

## The pivotal role of phenological stage enhanced by plant origin preparations in the process of rhizogenesis of *Rosa* 'Hurdal' stem cuttings

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### Abstract

Some biostimulants, including plant origin preparations, act similarly to plant hormones. Moreover, the supplementation of known and unknown rooting cofactors can stimulate rhizogenesis in cuttings. The aim of this research was to assess the response of difficult-to-root and long-rooting stem cuttings of the once-blooming old variety *Rosa* 'Hurdal' to preparations of plant origin. The hypothesis was that plant origin preparations could enhance rooting processes by inhibiting chlorophyll a/b degradation in leaves and postponing leaf senescence, simultaneously increasing the quality of cuttings. The one-bud stem cuttings were made in four phenological stages: (H1) flower buds closed, (H2) open flowers, (H3) just after petal fall, (H4) 7-14 days after petal shedding. They were treated with either standard commercial powder preparations containing 0.4% indolebutyric acid (IBA) or 0.2% naphthalene acetic acid (NAA) as well as with commercial plant origin preparations that this work will henceforth refer to as: Algae Extract, Organic Preparation, and Plant Extract. The cuttings were evaluated after 12 weeks of rooting them in two substrates: peat-perlite and peat-sand (v:v; 1:1). Mean root percentages for both substrates were noted after preparation from stage H1 (74.5 %), H2 (59.5 %), H3 (50.8 %) shoots. The H4 cuttings didn't root at all and were not considered further. The means for all phenology stages together were the highest by the use of 0.6 % Algae Extract, 0.012 % and 0.02 % Organic Preparation, 0.2 % and 0.4 % Plant Extract. The lowest means were reported for the control cuttings as well as NAA and IBA treatment. Plant origin preparations encouraged growth parameters but did not unequivocally inhibit the decrease of chlorophyll content in the cuttings' leaves. Rooting percentage depended on the quality of cuttings as well as chlorophyll a/b and soluble protein content in leaves in both rooting substrates.

**Keywords:** biostimulant; carotenoids; chlorophyll; IBA; leaf senescence; NAA; phenology; soluble proteins

### 1. Introduction

The propagation of woody ornamental plants, including roses, by stem cuttings harvested in flowering time is the most easy and economical method in nursery production [1]. However, roses are highly biodiverse, and considering their many species, subspecies, groups and cultivars [2] as well as diversification connected with anatomical [3], morphological and various phenotypical features [2], their propagation can be difficult and seems to be fallible when using the standard nursery methods [1,4].

Meanwhile, conservation and popularisation, especially of old garden roses, is important on grounds of preserving a wide genetic pool and increasing plant biodiversity in gardens as an

implementation of the provisions of the Convention on Biological Diversity (CBD) drawn up in Rio de Janeiro on June 5th, 1992. The object of this study is the once-blooming *Rosa* 'Hurdal', one of the valuable garden Old Roses, probably an Alba or Villosa Hybrid created by an unnamed breeder. Its winterhardiness as well as high tolerance to frost and fungal leaf diseases [2,5] predestines this variety to cultivation on own roots, as is preferred in extensive cultivation. However, the rooting ability of the cuttings of this rose is low and strictly dependent on the phenological stage of shoots [6,7]. Moreover, the rooting of one-leaf stem cuttings of this rose is long, lasting 12 weeks [6,8], as is observed in the case of other wild [9] and old rose taxa [4,6,8] in contrast to Modern Roses such as Tea Hybrids, the cuttings of which root in 4-6 weeks [10].

During their lengthy rhizogenesis, the stem cuttings of *Rosa* 'Hurdal' are especially exposed to stress factors caused by a decrease in chlorophyll and reduced content of carbohydrates in leaves [6]. The decrease of chlorophyll content and increase of carotenoids is one of the symptoms of leaf senescence processes [11,12]. Moreover, the decrease of chlorophyll content in the leaves of the 'Hurdal' rose, both during the process of rhizogenesis and after flowering in the stock plant, has been proven in previous research and recognized as one of the probable factors of cuttings failing to root when prepared from shoots during the fruiting phenological stage – 7-14 days after petal fall [6] (BBCH 71 702) [13].

The following eco-friendly plant origin commercial preparations are used in the present study on the 'Hurdal' rose: Bio Rhizotonic [14], Root Juice [15] and Bio Roots [16]. A detailed characterisation of these preparations is presented in Table 1. These preparations can be qualified as biostimulants containing humic substances. Their use increased the content of nutrients in plant tissues, had positive effects on metabolic processes, and has mostly been considered and proved to be efficient in relation to crops and vegetable plants [17,18,19], rarely ornamentals [20], including woody plants propagation [21,22,23]. When applied exogenously, some plant preparations have similar actions to groups of known plant hormones (auxins, gibberellins, cytokinins) [24,25]. Seaweed extracts in particular can also contain growth regulators that enhance rhizogenesis [26].

**Table 1.** Preparations used for rooting *Rosa* 'Hurdal' in the experiment.

Name in work	Preparation	Characteristics	Certifications
IBA	Ukorzeniacz A <sub>aqua</sub> (Himal, Poland)	0.4% IBA	–
NAA	Ukorzeniacz B <sub>aqua</sub> (Himal, Poland)	0.2% NAA	–
Algae Extract	Bio Rhizotonic [14]	seaweed-based, 100% organic; contains N-P-K 0.6-0.2-0.6, vitamins such as B1, B2 and other biologically active substances	Organic Materials Review Institute [27]
Organic preparation	Root Juice™ [15]	combination of humic acid and seaweed extracts, containing N-P-K 2-6-6	National Organic Program (NOP); Control Union Certified EU; Good Soil Quality Mark; Point Vert; Organic Materials Review Institute (OMRI); Clean Green Certified
Plant Extract	Bio Roots [16]	amino acids and oligosaccharins, fruit oil up to 1%; humic acids 1%; pectinate 1%; sodium alginate 3%; seaweed species 10%, organic matter 84%	European regulation EC No 834/2007 on organic agriculture [28]

The aim of this work was to assess the response of difficult-to-root and long-rooting stem cuttings of *Rosa* 'Hurdal' to preparations of plant origin. The hypothesis was that plant origin preparations could enhance rooting processes, inhibiting chlorophyll degradation and postponing leaf senescence and at the same time increasing the quality of cuttings. In previous research we proved the key roles of the phenological stage of shoots harvested for cuttings [6,29-31] and of the rooting substrate [8] in the rhizogenesis of this taxon. In this work, the results were analysed considering four phenological stages of shoots and two rooting substrates. The possibility of using organic plant origin preparations encourages eco-friendly plant reproduction in nurseries. These ideas are aligned with the Official Journal of the European Union and EU Council Directive [32-34], the National Organic Program USDA [35], and the Organic Materials Review Institute [27] limiting the use of chemicals in plant production.

## 2. Material and Methods

### 2.1. Plant material

The cuttings were harvested from shrubs of *Rosa* 'Hurdal' (Germany-Norway; origin unknown, *R. ×alba* × *R. villosa*, Figure 1a) growing in the National Collection of Rose Cultivars in the Polish Academy of Sciences Botanical Garden, Centre of Biological Diversity Conservation in Powsin, Warsaw, Poland.

The single-node leafy semi-hardwood shoot cuttings of *Rosa* 'Hurdal' [2] with an original leaf (leaf area 24–27 cm<sup>2</sup>) (Figure 1 b and c) were taken from the middle part of generative shoots. The harvesting time of shoots in four periods and four phenological stages (Figure 1d) according to BBCH scale in roses [13] lasted 16 days and is denoted in the work by the letter H: H1 – 27<sup>th</sup> May, 54 504 (flower buds still closed), H2 – 1<sup>st</sup> June, 69 605 (full flowering; at least 50% of flowers open), H3 – 6<sup>th</sup> June, 69 629 (end of flowering of second order stem: all petals fallen), H4 – 11<sup>th</sup> June, 70 701 (fruit set: beginning of berry growth on the main stem) (Figure 1d).

### 2.2. Rooting medium and experimental conditions

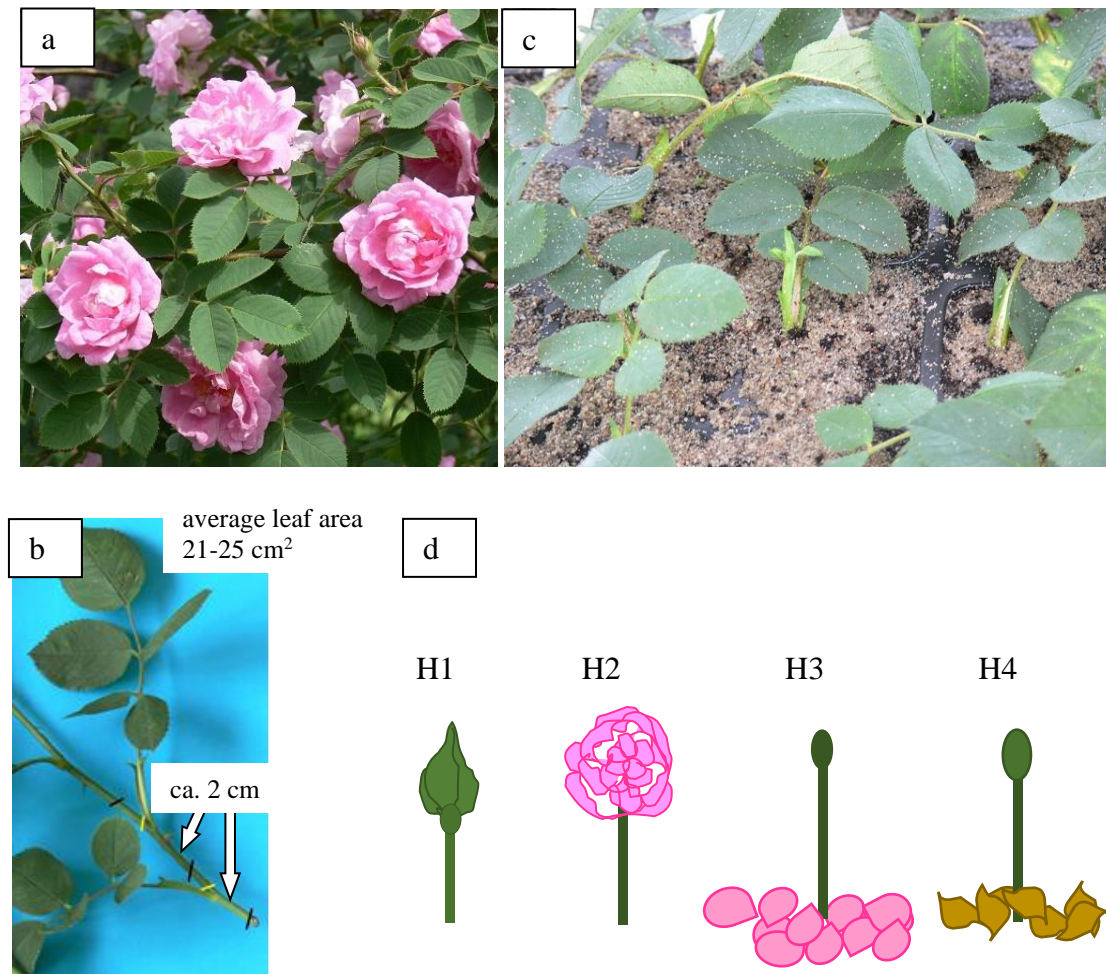
The cuttings were inserted in multipot trays (6.6 × 6.6 cm, 98 cm<sup>3</sup>) in two rooting substrates: (i) peat-perlite and (ii) peat-sand, mixture (v:v 1:1; pH 6-6.5). The peat was purchased from the Karaska company (Poland); very coarse-grained sand (1.25-2.00 mm grain size) originated from the River Vistula (Poland); and perlite was imported from Italy (Tables 2 and 3).

**Table 2.** Basic analysis of the growing media.

Growing media	PH in H <sub>2</sub> O	The content of assimilable components in mg/dm <sup>3</sup> of growing media							Salinity in g KCl/dm <sup>3</sup>
		N-NO <sub>3</sub>	N-NH <sub>4</sub>	P	K	Ca	Mg	Cl	
peat - perlite 1:1	6.3	7.0	5.7	10.0	20.0	646.7	45.3	15.0	0.15
peat - sand 1:1	6.3	5.0	16.3	11.7	23.3	686.7	44.0	18.0	0.17

**Table 3.** The physical properties of rooting media, peat-perlite and peat-sand, mixed at 1 : 1 ratio in the cutting propagation experiment.

Rooting medium	Organic matter content (%)	Dry bulk density (g·dm <sup>-3</sup> )	Container capacity (g·dm <sup>-3</sup> )	Total porosity (%)	Water space at 10 cm H <sub>2</sub> O (%)	Air space at 10 cm H <sub>2</sub> O (%)	Total water capacity (%)	Container water capacity (%)	Water easily accessible (%)
	m/m	(g·dm <sup>-3</sup> )	(g·dm <sup>-3</sup> )	(% vol)	(% vol)	(% vol)	(% vol)	(% vol)	(% vol)
Peat / perlite	12.4	752.9	744.9	69.1	57.9	11.2	69.1	61.2	26.6
Peat / sand	57.5	239.8	321.0	87.3	65.5	21.8	87.3	71.1	24.1



**Figure 1.** The Rosa 'Hurdal' (a), single-node leaf cutting prepared for experiment (b); planted cuttings (c); phenological stages of shoots for cuttings' preparation: as they figure in this work named and in BBCH scale (Meier et al. 2009) (d): H1 — flower buds closed (BBCH 54 504; flower buds still closed); H2 — flowers opened (69 605; full flowering, at least 50% of flowers open); H3 — immediately after petal shedding (69 629; end of flowering of second order stem: all petals fallen); H4 — 7\*14 days after shedding petals (70 701; fruit set: beginning of hip – berry – growth on the main stem).

