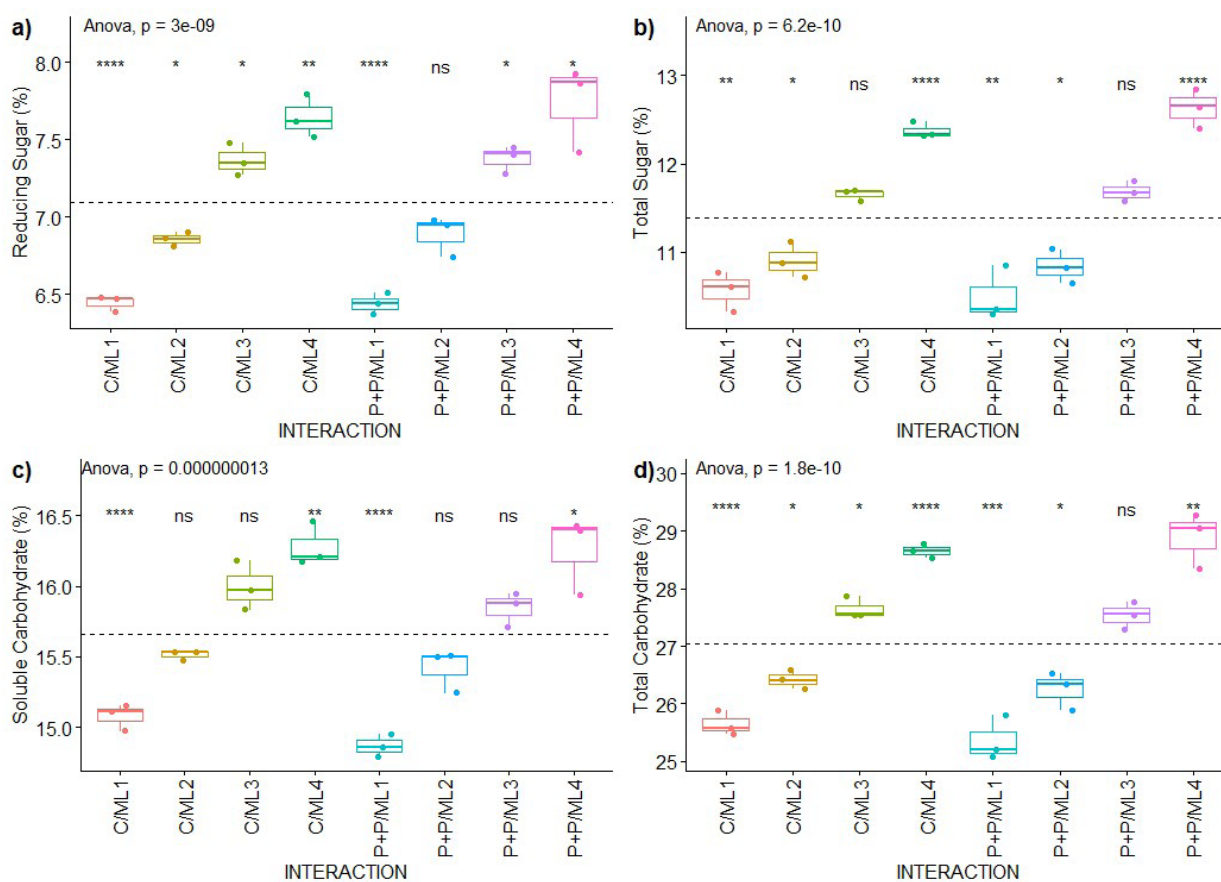


Fig. 6. The interactions of different moisture levels (ML) and substrates (C: cocopeat; P + P: peat + perlite) on carbohydrates of the snowdrop. Dashed line shows grand mean and dots indicate measurement value for each traits

was affected significantly ($p < 0.05$) by different moisture levels, while there was no significant differences between the substrates (Tab. 3). The lowest reduced sugar content of 6.45 g/100 g was recorded at the highest irrigation (ML_1), while the largest (7.69 g/100 g) at water deficit (ML_4). Therefore, the differences in the moisture levels significantly affected the reduced sugar content of snowdrop. Actually, if we add more water up to 85.86 mm in the ML_1 treatment to the root area, this will have a higher sugar content to the unit increment of water. It was clearly seen that, the moisture levels related to the applied water, also had a similar effect on the total sugar and starch content as being in as for the reduced sugars. On the other hand, the obtained results were indicate that the amount of starch was higher than that of sucrose and other poly-

saccharides. These variations in carbohydrate accumulation are overall characteristics of geophytes. It is a great advantage to be able to store starch, the product of photosynthesis and use it as an energy source when the water is scarce in the dormant periods.

According to Rosa et al. [2009], variations in environmental factors may lead to a significant decrease in the efficiency of photosynthesis in source tissues and thus, reduce the supply of soluble sugars to sink tissues. Our observations were pointed out that the carbohydrate accumulation within the snowdrop bulb increased as the the moisture level in the effective root depth was decreased by the amount of water applied. This can be seen as a critical physiological parameter since it regulates the growth and development of the bulb as a storage organ.