The treatment of cuttings in the experiment is presented in detail in Table 4. According to this schema, the basal parts (1 cm) of the stems were dipped in rooting powder containing 0.4% IBA or 0.2% NAA. The remaining cuttings were watered with 10 cm<sup>3</sup> per pot (98 cm<sup>3</sup>) with a solution of plant origin preparation or, in the control combination, water. On the 10<sup>th</sup> and 20<sup>th</sup> day of rooting, the cuttings were watered with 10 cm<sup>3</sup> water or Algae Extract solution per pot (98 cm<sup>3</sup>) (Table 4).

The experiment was conducted in a foil tunnel in the commercial nursery of M&M Kryt in Wola Prazmowska (51.56°N, 20.28°E), Poland. An electronically controlled misting system maintained optimal climatic conditions (air temperature 23-25°C, ambient relative humidity 80-90%). The cuttings were covered with a shading material that limited the photosynthetic photon flux density to 130  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . The cuttings were protected against fungal diseases by applying fungicides every 7-9 days during the rooting period: Previcur Energy 840 SL (propamocarb 47.28%,



fosetyl 27.65%), Amistar® 250 SC (azoxystrobin 250 g·dm<sup>-3</sup>), Score 250 SC (difenoconazole 250 g·dm<sup>-3</sup>).

**Table 4.** The treatment of cuttings of *Rosa* 'Hurdal' at each phenological phase of the experiment.

No. of treatments	The treatment of cuttings		Name in tables and figures
1	Control		Control
2	0.4% IBA; at the time of planting		IBA
3	0.2% NAA; at the time of planting		NAA
4	0.4 % Algae Extract	watering (10 ml) after	RH 0.4x1
5	0.6 % Algae Extract	planting	RH 0.6x1
6	0.4 % Algae Extract	watering (10 ml) after	RH 0.4x2
7	0.6 % Algae Extract	planting and 10 days later	RH 0.6x2
8	0.4 % Algae Extract	watering (10 ml) after	RH 0.4x3
9	0.6 % Algae Extract	planting, 10 and 20 days later	RH 0.6x3
10	0.1 % Organic preparation		RJ 0.1
11	0.2 % Organic preparation		RJ 0.2
12	0.4 % Organic preparation	watering (10 ml) after	RJ 0.4
13	0.012 % Plant Extract	planting	BR 0.012
14	0.02 % Plant Extract		BR 0.02

### 2.3. Evaluation of the cuttings' growth

The cuttings were dug up after 12 weeks, in the first days of August. The rooted cuttings were cleaned carefully from the medium and evaluated.

The percentage of rooted cuttings was calculated (%) in relation to the number of planted cuttings, while the percentage of cuttings with retained stock plant leaf and the percentage of cuttings that formed a new shoot were calculated in relation to the number of cuttings with roots.

The fresh weight (g) of rooted cuttings was estimated using an analytical balance (PS 6000/C/2, RADWAG, Poland). The total leaf area (cm<sup>2</sup>, original leaf with all leaves on the new shoot) was scanned with a leaf area meter (AM 300, ADC BioScientific Ltd., Hoddesdon, UK). Due to the length of young new shoots not exceeding 1 cm for most of the cuttings, this parameter was not considered or measured.

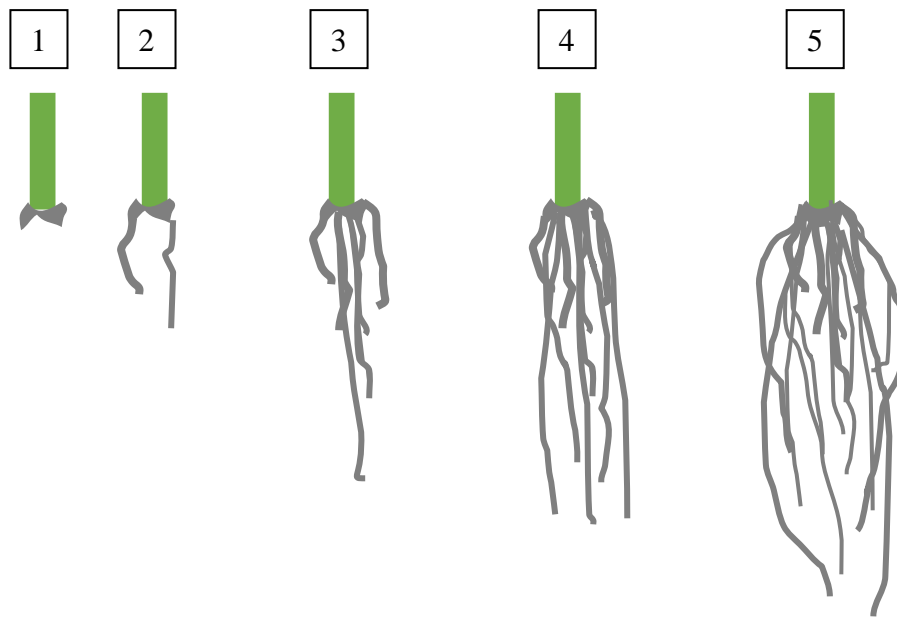
The quality of the root system was estimated as the degree of rooting on a 5-point scale: 1 – only a callus, without formed roots; 2 – at least one short root; 3 – a few longer roots; 4 – roots visibly developed, differentiated in length; 5 – roots strong, starting forming a root ball, well developed (Figure 2). Cuttings with the value '1' were rejected and not evaluated further. They figure in the experiment and in the statistical evaluation of the degree of rooting as unrooted.

### 2.3. Protein and pigment analyses

For biochemical analyses, the remaining stock plant leaves of rooted cuttings were sampled after 12 weeks of the rhizogenesis process. Additionally, the leaves were taken from stock plants on the days of evaluation of the rooting cuttings in August. All leaf samples were frozen and stored at a temperature of –18 °C. Research was conducted in the laboratories of PAS Botanical Garden CBDC in Powsin.

Protein extract was used to estimate the content of soluble proteins [36]. A frozen leaf sample (about 100 mg) was grinded in liquid nitrogen with a mortar and pestle. Proteins were extracted with 1 ml of extraction buffer (0.063M TRIS, 2% SDS, 5% β-mercaptoethanol, 10% glycerol) for 30 minutes [36]. The extraction slurry was transferred to Eppendorf's tube and proteins were denaturated in a water bath (95°C, 5 min). After centrifugation at 10 000xg for 20

min. at room temperature, 300 µl of supernatant was taken to a new tube and frozen at -40°C for future assessment.



**Figure 2.** The quality of the root system (degree of rooting) on a 5-point valuation scale: 1 – only callus, without formed roots; 2 – at least one short root; 3 – a few longer roots; 4 – roots visibly developed, differentiated by length; 5 – strong roots, starting to form a root ball, well developed.

Total soluble protein content was evaluated according to Ghosh with minor modifications [37]. Aliquot of 10 µl of proteins extract was loaded on squared pieces (8x8 mm) of filter paper (Whatman 3MM) placed in cells of plastic cultivation plates. After 15 min. drying at room temperature, paper pieces were saturated with 15 µl of 0.2% CBB R-250 solution in 40% methanol and 10% acetic acid. After 20 minutes, the nonbonded dye was washed off three times with 0.5 ml of 20% methanol and 10% acetic acid solution and dried at 35°C in an oven. Dried papers were transferred to a centrifuge tube (2 ml), and the protein-dye complex was eluted with 2 ml of 1% SDS solutions in an ultrasonic washer for 1 hour. Protein content was assessed spectrophotometrically at 590 nm. The amount of protein was calculated using a standard curve created for BSA solutions.

For the assessment of the content of chlorophyll a/b and carotenoids, the leaf tissue sample of 0.1 g was grinded to a powder by hand in a mortar in liquid nitrogen. The extraction was conducted with 5 mL of 99.5% acetone by adding a pinch of calcium carbonate to neutralize the organic acids. The extracts were completed to a total volume of 25 mL with acetone and filtered through soft blotting paper. The assessment of the content of the pigments was made spectrophotometrically measuring the absorbance at wavelengths of 750, 662, 645, and 470 nm (GeneQuant 1300, GE). The calculations were made using the following formulas [38]:

$$\begin{aligned}\text{Chlorophyll a} &= (12.7 \times A_{663} - 2.7 \times A_{645}) \times V \times (1000 W)^{-1} \\ \text{Chlorophyll b} &= (22.9 \times A_{645} - 4.7 \times A_{663}) \times V \times (1000 W)^{-1} \\ \text{Carotenoids} &= (1000 \times M_{470} - 1.9 \times \text{chlorophyll a} - 63.14 \times \text{chlorophyll b}) \times 214^{-1}\end{aligned}$$

where A – absorbance at a given wavelength, V – total volume of the extract (cm<sup>3</sup>), and W – sample weight (g).

## 2.4. Statistical analysis

The experiment was constructed as a randomised complete block design (RCBD) [39] for each maturity phase and involved 112 combinations for three variables (treatment, phenological stage, medium). Each combination of treatment in four phenological stages of shoots (Figure 1) included four replicas of 10 cuttings. A total of 4480 cuttings was planted.

The values in percentage were transformed by using the function  $\text{ARCSIN}(x)^{1/2}$  according to Bliss or  $y = x^2 + (x^2 + 1)^2$  to compare the means and carry out analyses [39]. The data of biochemical analyses and growth parameters were analysed by using three or two-factorial analysis of variance (ANOVA), and Duncan's Honest Significant Difference (HSD) test was used to determine the significance of differences between the means ( $p \leq 0.05$ ) for three or two variables – phenological stage, treatment with rooting enhancer, and medium.

Correlations between the data parameters of growth, pigments and soluble proteins content in cuttings were carried out [39]. The results are shown together for each phenological phase and rooting substrate. The correlations between rooting percentage and evaluated parameters in relation to rooting enhancers were presented separately for each stage rooted in both substrates.

STATISTICA 10 software package (Statsoft Polska, Cracow, Poland) was used for statistical analysis.

## 3. Results

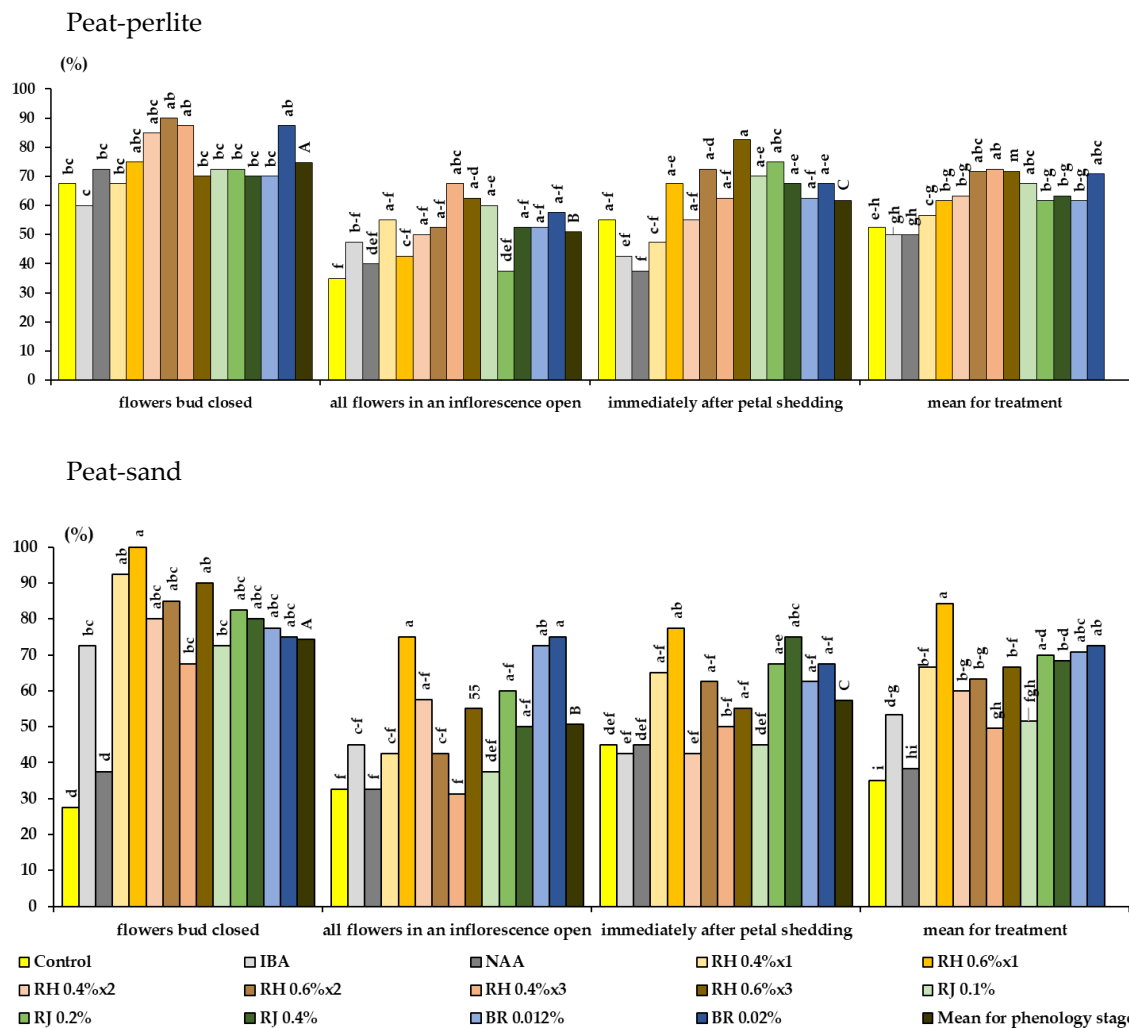
### 3.1. The pivotal role of phenology stage in the rooting of *Rosa* 'Hurdal' cuttings

The research showed a dominant role of the phenological stage of shoots in rooting the cuttings of *Rosa* 'Hurdal' due to its influence on the effectiveness of propagation and the quality of cuttings.

Higher mean rooting percentage of the stem cuttings of *Rosa* 'Hurdal' were noticed after preparing them from shoots with flower buds closed (H1; 74.5 %); the means were significantly lower after attempts to root cuttings harvested from shoots with opened flowers (H2; 59.5 %) or immediately after petal fall (H3; 50.8 %) (Table 5). The cuttings prepared from shoots 7-14 days after petal fall didn't root at all and were not taken into further consideration. The means for all phenology stages together were the highest by the use of 0.6 % Algae Extract, 0.012 % and 0.02 % Plant Extract, 0.2 % and 0.4 % Organic Preparation. The lowest means were noticed for the control cuttings as well as NAA and IBA treatment (Table 6). The rooting substrate did not affect this mean for all phenology stages of shoots (Table 5).

The highest rooting percentage of stem cuttings harvested from shoots with flower buds closed (H1) was obtained after single treatment with 0.6% Algae Extract and rooting in peat-sand substrate (100 %). A high rooting percentage was also obtained in H1 by single use of 0.4 % and three-time use of 0.6 % Algae Extract in peat-sand (92.5 % and 90 %, respectively) as well as two-time use of 0.4 % and 0.6 % Algae Extract, 0.02 % Plant Extract in peat-perlite (90.0 %, 87.5 %, 87.5 %, respectively). In the case of H2 cuttings, the highest rooting percentage was obtained by the single use of 0.6% Algae Extract and 0.02 % Plant Extract and rooting in peat-sand substrate (both 75.0 %). High means for H3 cuttings were noted by the use of three-time 0.6 % Algae Extract and rooting in peat-perlite (82.5 %) as well as single treatment with 0.6% Algae Extract and rooting in peat-sand substrate (77.5 %). The lowest rooting percentage for H1 and H2 were obtained in control cuttings or after the use of NAA and rooting in peat-sand substrate (27.5 % and 37.5 % respectively, and both 32.5 % for H2). The low means for H3 were obtained by the use of NAA and IBA preparations in both rooting media (Figure 3).

The highest mean rooting degree of the stem cuttings of *Rosa* 'Hurdal' was noticed after preparing them from shoots H1 and H3 as compared to H2 (Table 5). The means for all phenology stages together were the highest by the use of one-time 0.6 % Algae Extract and 0.012 % Plant Extract, 0.2 % (Table 6).



**Figure 3.** Rooting percentage in *Rosa* 'Hurdals' cuttings prepared from three phenological stages (H1-H3) of shoots and rooted in two rooting substrates after treatment by rooting enhancers. H1 — flower buds closed (BBCH 54 504; flower buds still closed); H2 — flowers opened (69 605; full flowering, at least 50% of flowers open); H3 — immediately after petal shedding (69 629; end of flowering of second order stem: all petals fallen); and treated: Control – the control cuttings; IBA – 0.4% IBA (Ukorzeniacz Aaqua); NAA – 0.2% NAA (Ukorzeniacz Baqua); RH – Algae extract: watering with 0.4% or 0.6% Bio Rhizotonic (10 ml) 1, 2 or 3-time (after planting and 10 and 20 days later); RJ – Organic Preparation – watering with 0.1%, 0.2% or 0.4% Root Juice™ (10 ml) after planting; BR – Plant Extract – watering with 0.012% or 0.02% Bio Roots (10 ml) after planting. Different small letters indicate significant interactions between rooting substrate and treatment in each phenological stage separately (two-way ANOVA). Different capital letters indicate significant differences between phenological stages. The Duncan's test ( $\alpha=0.05$ ) was used.

In the case of cuttings from H1, the highest values in rooting degree were obtained by the single use of 0.4 % and 0.6 % Algae Extract and 0.2 % Organic Preparation rooted in peat-sand, while the lowest were reported after the use of NAA rooting in both substrates. The H2 cuttings had the highest rooting degree after the use of Plant Extract and single-use 0.6 % Algae Extract rooted in peat-sand substrate, while the lowest was found in the control in both media and after the use of NAA for cuttings rooted in peat-perlite. In the case of H3 cuttings, the use of 0.6 % Algae Extract provided higher means in both rooting media, while the use of NAA and IBA resulted in lower means in both rooting substrates, similarly to control cuttings rooted in peat-perlite. The



rooting degree was higher for cuttings rooted in peat-sand medium for H1 and H2 but lower for H3 (Figure 4).

The highest mean weight of rooted *Rosa* 'Hurdal' stem cuttings was observed after harvesting them from shoots with flower buds closed (H1; 1.1 g) and was significantly lower after harvesting the H3 cuttings (0.8 g) and H2 shoots (0.4 g) (Table 5). The means for all phenology stages together were the highest for one-time use of 0.4 % and 0.6 % Algae Extract, two-time use of 0.6 % Algae Extract, and 0.4 % Organic Preparation™ (Table 6). The lowest mean was noticed for the control cuttings. The rooting substrate did not affect the mean cutting weight of shoots in all phenological stages (Table 5).

The weight of rooted cuttings of H1 shoots was higher after the single use of Algae Extract rooted in peat-sand and after single watering by 0.6 % Algae Extract for H2 and H3 in both substrates. Moreover, a high mean was obtained for H2 cuttings using 0.02 % Plant Extract. The rooting substrate did not affect this mean for H1 cuttings, while for H2 cuttings it was higher in peat-sand and for H3 – in peat-perlite (Figure 5).

The highest mean percentage of *Rosa* 'Hurdal' cuttings with retained stock plant leaf was obtained after cutting from H3 shoots (H1; 95.8 %) and was significantly lower after harvesting H2 cuttings (83.8 %) and H3 shoots (77.1 %) (Table 5). The means for all phenology stages together were the highest by the use of one-time 0.6 % Algae Extract, 0.4 % Organic Preparation, and 0.012 % Plant Extract. The lowest means were noticed for cuttings treated with IBA preparation and for the control cuttings (Table 6). The rooting substrate did not affect all phenology stages of shoots in terms of the mean percentage of cuttings with retained stock plant leaf (Table 5).

**Table 5.** The growth parameters, pigments, and soluble protein content of cuttings in *Rosa* 'Hurdal' for different phenological stages and rooting substrates taken together.

Mean	Phenological stage of shoots			Rooting substrate	
	flower buds closed (H1)	all flowers in an inflorescence open (H2)	immediately after petal shedding (H3)	peat-perlite	peat-sand
Percentage of rooted cuttings (%)	74.5 a <sup>2</sup>	50.8 c	59.6 b	62.5 a	60.7 a
Rooting degree <sup>1</sup>	3.8 a	3.2 b	3.8 a	3.5 a	3.7 a
Weight of rooted cuttings (g)	1.1 a	0.4 c	0.8 b	0.7 a	0.8 a
Percentage of cuttings with retained stock plant leaf (%)	95.8 a	83.7 b	77.1 c	85.3 a	85.8 a
Percentage of cuttings with new shoot (%)	6.1 a	7.6 a	9.0 a	5.9 b	9.3 a
Total leaf area (cm <sup>2</sup> )	24.4 a	21.4 b	18.5 c	21.1 a	21.8 a
Chlorophyll a	0.43 b	0.49 a	0.50 a	0.50 a	0.45 b
Chlorophyll b	0.19 b	0.22 a	0.22 a	0.22 a	0.20 b
Carotenoids	0.37 b	0.43 b	0.43 b	0.43 a	0.39 b
Soluble proteins	42.5 c	47.7 b	51.3 a	49.3 a	45.0 b

<sup>1</sup>The degree of rooting on a 5-point scale: 1 – only a callus, without formed roots; 2 – at least one short root; 3 – a few longer roots; 4 – roots visibly developed, differentiated by the length; 5 – strong roots starting forming a root ball, well developed;

<sup>2</sup>Different letters indicate significant differences between phenological stages or rooting substrate. The Duncan's test ( $\alpha = 0.05$ ) was used.

**Table 6.** Growth parameters, pigments, and soluble protein content of cuttings in *Rosa* 'Hurdal' together with type of treatment in the experiment.

Mean	Percentage of rooted cuttings (%)	Rooting degree <sup>1</sup>	Weight of rooted cuttings (g)	Percentage of cuttings with retained stock plant leaf (%)	Percentage of cuttings with new shoot (%)	Total leaf area (cm <sup>2</sup> )	Chlorophyll a (mg·g <sup>-1</sup> FW)	Chlorophyll b (mg·g <sup>-1</sup> FW)	Carotenoids (mg·g <sup>-1</sup> FW)	Soluble proteins (μg·mg <sup>-1</sup> FW)
Control <sup>2</sup>	43.8 e <sup>3</sup>	3.1 de	0.65 e	78.4 cd	3.4 b	19.3 b	0.43 fg	0.18 h	0.36 fg	47.2 f
IBA	51.7 de	3.4 cd	0.75 a-d	77.2 d	3.4 b	21.0 ab	0.47 c-f	0.20 e-h	0.39 d-g	50.1 d
NAA	44.2 e	2.9 e	0.71 cde	81.6 bcd	3.4 b	21.0 ab	0.45 d-g	0.19 fh	0.39 d-g	53.5 c
RH 0.4x1	61.7 bc	3.7 abc	0.82 ab	84.6 bcd	9.3 ab	21.0 ab	0.45 d-g	0.19 fh	0.38 e-g	44.7 g
RH 0.6x1	72.9 a	4.0 a	0.84 a	98.3 a	7.2 ab	22.8 b	0.48 c-f	0.21 d-g	0.42 cde	48.1 ef
RH 0.4x2	61.7 bc	3.6 abc	0.76 a-d	88.6 a-d	6.1 ab	20.8 ab	0.51 c	0.23 cde	0.44 cd	47.4 f
RH 0.6x2	67.5 abc	3.7 abc	0.74 a-e	84.4 bcd	8.3 ab	20.8 ab	0.50 cd	0.25 c	0.46 c	39.8 h
RH 0.4x3	61.0 c	3.5 bcd	0.68 de	86.9 bcd	5.0 b	22.8 a	0.50 cde	0.22 c-f	0.44 cd	45.2 g
RH 0.6x3	69.2 abc	3.8 abc	0.80 abc	83.8 bcd	8.6 ab	22.5 ab	0.41 g	0.18 h	0.35 g	44.2 g
RJ 0.1	59.6 cd	3.4 cd	0.72 b-e	82.5 bcd	9.1 ab	21.9 ab	0.50 cde	0.22 c-f	0.43 cde	49.4 ed
RJ 0.2	65.8 abc	3.7 abc	0.72 b-e	82.1 bcd	8.8 ab	21.0 ab	0.45 d-g	0.20 e-h	0.40 d-g	45.0 g
RJ 0.4	65.8 abc	3.8 abc	0.75 a-d	90.1 abc	13.2 a	22.8 a	0.50 cde	0.23 cde	0.44 cd	47.7 f
BR 0.012	66.3 abc	3.9 ab	0.74 a-e	91.8 ab	13.0 a	20.8 ab	0.50 cde	0.24 cd	0.44 cd	50.3 d
BR 0.02	71.7 ab	3.7 abc	0.76 a-d	87.5 a-d	7.7 ab	21.3 ab	0.47 c-f	0.20 e-h	0.41 def	46.8 f
Before rooting in stockplant in August	–	–	–	–	–	–	1.15 a	0.51 a	1.14 a	66.5 b
	–	–	–	–	–	–	0.81 b	0.44 b	0.82 b	64.9 b

<sup>1</sup>The degree of rooting on a 5-point scale: 1 – only a callus, without formed roots; 2 – at least one short root; 3 – a few longer roots; 4 – roots visibly developed, differentiated by the length; 5 – strong roots starting forming a root ball, well developed;

<sup>2</sup>Control – the control cuttings; IBA – 0.4% IBA (Ukorzeniacz Aaqua); NAA – 0.2% NAA (Ukorzeniacz Baqua); RH – Algae extract: watering with 0.4% or 0.6% Bio Rhizotonic (10 ml) 1, 2 or 3-time (after planting and 10 and 20 days later); RJ – Organic Preparation – watering with 0.1%, 0.2% or 0.4% Root Juice™ (10 ml) after planting; BR – Plant Extract – watering with 0.012% or 0.02% Bio Roots (10 ml) after planting.

<sup>3</sup>Different letters indicate significant differences between treatment. The Duncan's test ( $\alpha = 0.05$ ) was used.

In the case of H1 cuttings, the percentage of cuttings with retained stock plant leaf was high, while the use of 0.2 % Organic Preparation™ and rooted peat-perlite caused the lowest (73.4 %) mean. This parameter in H2 cuttings was higher after the use of 0.2 % and 0.4 % Organic Preparation as well as Plant Extract. The weight of cuttings of H3 shoots was higher after the single use of 0.6 % Algae Extract and rooting in peat-sand (Figure 6).