Table 3. Effects of different moisture levels and substrates on reducing sugar content (g/100 g), on total sugar (g/100 g) content, on starch (g/100 g) content, on total carbohydrate (%) content of the snowdrop

Reducing sugar (g/100 g)					
Substrates	Moisture levels				Mean
	ML ₁	ML ₂	ML ₃	ML ₄	
Peat + Perlite	6.44 d	6.89 c	7.38 b	7.74 a	7.11 A
Cocopeat	6.45 d	6.86 c	7.37 b	7.65 a	7.08 A
Mean	6.45 D	6.87 C	7.37 B	7.69 A	
LSD (0.05)	substrate: 0.12	moisture levels: 0.17		s × ml: 0.24	
Total soluble sugar (g/100 g)					
Substrates	Moisture levels				Mean
	ML ₁	ML ₂	ML ₃	ML ₄	
Peat + Perlite	10.50 e	10.83 d	11.68 c	12.63 b	11.41A
Cocopeat	10.56 e	10.91 d	11.65 c	12.38 a	11.38 A
Mean	10.53 D	10.87 C	11.67 B	12.50 A	
LSD (0.05)	substrate: 0.12	moisture levels: 0.17		s × ml: 0.62	
Starch (g/100 g)					
Substrates	Moisture levels				Mean
	ML ₁	ML ₂	ML ₃	ML ₄	
Peat + Perlite	14.87 e	15.42 d	15.85 c	16.25 ab	15.60 A
Cocopeat	15.08 e	15.51 d	16.00 bc	16.28 a	15.72 A
Mean	14.97 D	15.46 C	15.92 B	16.27 A	
LSD (0.05)	substrate: 0.14	moisture levels: 0.20		s × ml: 0.28	
Total carbohydrate (%)					
Substrates	Moisture levels				Mean
	ML ₁	ML ₂	ML ₃	ML ₄	
Peat + perlite	25.36 d	26.25 c	27.53 b	28.89 a	27.00 A
Cocopeat	25.64 d	26.42 c	27.65 b	28.66 a	27.09 A
Mean	25.50 D	26.33 C	27.59 B	28.77 A	
LSD (0.05)	substrate: 0.22	moisture levels: 0.33		s × ml: 0.46	

Capital letters show the significant differences between the means of substrates in the same column and show the significant differences between the means of moisture levels in the same row; small letters show the significant differences between moisture levels on the same column and same row, LSD, $p < 0.05$

Table 4. The correlation coefficients between plant development characteristics and carbohydrate contents

	Plant height	Bulb circumference	Bulb height	Bulb fresh mass	Reducing sugar	Total soluble sugar	Starch content	Total carbohydrate
Plant height	1.00							
Bulb circumference	0.63 ^{ns}	1.00						
Bulb height	0.54 ^{ns}	0.86*	1.00					
Bulb fresh mass	0.59 ^{ns}	0.94*	0.81*	1.00				
Reducing sugar	-0.61 ^{ns}	-0.94*	-0.79*	-0.90*	1.00			
Total soluble sugar	-0.54 ^{ns}	-0.92*	-0.84*	-0.88*	0.95*	1.00		
Starch content	-0.64 ^{ns}	-0.91*	-0.80*	-0.87*	0.98*	0.93*	1.00	
Total carbohydrate	-0.59 ^{ns}	-0.93*	-0.84*	-0.89*	0.98*	0.99*	0.97*	1.00

* – %95 significant, ns – not significant

Many researchers have stated that carbohydrates in different plants has a function as an osmoregulator in increasing their durability to cold and drought. Some studies reported that starch is used as an energy source rather than carbohydrates in the plant of *Lachenalia minima* and water movement plays a key role in the storage of sucrose [Vijn and Smeekens 1999, Orthen 2001]. Carbohydrate content in the bulb of *Galanthus nivalis* are affected by the different moisture levels [Orthen and Whermeyer 2004].

Researchers reported that the water stress was effected carbohydrate content on *C. hederifolium* [Yıldırım et al. 2009]. Our results indicate that the means for highest reducing sugar (7.69 g/100 g), total soluble sugar (12.50 g/100 g), starch (16.27 g/100 g) and total carbohydrate (28.77 g/100 g) levels were obtained from the severe stress treatment (ML₄) (Tab. 3).

Relations between plant growth characteristics and carbohydrate reserves

The correlations of the carbohydrate content and plant development parameters are shown in Table 4. According to the correlation coefficients, the strongest relationship ($r = 0.94$) in the snowdrop was found between the bulb circumference size and reduced sugar content. It can be seen that there was a positive correlation between total carbohydrate ($r = 0.95$) and starch ($r = 0.98$) and also between reduced and total soluble sugar content ($r = 0.95$), while there was

a negative relationship between carbohydrate content and plant growth characteristics. On the other hand, there was no significant correlation between the plant height and carbohydrate contents. Our results correspond with those reported *Cyclamen* spp. for which there was a positive correlation between water content of the leaf size and chlorophyll content, while the correlation was negative between the leaf size and total sugar content [Akçal 2012].

CONCLUSIONS

Our study in snowdrop (*Galanthus elwesii* Hook.) clearly show that plant growth characteristics and carbohydrate accumulation were significantly affected by variable moisture levels. On the other hand, both of the substrates (cocopeat and peat + perlite) had a noticeable effect on the growth parameters and carbohydrate accumulation. In other words, total soluble sugars and starch content increased related to induced moisture levels in different substrates. The overall results reveal that the circumference size, the height, the fresh mass of bulbs and the plant height decreased in response to a decrease in the amount of applied water. In contrast, an increase in the contents of all types of carbohydrates in bulbs, was observed with the decrease in substrate moisture. This results indicates that snowdrop tolerate the moderate-water stress induced by different irrigation levels. This is the proof

for snowdrop became survival by using carbohydrate reserves under drought conditions, so it seems to be suitable for landscape purposes in semi-arid regions. In this study, the process detailed for the snowdrop can be considered as an effective crop strategy for the abiotic stress management in bulbous ornamentals.

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