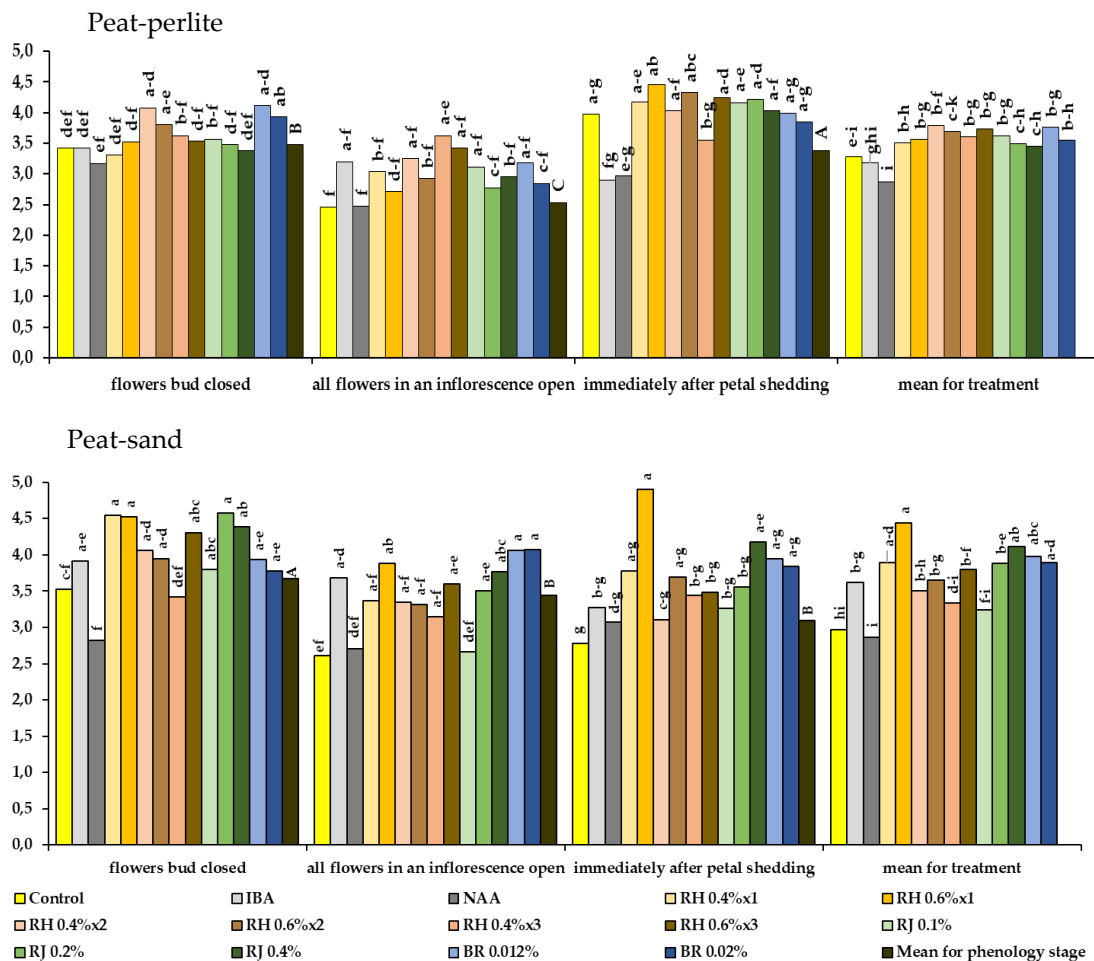
The mean percentage of *Rosa* 'Hurdal' cuttings with new shoots was similar when prepared from all three phenological stages (Table 5). The means for all phenology stages together were the highest by the use of 0.4 % Organic Preparation and 0.012 % Plant Extract, while the lowest means were noticed for cuttings treated with IBA preparation and for the control cuttings (Table 6). The percentage of cuttings with new shoots was higher for cuttings rooted in peat-sand medium (Table 5).

The percentage of cuttings with new shoots was highest by the use of 0.2 % Organic Preparation in the case of H1 cuttings and after the single use of 0.6 % Algae Extract and rooting in peat-sand for H2 and H3. The rooting substrate did not affect this mean for H1 and H3 cuttings, while for H2 cuttings it was higher in peat-sand (Figure 7).

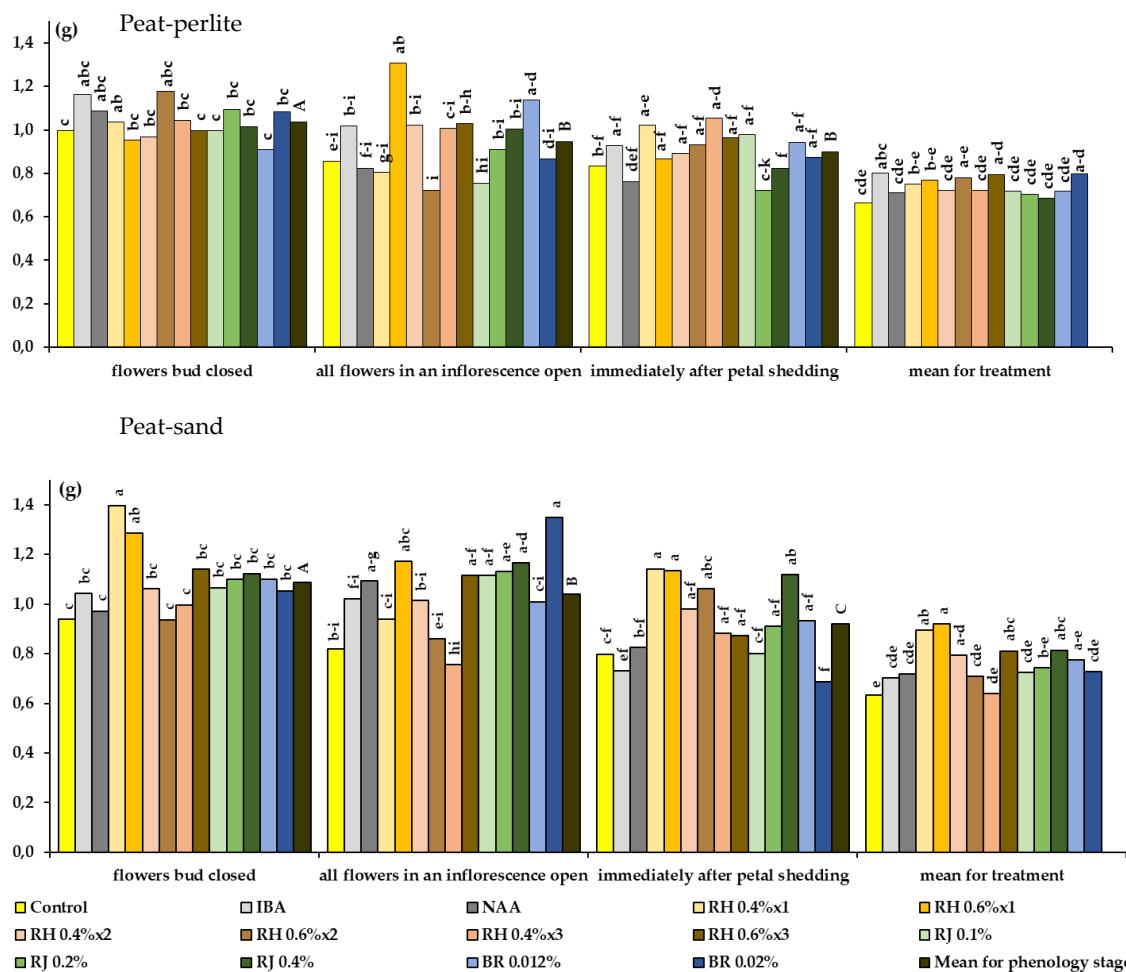
The highest mean of total leaf area in *Rosa* 'Hurdal' stem cuttings was noticed after harvesting from shoots with flower buds closed (H1; 24.4 cm<sup>2</sup>) and was significantly lower after preparing the cuttings from H2 (21.4 cm<sup>2</sup>) and from H3 shoots (18.5 cm<sup>2</sup>) (Table 5). The means for all phenology stages together were highest by the one-time use of 0.6 % or two-time use of 0.4 % and 0.6 % Algae Extract, and 0.4 % Organic Preparation, compared to the control cuttings. (Table

6) The rooting substrate did not affect the mean weight of cuttings for all phenology stages of shoots (Table 5).

The total leaf area of cuttings of H1 shoots was higher after the use of IBA; one-time watering with 0.4 % or triple watering with 0.6 % Algae Extract, rooted in peat-perlite; and NAA rooted in both substrates, compared to the use of 0.02% Plant Extract for cuttings rooted in peat-sand. The highest mean for H2 cuttings was obtained by the one-time use of 0.6 % Algae Extract and rooting in peat-perlite as well as three-time use of 0.4 % Algae Extract and rooting in peat-sand. In the case of H3 cuttings the analysis did not show any significant interaction between treatment and substrate (Figure 8).



**Figure 4.** Rooting degree in *Rosa* 'Hurdal' cuttings prepared from three phenological stages (H1-H3) of shoots and rooted in two rooting substrates after treatment by rooting enhancers. H1 — flower buds closed (BBCH 54 504; flower buds still closed); H2 — flowers opened (69 605; full flowering, at least 50% of flowers open); H3 — immediately after petal shedding (69 629; end of flowering of second order stem: all petals fallen); and treated: Control – the control cuttings; IBA – 0.4% IBA (Ukorzeniacz Aaqua); NAA – 0.2% NAA (Ukorzeniacz Baqua); RH – Algae extract: watering with 0.4% or 0.6% Bio Rhizotonic (10 ml) 1, 2 or 3-time (after planting and 10 and 20 days later); RJ – Organic Preparation – watering with 0.1%, 0.2% or 0.4% Root Juice™ (10 ml) after planting; BR – Plant Extract – watering with 0.012% or 0.02% Bio Roots (10 ml) after planting. Different small letters indicate significant interactions between rooting substrate and treatment in each phenological stage separately (two-way ANOVA). Different capital letters indicate significant differences between phenological stages. The Duncan's test ( $\alpha=0.05$ ) was used.



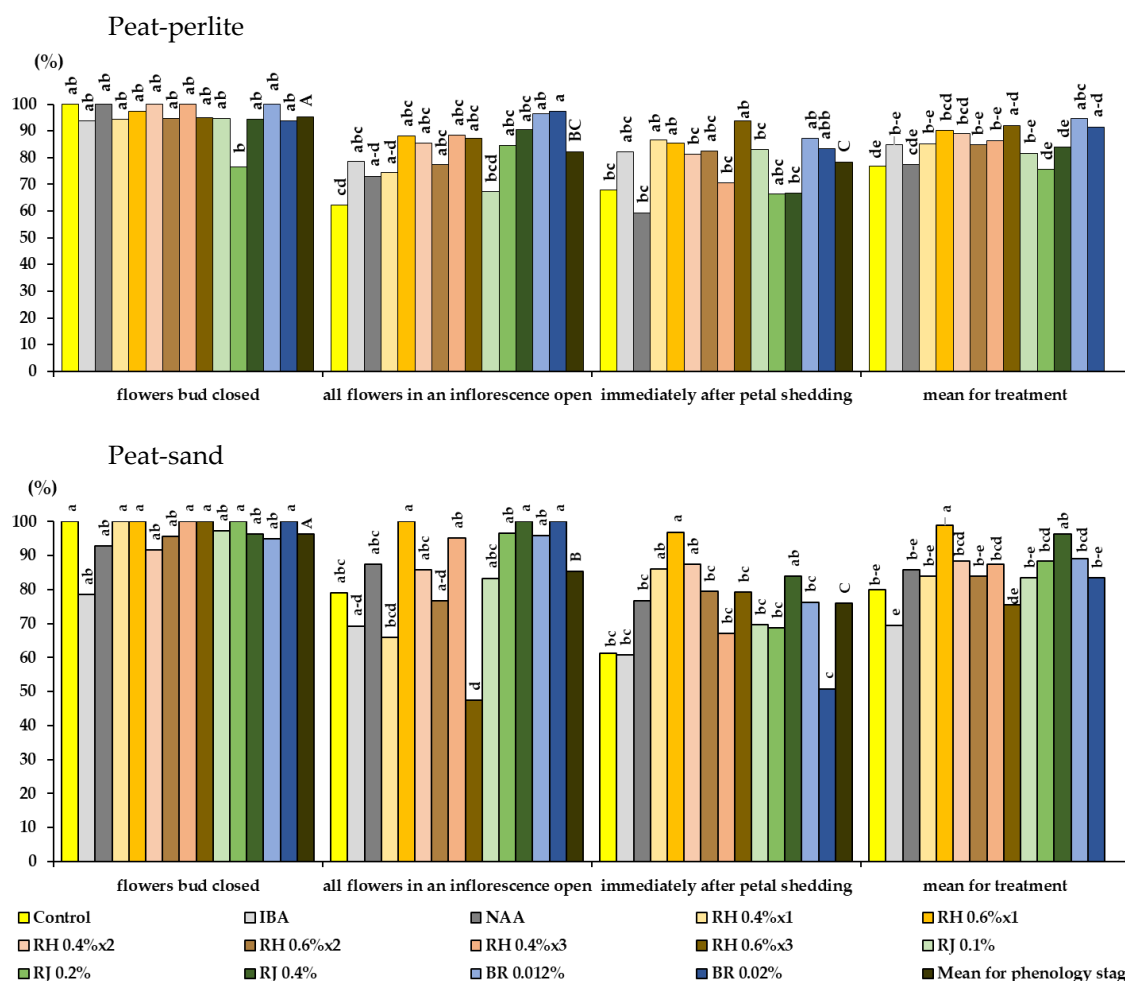
**Figure 5.** Weight of rooted cuttings in *Rosa* 'Hurdal' prepared from three phenological stages (H1-H3) of shoots and rooted in two rooting substrates after treatment by rooting enhancers. H1 — flower buds closed (BBCH 54 504; flower buds still closed); H2 — flowers opened (69 605; full flowering, at least 50% of flowers open); H3 — immediately after petal shedding (69 629; end of flowering of second order stem: all petals fallen); and treated: Control – the control cuttings; IBA – 0.4% IBA (Ukorzeniacz Aaqua); NAA – 0.2% NAA (Ukorzeniacz Baqua); RH – Algae extract: watering with 0.4% or 0.6% Bio Rhizotonic (10 ml) 1, 2 or 3-time (after planting and 10 and 20 days later); RJ – Organic Preparation – watering with 0.1%, 0.2% or 0.4% Root Juice™ (10 ml) after planting; BR – Plant Extract – watering with 0.012% or 0.02% Bio Roots (10 ml) after planting. Different small letters indicate significant interactions between rooting substrate and treatment in each phenological stage separately (two-way ANOVA). Different capital letters indicate significant differences between phenological stages. The Duncan's test ( $\alpha=0.05$ ) was used.

### 3.2. The pivotal role of the phenological stage of shoots in the progressive aging processes of leaves during the rhizogenesis of *Rosa* 'Hurdal' cuttings

The content of chlorophyll a/b, carotenoids and proteins in the leaves of cuttings decreased during the rhizogenesis process and were lower in rooted cuttings both before rooting and in stock plant leaves in August at the time of harvesting the cuttings. These phenomena were observed in cuttings of all phenological phases (Table 5; Figure 9-12).

The mean chlorophyll a/b and carotenoid contents in leaves of *Rosa* 'Hurdal' cuttings were similar when prepared from H2 and H3 shoots and higher than in phenological stage H1. The protein content was higher in H3 than in H2 and H1. The content of pigments and proteins was

higher for the cuttings rooted in peat-perlite medium when considering the results for all phenological phases together (Table 5).



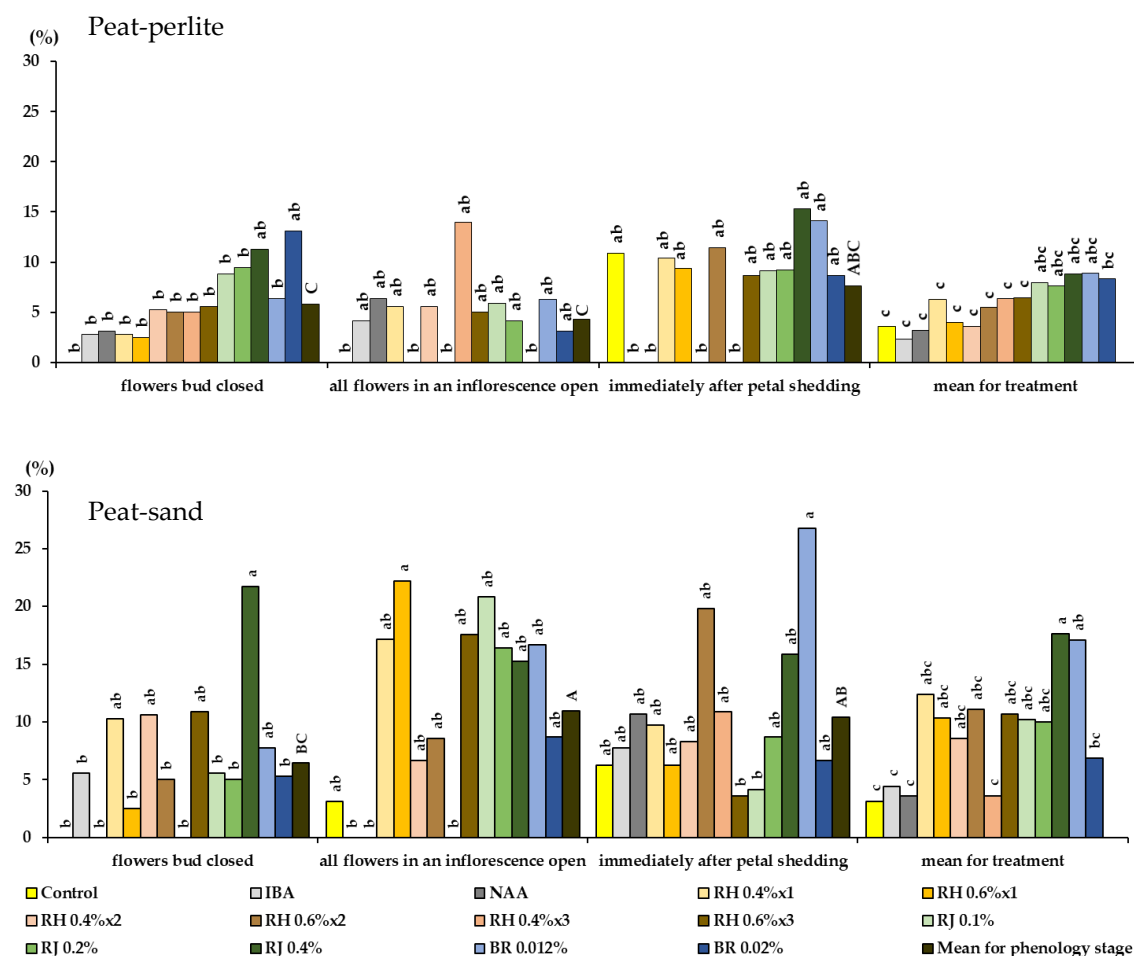
**Figure 6.** Percentage of rooted cuttings with retained stock plant leaf (%) in *Rosa* 'Hurdal' prepared from three phenological stages (H1-H3) of shoots and rooted in two rooting substrates after treatment by rooting enhancers. H1 — flower buds closed (BBCH 54 504; flower buds still closed); H2 — flowers opened (69 605; full flowering, at least 50% of flowers open); H3 — immediately after petal shedding (69 629; end of flowering of second order stem: all petals fallen); and treated: Control – the control cuttings; IBA – 0.4% IBA (Ukorzeniacz Aaqua); NAA – 0.2% NAA (Ukorzeniacz Baqua); RH – Algae extract: watering with 0.4% or 0.6% Bio Rhizotonic (10 ml) 1, 2 or 3-time (after planting and 10 and 20 days later); RJ – Organic Preparation – watering with 0.1%, 0.2% or 0.4% Root Juice™ (10 ml) after planting; BR – Plant Extract – watering with 0.012% or 0.02% Bio Roots (10 ml) after planting. Different small letters indicate significant interactions between rooting substrate and treatment in each phenological stage separately (two-way ANOVA). Different capital letters indicate significant differences between phenological stages. The Duncan's test ( $\alpha=0.05$ ) was used.

In the case of H1 cuttings, the chlorophyll a content in leaves increased after two-time treatment with Algae Extract and 0.2 % IBA on cuttings rooted in peat-perlite and 0.02 % Plant Extract rooted in peat-sand compared with cuttings rooted in peat-sand in the control and cuttings treated twice and three times with Algae Extract and 0.02 % Plant Extract. In the case of H2 cuttings, the chlorophyll a content in leaves increased after one-time treatment with 0.4 % and two-time treatment with 0.6 % Algae Extract rooted in peat-perlite compared with cuttings rooted in peat-sand and treated twice or three times with 0.6 % Algae Extract and rooted in peat-perlite as well as with those treated with 0.4 % Algae Extract, 0.1% Organic Preparation and 0.02 % Plant Extract.

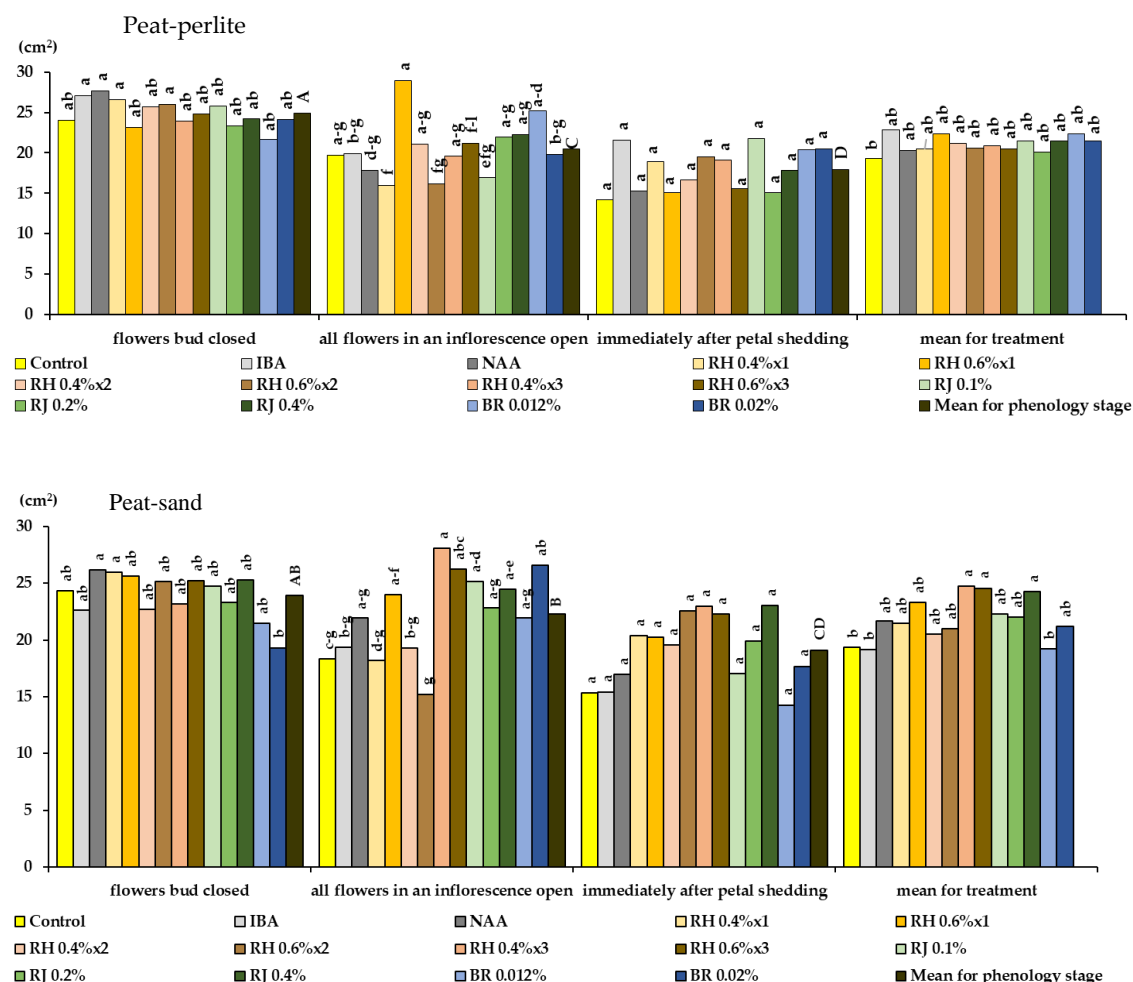


The highest means for H3 cuttings were obtained by the use of 0.1 Organic Preparation and rooting in peat-perlite as well as 0.012% Algae Extract and rooting in peat-sand. (Figure 9).

The chlorophyll b content in leaves of rooted H1 cuttings were higher after two-time watering with 0.4 % and 0.6 % Algae Extract for cuttings rooted in peat-perlite substrate. In the case of H2 cuttings, this mean was highest after the use of 0.4 Organic Preparation and two-time use of 0.6 % Algae Extract combined with rooting in peat-perlite substrate as well as after one- and two-time use of 0.6 % Algae Extract and rooting in peat-sand medium. In the case of H3 cuttings, the highest means were obtained after the use of 0.4 % Algae Extract, 0.1% and 0.4% Organic Preparation twice and rooting in peat-perlite (Figure 10).



**Figure 7.** Percentage of rooted cuttings that have created a new shoot (%) in *Rosa* 'Hurdal' prepared from three phenological stages (H1-H3) of shoots and rooted in two rooting substrates after treatment by rooting enhancers. H1 — flower buds closed (BBCH 54 504; flower buds still closed); H2 — flowers opened (69 605; full flowering, at least 50% of flowers open); H3 — immediately after petal shedding (69 629; end of flowering of second order stem: all petals fallen); and treated: Control – the control cuttings; IBA – 0.4% IBA (Ukorzeniacz Aaqua); NAA – 0.2% NAA (Ukorzeniacz Baqua); RH – Algae extract: watering with 0.4% or 0.6% Bio Rhizotonic (10 ml) 1, 2 or 3-time (after planting and 10 and 20 days later); RJ – Organic Preparation – watering with 0.1%, 0.2% or 0.4% Root Juice™ (10 ml) after planting; BR – Plant Extract – watering with 0.012% or 0.02% Bio Roots (10 ml) after planting. Different small letters indicate significant interactions between rooting substrate and treatment in each phenological stage separately (two-way ANOVA). Different capital letters indicate significant differences between phenological stages. The Duncan's test ( $\alpha=0.05$ ) was used.

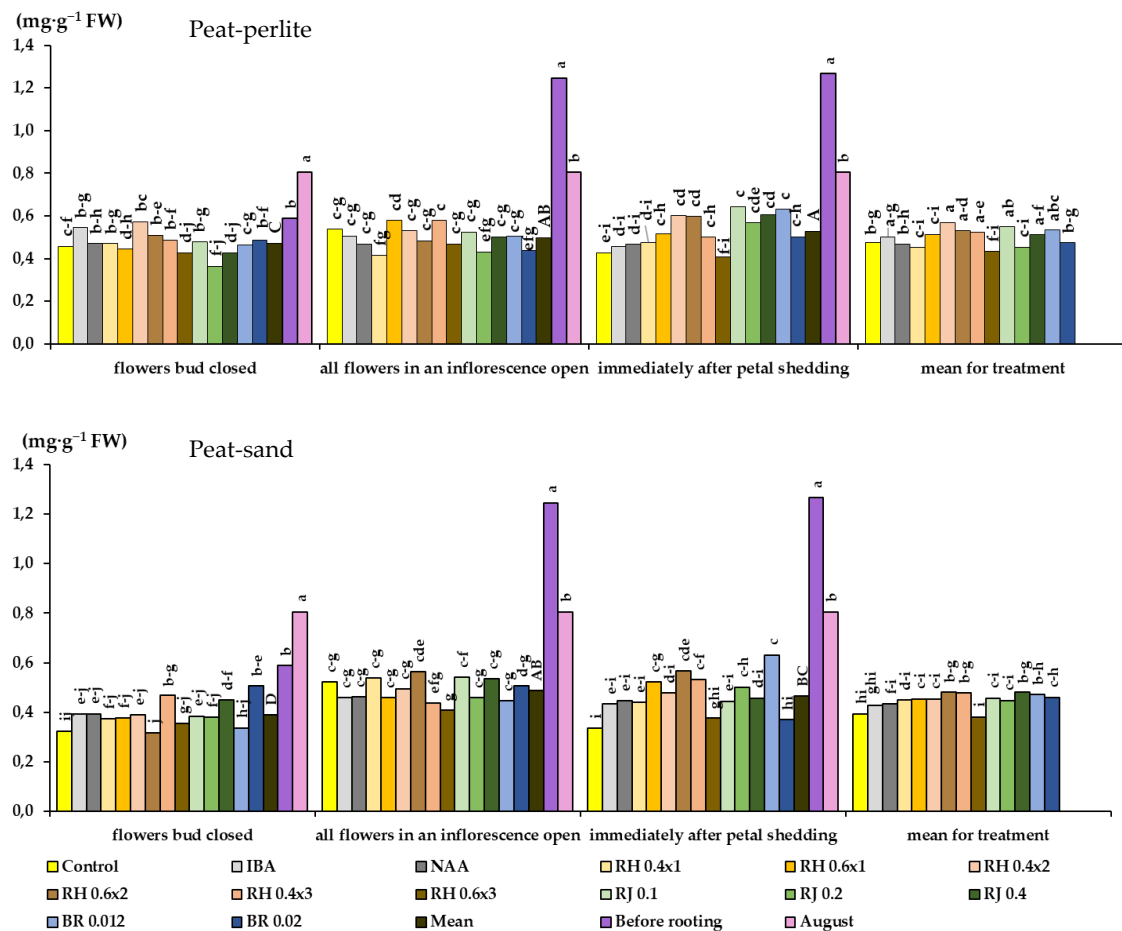


**Figure 8.** The total leaf area (cm<sup>2</sup>) in rooted cuttings of *Rosa* 'Hurdal' prepared from three phenological stages (H1-H3) of shoots and rooted in two rooting substrates after treatment by rooting enhancers. H1 — flower buds closed (BBCH 54 504; flower buds still closed); H2 — flowers opened (69 605; full flowering, at least 50% of flowers open); H3 — immediately after petal shedding (69 629; end of flowering of second order stem: all petals fallen); and treated: Control – the control cuttings; IBA – 0.4% IBA (Ukorzeniacz Baqua); NAA – 0.2% NAA (Ukorzeniacz Baqua); RH – Algae extract: watering with 0.4% or 0.6% Bio Rhizotonic (10 ml) 1, 2 or 3-time (after planting and 10 and 20 days later); RJ – Organic Preparation – watering with 0.1%, 0.2% or 0.4% Root Juice™ (10 ml) after planting; BR – Plant Extract – watering with 0.012% or 0.02% Bio Roots (10 ml) after planting. Different small letters indicate significant interactions between rooting substrate and treatment in each phenological stage separately (two-way ANOVA). Different capital letters indicate significant differences between phenological stages. The Duncan's test ( $\alpha=0.05$ ) was used.

The rooting substrate didn't affect the chlorophyll a/b content in leaves of rooted cuttings for H2 cuttings, while for H1 and H3 cuttings it was higher in peat-perlite (Figure 11 and 12).

The carotenoid content in leaves of rooted H1 cuttings was higher after watering twice with 0.4 % and 0.6 % Algae Extract and rooting in peat-perlite substrate. In the case of H2 cuttings, this mean was higher after the use of 0.4 % Organic Preparation and two-time use of 0.6 % Algae Extract combined with rooting in peat-sand substrate as well as one-time use of 0.6 % Algae Extract and rooting in peat-perlite medium. In the case of H3 cuttings, the highest means were obtained after the use of 0.02 % Plant Extract and rooting in peat-sand as well as all treatments with Organic Preparation, 0.02 % Plant Extract and 0.4 % Algae Extract when rooted in peat-perlite (Figure 12).

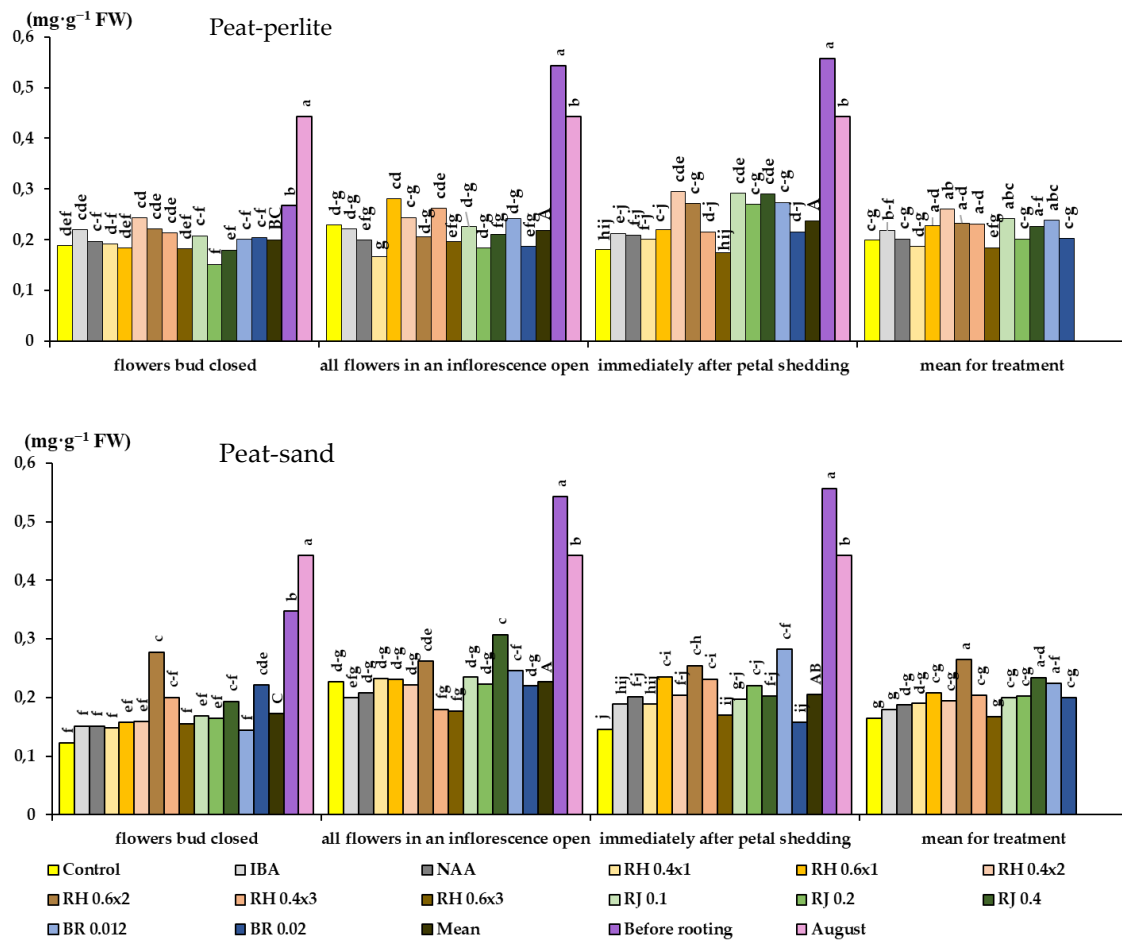
The rooting substrate did not affect the chlorophyll a/b and carotenoid content in the leaves of rooted cuttings for H2 cuttings, while for H1 and H3 cuttings, the means were higher in peat-perlite (Figure 9-11).



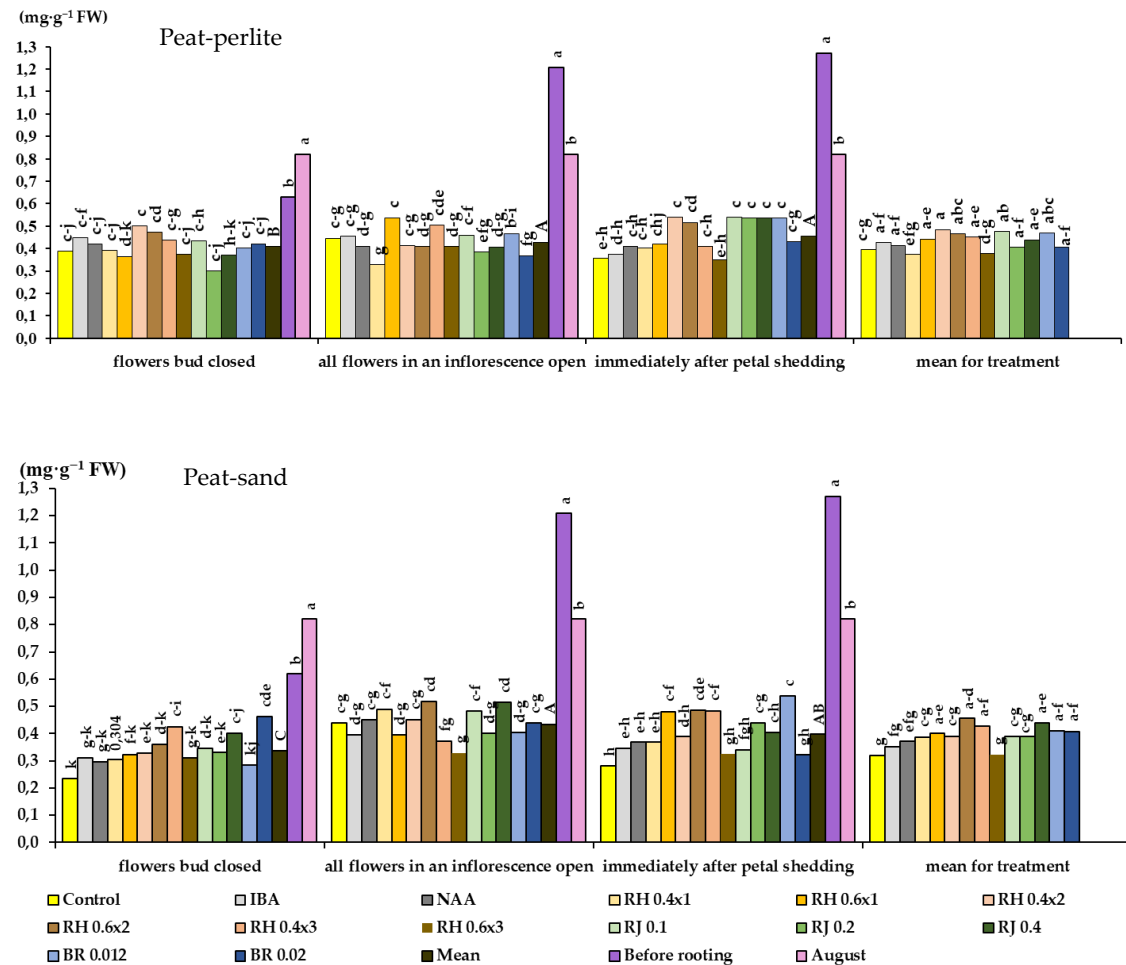
**Figure 9.** The chlorophyll a content (mg·g<sup>-1</sup> FW) in leaves of rooted cuttings of *Rosa* 'Hurdals' prepared from three phenological stages (H1-H3) of shoots and rooted in two rooting substrates after treatment by rooting enhancers. H1 — flower buds closed (BBCH 54 504; flower buds still closed); H2 — flowers opened (69 605; full flowering, at least 50% of flowers open); H3 — immediately after petal shedding (69 629; end of flowering of second order stem: all petals fallen); and treated: Control – the control cuttings; IBA – 0.4% IBA (Ukorzeniacz Aaqua); NAA – 0.2% NAA (Ukorzeniacz Baqua); RH – Algae extract: watering with 0.4% or 0.6% Bio Rhizotonic (10 ml) 1, 2 or 3-time (after planting and 10 and 20 days later); RJ – Organic Preparation – watering with 0.1%, 0.2% or 0.4% Root Juice™ (10 ml) after planting; BR – Plant Extract – watering with 0.012% or 0.02% Bio Roots (10 ml) after planting. Different small letters indicate significant interactions between rooting substrate and treatment in each phenological stage separately (two-way ANOVA). Different capital letters indicate significant differences between phenological stages. The Duncan's test ( $\alpha=0.05$ ) was used.

The protein content in leaves of rooted H1 cuttings was higher after watering the cuttings with 0.4 % Organic Preparation and 0.02 % Plant Extract and rooting in peat-perlite substrate. In the case of cuttings rooted in peat-sand, the highest means were noticed for cuttings treated with 0.012 % Plant Extract and 0.4 % Organic Preparation. In the case of H2 cuttings rooted in peat-perlite medium these means were higher after the use Plant Extract, also when compared to protein content in stock plant leaves before rooting. In the case of H3 cuttings rooted in peat-sand medium, this mean was the highest after the use of 0.4 % NAA and 0.2 % IBA, also when compared to the

content in stock plant leaves in August. The rooting substrate did not affect the protein content in leaves of rooted H3 cuttings; for H1 cuttings it was highest in peat-sand, for H2 – in peat-perlite (Figure 12).

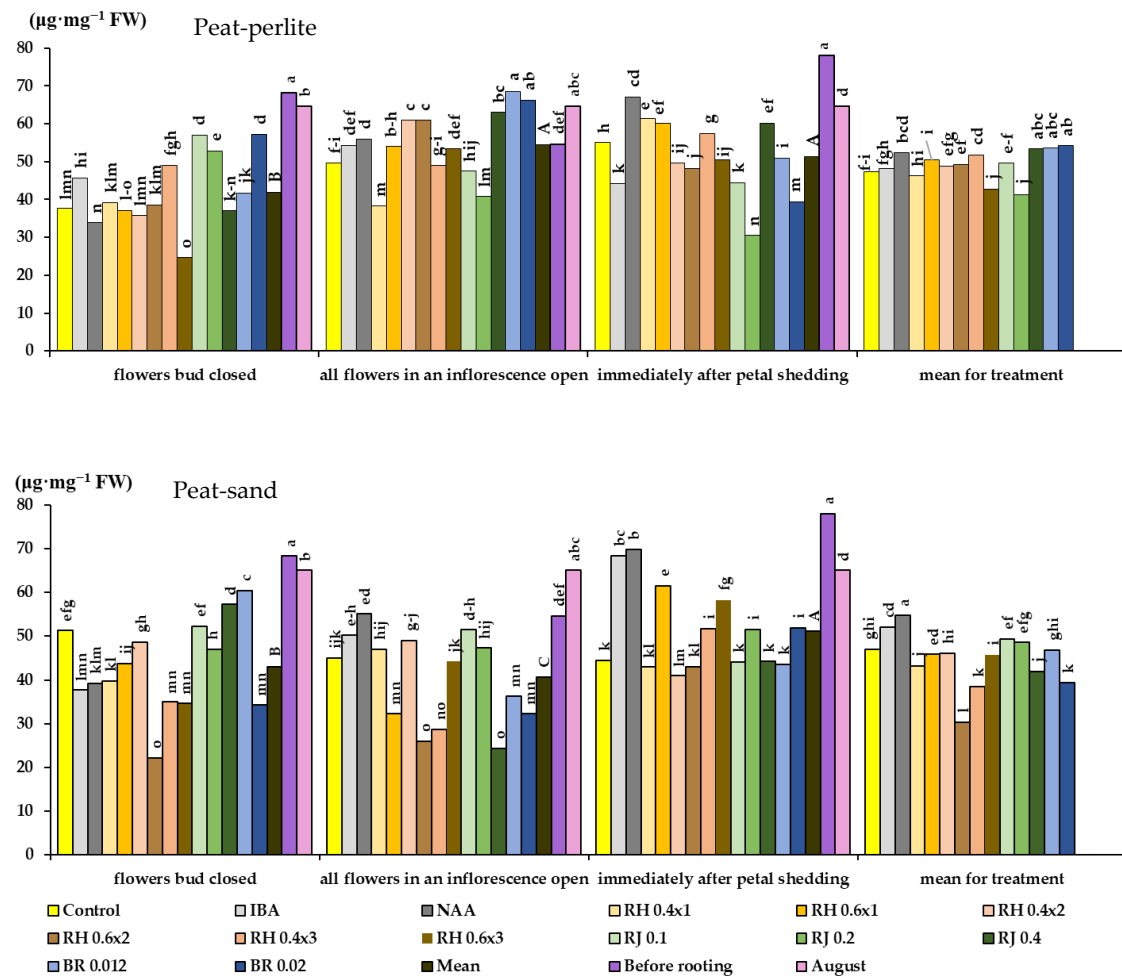


**Figure 10.** The chlorophyll b content ( $\text{mg}\cdot\text{g}^{-1}$  FW) in leaves of rooted cuttings of *Rosa* 'Hurdals' prepared from three phenological stages (H1-H3) of shoots and rooted in two rooting substrates after treatment by rooting enhancers. H1 — flower buds closed (BBCH 54 504; flower buds still closed); H2 — flowers opened (69 605; full flowering, at least 50% of flowers open); H3 — immediately after petal shedding (69 629; end of flowering of second order stem: all petals fallen); and treated: Control – the control cuttings; IBA – 0.4% IBA (Ukorzeniacz A<sub>aqua</sub>); NAA – 0.2% NAA (Ukorzeniacz B<sub>aqua</sub>); RH – Algae extract: watering with 0.4% or 0.6% Bio Rhizotonic (10 ml) 1, 2 or 3-time (after planting and 10 and 20 days later); RJ – Organic Preparation – watering with 0.1%, 0.2% or 0.4% Root Juice™ (10 ml) after planting; BR – Plant Extract – watering with 0.012% or 0.02% Bio Roots (10 ml) after planting. Different small letters indicate significant interactions between rooting substrate and treatment in each phenological stage separately (two-way ANOVA). Different capital letters indicate significant differences between phenological stages. The Duncan's test ( $\alpha=0.05$ ) was used.



**Figure 11.** The carotenoids content (mg·g<sup>-1</sup> FW) in leaves of rooted cuttings of *Rosa* 'Hurdals' prepared from three phenological stages (H1-H3) of shoots and rooted in two rooting substrates after treatment by rooting enhancers. H1 — flower buds closed (BBCH 54 504; flower buds still closed); H2 — flowers opened (69 605; full flowering, at least 50% of flowers open); H3 — immediately after petal shedding (69 629; end of flowering of second order stem: all petals fallen); and treated: Control – the control cuttings; IBA – 0.4% IBA (Ukorzeniacz A<sub>aqua</sub>); NAA – 0.2% NAA (Ukorzeniacz B<sub>aqua</sub>); RH – Algae extract: watering with 0.4% or 0.6% Bio Rhizotonic (10 ml) 1, 2 or 3-time (after planting and 10 and 20 days later); RJ – Organic Preparation – watering with 0.1%, 0.2% or 0.4% Root Juice™ (10 ml) after planting; BR – Plant Extract – watering with 0.012% or 0.02% Bio Roots (10 ml) after planting. Different small letters indicate significant interactions between rooting substrate and treatment in each phenological stage separately (two-way ANOVA). Different capital letters indicate significant differences between phenological stages. The Duncan's test ( $\alpha=0.05$ ) was used.





**Figure 12.** The soluble proteins content ( $\mu\text{g}\cdot\text{mg}^{-1}$  FW) in leaves of rooted cuttings of *Rosa* 'Hurdals' prepared from three phenological stages (H1-H3) of shoots and rooted in two rooting substrates after treatment by rooting enhancers. H1 — flower buds closed (BBCH 54 504; flower buds still closed); H2 — flowers opened (69 605; full flowering, at least 50% of flowers open); H3 — immediately after petal shedding (69 629; end of flowering of second order stem: all petals fallen); and treated: Control – the control cuttings; IBA – 0.4% IBA (Ukorzeniacyz A<sub>aqua</sub>); NAA – 0.2% NAA (Ukorzeniacyz B<sub>aqua</sub>); RH – Algae extract: watering with 0.4% or 0.6% Bio Rhizotonic (10 ml) 1, 2 or 3-time (after planting and 10 and 20 days later); RJ – Organic Preparation – watering with 0.1%, 0.2% or 0.4% Root Juice™ (10 ml) after planting; BR – Plant Extract – watering with 0.012% or 0.02% Bio Roots (10 ml) after planting. Different small letters indicate significant interactions between rooting substrate and treatment in each phenological stage separately (two-way ANOVA). Different capital letters indicate significant differences between phenological stages. The Duncan's test ( $\alpha=0.05$ ) was used.

### 3.3. Interdependence of qualitative parameters of cuttings and rooting percentage

The correlation analysis of the rooted single-node stem cuttings of *Rosa* 'Hurdal' derived from H2-H3 shoots and rooted in peat-perlite or peat-sand showed a strict relationship between increased rooting and a high percentage of cuttings with retained stock plant leaf. The rooting

degree decreased with the increase of total leaf area in H1 cuttings rooted in peat-perlite substrate, however the same parameter positively correlated with an increase in the percentage of cuttings with retained stock plant leaf (H2, H3/peat-perlite, H2, H3/peat-sand), the percentage of cuttings with young shoots (H1/peat-sand), the weight of cuttings (H3/peat-perlite; H1, H2, H3/peat-sand), and chlorophyll b and carotenoids content (H3/peat-sand). The weight of cuttings was positively correlated with the total leaf area (H1, H2/peat-perlite) and the percentage of cuttings with retained stock plant leaf (H3/peat-perlite; H3/peat-sand) (Tables 7-18).

The chlorophyll a/b, carotenoid and protein content in the leaves of cuttings before rooting did not correlate with any measured parameters in H1 cuttings rooted in peat-perlite or in peat-sand substrate (Tables 7 and 10), in H3 rooted in peat-perlite (Table 7), or in H2 rooted in peat-sand (Table 11). The increase of chlorophyll a/b and carotenoid content in leaves of cuttings before rooting was correlated with a decrease in the value of these parameters in H2 cuttings rooted in peat-perlite (Table 8) and of the total leaf area of H3 cuttings rooted in peat-sand (Table 12). The content of chlorophyll a/b and carotenoids measured in leaves of rooted cuttings were strictly connected (Tables 7-12)

Correlation analyses of the rooted cuttings treated with rooting enhancers derived from the shoots of three phenological stages (H1-H3) showed a relationship between rooting percentage following treatments in two rooting media and the pigments and soluble protein content in leaves of cuttings before rooting. The changes in growth and biochemical parameters caused an increase in rooting percentage (Suppl. Mat. 1-6).

**Table 7.** Correlation matrices between growth parameters, pigments, and soluble proteins content for *Rosa* 'Hurdal' cuttings harvested from shoots with flower buds closed (H1) and rooted in peat-perlite substrate.

Variable	Rooting degree	Weight of cuttings	Percentage of cuttings with retained stock plant leaf	Percentage of cuttings with young shoot	Total leaf area	Chlorophyll a	Chlorophyll b	Carotenoids	Soluble proteins	Chlorophyll a – before rooting	Chlorophyll b – before rooting	Carotenoids – before rooting	Proteins – before rooting
	1	2	3	4	5	6	7	8	9	10	11	12	13
2	0.114	1.000											
3	0.168	0.046	1.000										
4	0.220	-0.153	-0.077	1.000									
5	-0.281*	0.345**	-0.077	-0.289*	1.000								
6	0.112	-0.005	0.262	0.026	0.110	1.000							
7	0.143	0.002	0.250	0.064	0.077	0.979*****	1.000						
8	0.095	0.012	0.260	0.042	0.112	0.964*****	0.988*****	1.000					
9	0.106	0.087	-0.170	0.251	-0.085	0.019	0.031	0.008	1.000				
10	-0.081	-0.050	0.121	0.256	-0.045	0.001	-0.007	-0.004	0.004	1.000			
11	0.002	-0.152	0.033	0.190	0.155	0.119	0.101	0.094	0.150	0.432**	1.000		
12	-0.068	-0.022	0.116	0.245	-0.068	-0.009	-0.016	-0.016	-0.011	0.992*****	0.335*	1.000	
13	-0.202	-0.133	0.152	0.187	-0.019	-0.030	-0.028	0.009	-0.017	0.760****	0.279*	0.709****	1.000

\*Marked correlations are significant at  $p < 0.05$ . Correlation significance: \*0.265-0.349 – very low; \*\*0.350-0.499 – low; \*\*\*0.500-0.599 – restrained; \*\*\*\*0.600-0.899 – high; \*\*\*\*\*>0.900 – very high.

**Table 8.** Correlation matrices between growth parameters, pigments, and soluble proteins content for *Rosa* 'Hurdal' cuttings harvested from shoots with flowers opened (H2) and rooted in peat-perlite substrate.

Variable	Rooting degree	Weight of cuttings	Percentage of cuttings with retained stock plant leaf	Percentage of cuttings with young shoot	Total leaf area	Chlorophyll a	Chlorophyll b	Carotenoids	Soluble proteins	Chlorophyll a – before rooting	Chlorophyll b – before rooting	Carotenoids – before rooting	Proteins – before rooting
	1	2	3	4	5	6	7	8	9	10	11	12	13
2	0.113	1.000											
3	0.301*	0.201	1.000										
4	0.148	0.095	-0.072	1.000									
5	-0.074	0.351****	0.153	0.079	1.000								
6	0.206	0.052	0.119	0.054	0.259	1.000							
7	0.111	0.123	0.122	0.033	0.401**	0.918*****	1.000						
8	0.174	0.096	0.098	0.092	0.347*	0.901*****	0.892****	1.000					
9	0.048	0.197	0.372**	-0.130	0.221	0.063	0.119	0.053	1.000				
10	-0.156	0.056	-0.016	-0.185	-0.017	-0.273*	-0.294*	-0.279*	0.085	1.000			
11	-0.171	0.047	-0.027	-0.202	-0.034	-0.283*	-0.290*	-0.290*	0.071	0.986*****	1.000		
12	-0.165	0.055	-0.020	-0.192	-0.022	-0.279*	-0.293*	-0.285*	0.078	0.997*****	0.995*****	1.000	
13	-0.017	0.041	0.018	-0.084	0.022	-0.140	-0.215	-0.142	0.135	0.741****	0.628****	0.690****	1.000

\*Marked correlations are significant at  $p < 0.05$ . Correlation significance: \*0.265-0.349 – very low; \*\*0.350-0.499 – low; \*\*\*0.500-0.599 – restrained; \*\*\*\*0.600-0.899 – high; \*\*\*\*\*>0.900 – very high.

**Table 9.** Correlation matrices between growth parameters, pigments, and soluble proteins content for *Rosa* 'Hurdal' harvested from cuttings from shoots immediately after shedding petals (H3) and rooted in peat-perlite substrate.

Variable	Rooting degree	Weight of cuttings	Percentage of cuttings with retained stock plant leaf	Percentage of cuttings with young shoot	Total leaf area	Chlorophyll a	Chlorophyll b	Carotenoids	Soluble proteins	Chlorophyll a – before rooting	Chlorophyll b – before rooting	Carotenoids – before rooting	Proteins – before rooting
	1	2	3	4	5	6	7	8	9	10	11	12	13
2	0.229	1.000											
3	0.287*	0.965*****	1.000										
4	0.206	-0.084	-0.078	1.000									
5	-0.154	0.108	0.151	0.045	1.000								
6	0.176	0.043	0.098	0.021	0.269*	1.000							
7	0.151	0.020	0.066	-0.023	0.210	0.960*****	1.000						
8	0.184	0.035	0.078	0.046	0.206	0.952*****	0.966*****	1.000					
9	-0.108	-0.046	-0.081	-0.034	-0.138	-0.153	-0.202	-0.223	1.000				
10	-0.131	0.160	0.143	-0.132	-0.102	0.021	0.105	-0.002	-0.037	1.000			
11	-0.131	0.160	0.142	-0.132	-0.102	0.021	0.105	-0.003	-0.037	0.999*****	1.000		
12	-0.131	0.158	0.141	-0.133	-0.101	0.021	0.105	-0.004	-0.037	0.999*****	0.999*****	1.000	
13	-0.126	0.180	0.207	-0.046	-0.093	0.017	0.074	0.065	-0.040	0.612****	0.608****	0.599***	1.000

\*Marked correlations are significant at  $p < 0.05$ . Correlation significance: \*0.265-0.349 – very low; \*\*0.350-0.499 – low; \*\*\*0.500-0.599 – restrained; \*\*\*\*0.600-0.899 – high; \*\*\*\*\*>0.900 – very high.

**Table 10.** Correlation matrices between growth parameters, pigments, and soluble protein content for *Rosa* 'Hurdal' cuttings harvested from shoots with flower buds closed (H1) and rooted in peat-sand substrate.

Variable	Rooting degree	Weight of cuttings	Percentage of cuttings with retained stock plant leaf	Percentage of cuttings with young shoot	Total leaf area	Chlorophyll a	Chlorophyll b	Carotenoids	Soluble proteins	Chlorophyll a – before rooting	Chlorophyll b – before rooting	Carotenoids – before rooting	Proteins – before rooting
	1	2	3	4	5	6	7	8	9	10	11	12	13
2	0.554***	1.000											
3	0.037	0.067	1.000										
4	0.393**	0.021	0.087	1.000									
5	-0.030	0.201	-0.311*	-0.055	1.000								
6	-0.031	-0.025	0.008	0.133	-0.124	1.000							
7	0.068	-0.183	0.066	0.083	-0.107	0.532***	1.000						
8	0.075	-0.094	0.058	0.164	-0.147	0.888****	0.807****	1.000					
9	0.116	0.109	-0.028	0.197	-0.017	-0.004	-0.437**	-0.190	1.000				
10	-0.019	0.001	-0.082	0.131	0.161	0.110	0.042	0.064	0.064	1.000			
11	-0.008	0.109	0.043	0.066	0.023	0.207	0.044	0.164	0.040	0.431**	1.000		
12	-0.016	-0.013	-0.113	0.141	0.185	0.083	0.029	0.045	0.060	0.992****	0.334*	1.000	
13	-0.036	0.004	0.119	0.001	-0.048	0.143	0.114	0.068	0.067	0.760****	0.278*	0.709****	1.000

\*Marked correlations are significant at  $p < 0.05$ . Correlation significance: \*0.265–0.349 – very low; \*\*0.350–0.499 – low; \*\*\*0.500–0.599 – restrained; \*\*\*\*0.600–0.899 – high; \*\*\*\*\*>0.900 – very high.

**Table 11.** Correlation matrices between growth parameters, pigments, and soluble protein content for *Rosa* 'Hurdal' cuttings harvested from shoots with flowers opened (H2) and rooted in peat-sand substrate.

Variable	Rooting degree	Weight of cuttings	Percentage of cuttings with retained stock plant leaf	Percentage of cuttings with young shoot	Total leaf area	Chlorophyll a	Chlorophyll b	Carotenoids	Soluble proteins	Chlorophyll a – before rooting	Chlorophyll b – before rooting	Carotenoids – before rooting	Proteins – before rooting
	1	2	3	4	5	6	7	8	9	10	11	12	13
2	0.529***	1.000											
3	0.317*	0.198	1.000										
4	0.252	0.172	0.078	1.000									
5	0.184	0.243	0.248	0.002	1.000								
6	-0.077	-0.018	0.047	-0.075	-0.226	1.000							
7	0.091	0.155	0.256	0.006	-0.078	0.725****	1.000						
8	-0.075	-0.024	0.065	-0.067	-0.346**	0.903****	0.717****	1.000					
9	-0.327*	-0.006	-0.318*	-0.066	-0.131	-0.140	-0.288*	-0.125	1.000				
10	0.011	-0.145	-0.038	-0.022	-0.187	-0.062	-0.023	-0.060	-0.019	1.000			
11	0.010	-0.149	-0.038	-0.026	-0.199	-0.039	0.018	-0.033	-0.011	0.986****	1.000		
12	0.009	-0.146	-0.037	-0.025	-0.191	-0.052	-0.003	-0.047	-0.015	0.997****	0.995****	1.000	
13	0.027	-0.098	-0.035	0.017	-0.106	-0.142	-0.197	-0.159	-0.047	0.741****	0.628****	0.690****	1.000

\*Marked correlations are significant at  $p < 0.05$ . Correlation significance: \*0.265–0.349 – very low; \*\*0.350–0.499 – low; \*\*\*0.500–0.599 – restrained; \*\*\*\*0.600–0.899 – high; \*\*\*\*\*>0.900 – very high.

**Table 12.** Correlation matrices between growth parameters, pigments, and soluble protein content for *Rosa* 'Hurdal' cuttings harvested from shoots immediately after shedding petals (H3) and rooted in peat-sand substrate.

Variable	Rooting degree	Weight of cuttings	Percentage of cuttings with retained stock plant leaf	Percentage of cuttings with young shoot	Total leaf area	Chlorophyll a	Chlorophyll b	Carotenoids	Soluble proteins	Chlorophyll a – before rooting	Chlorophyll b – before rooting	Carotenoids – before rooting	Proteins – before rooting
	1	2	3	4	5	6	7	8	9	10	11	12	13
2	0.628****	1.000											
3	0.633****	0.929****	1.000										
4	0.223	0.135	0.066	1.000									
5	0.133	0.274*	0.232	0.011	1.000								
6	0.253	0.254	0.242	0.223	0.102	1.000							
7	0.266*	0.237	0.197	0.234	0.061	0.965****	1.000						
8	0.317*	0.268*	0.228	0.259	0.114	0.953****	0.960****	1.000					
9	-0.011	-0.093	-0.040	-0.144	-0.086	-0.159	-0.116	-0.121	1.000				
10	0.027	0.103	-0.006	0.002	-0.346**	0.015	0.091	0.113	-0.024	1.000			
11	0.028	0.103	-0.006	0.002	-0.346**	0.014	0.090	0.111	-0.024	0.999****	1.000		
12	0.030	0.104	-0.005	0.004	-0.346**	0.011	0.087	0.108	-0.024	0.999****	0.999****	1.000	
13	-0.113	-0.018	-0.056	-0.075	-0.204	0.224	0.271*	0.295*	-0.010	0.612****	0.608****	0.599***	1.000

\*Marked correlations are significant at  $p < 0.05$ . Correlation significance: \*0.265-0.349 – very low; \*\*0.350-0.499 – low; \*\*\*0.500-0.599 – restrained; \*\*\*\*0.600-0.899 – high; \*\*\*\*\*0.900 – very high.

#### 4. Discussion and conclusions

Rhizogenesis in cuttings is affected by endogenous and exogenous processes [1]. Additionally, seasonal changes in plant tissues connected with the decrease of chlorophyll content and increase in carotenoids are typical for plants in the temperate zone [11,12]. The rooting ability of cuttings, and also the action of rooting enhancers, changes accordingly to the phenological stage of the plants [40]. Within a few weeks, the flowering shoots of once-blooming roses change not only in phenological stage but also in terms of biologically active components [6] and anatomical structure [7]. These phenomena are important especially in the case of once-blooming roses, because it is the phenological stage of harvesting the cuttings, rather than the period, that should be properly defined [7,41]. For this reason, the period when the shoots, having reached their highest natural rooting ability, should be harvested for cuttings is very short – about 15 days [6,7]. While once-blooming roses flower only in June, the young shoots of rose cultivars with repeating flowering can be harvested a few times throughout the summer despite undergoing similar processes. Previous research showed that among the four phenological stages of flowering *Rosa* 'Hurdal' shoots, cuttings made during the last stage – 10-14 days after petal shedding – did not root [6,7,29,30]. In this experiment, none of the rooting enhancers changed this result, and neither did the use of two rooting substrates (Figure 3). In research on the 'Maiden's Blush' rose, the rooting enhancers, including plant origin preparations, did not show any unambiguous impact on rooting percentage in cuttings or affect the low natural rooting ability in shoots harvested for cuttings 7–14 days after shedding petals [31].

Leaves have been proven to have a key role in the rooting of the one-leaf rose stem cuttings of 'Madelon' (Tea Hybrid) [10,42]. According to previous research [6], the leaf could also play a pivotal role for the 'Hurdal' rose due to the length of the process of rhizogenesis. The limited reserves contained in a small-sized shoot and leaf are the deciding factor of the survival and growth of softwood and semi-hardwood cuttings in woody plants [43]. In this experiment, both the



increase of quality of the cuttings (the weight of cuttings and the percentage of rooted cuttings with retained stock plant leaf, new shoot and total leaf) and the increase of photosynthetically active pigments and soluble proteins were connected with an increase of rooting percentage independently of the rooting substrate (Tables 13-18).

The use of preparations containing indole butyric acid (IBA) or naphthalene acetic acid (NAA) is a standard procedure in rooting cuttings. The possibilities of applying biostimulants in rooting cuttings of various ornamental plants have been studied [44,30]. Among them, algae preparations may contain auxins or different concentrations of similar substances [17,44] that can stimulate rhizogenesis [26]. Biostimulants, and among them plant origin preparations, affected the content of chlorophyll a, b, carotenoids [31,45] and carbohydrates in roses [30] along with rooting percentage and quality of cuttings of roses [30,45] as well as other woody ornamentals [21-23]. It has also been proven that plant origin preparations positively affect the quality of cuttings, indirectly increasing their rooting percentage (Tables 13-18).

It was confirmed in this research that preparations had different effects on the 'Hurdal' rose, and these depended on the phenological stage. The control cuttings rooted at a higher percentage in peat-perlite than in peat-sand. However, the use of proper rooting enhancers, especially plant origin preparations, independently of rooting substrate increased the rooting ability and rooting percentage in the H1-H3 phenological stage cuttings (Figure 3) and improved their quality in terms of degree of rooting (Figure 4), weight of rooted cuttings (Figure 5), percentage of cuttings with retained stock plant leaf (Figure 6) and new shoots (Figure 7), and also the total leaf area (Figure 8). The increase of quality of cuttings after the use of plant origin preparations is in line with other research on old rose taxa stem cuttings [45]. Moreover, the rooting enhancers had an unambiguous influence on growth parameters (Figures 3-8). Especially NAA decreased rooting percentage and rooting degree in H3 rooted in peat-perlite (Figures 3 and 4), which can be connected with the tendency to callus overgrowth showed in research on *Rosa helena* 'Semiplena' [46] and was proved in research on *Rosa* species leaf cuttings [47].

The process of rooting old roses is usually long and fallible [4]. The 'Hurdal' cuttings rooted in 12 weeks, but the rooting degree suggested (Figure 4) that the process could take longer in nursery production. Prolonged adventitious root formation exposes cuttings to similar stresses as cut flowers [48,49]. One of the reasons for low rooting ability can be the decrease of chlorophyll a/b, carotenoids and soluble proteins content in leaves as dynamic natural seasonal processes of leaf senescence progress [6]. However, the decrease of pigment content during the process of rhizogenesis was lower at the time of evaluation of the rooted cuttings, and the content of soluble proteins was similar to that observed in stock plant leaves in August. This confirmed that the 'Hurdal' rose is susceptible to senescence processes and also to a natural seasonal decrease of these components earlier than other varieties included in surveys [6]. The rooting enhancers variously affected the increase of pigments and soluble proteins content in leaves (Figure 9-11).

This experiment on the 'Hurdal' rose proved, in line with previous research on once-blooming roses propagated by stem cuttings, that the effectiveness of the method can often be fallible [4] and should be verified and modified for each individual cultivar before introduction to practice [6,45].

Plant origin preparations have an unquestionably favorable impact on the rooting percentage of the 'Hurdal' rose, and they may successfully replace traditional rooting powders containing IBA or NAA in encouraging the rhizogenesis process in difficult-to-root roses. However, their effect did not involve increasing the content of photosynthetically active pigments or soluble proteins in leaves of rooted cuttings. Also, none of the preparations inhibited dead processes of cuttings prepared from shoots 7-14 days after petal fall. Plant origin preparations positively influenced the quality and quantity of cuttings, e.g. the rooting degree, weight of cuttings, percentage of cuttings with retained stock plant leaf and new shoots, and the leaf area. However, the action of plant preparations is dependent on the phenological stage of shoots cut for cuttings as well as on the substrate. Plant preparations of natural origin can be recommended in

ecological cultivation and plant nursery propagation of the 'Hurdal' rose (91/414/EEC, 2009/128/WE; OJEU), as also proved by previous research on the long and difficult-to-root Gallica rose 'Duchesse d'Angoulême' [45] and Alba 'Maiden's Blush' [31]. The action of biostimulants, including plant origin preparations, can most likely be attributed to the supplementation of unknown rooting cofactors and IAA-like substances present in biopreparations as well as other factors such as improved nutrition of cuttings [19].

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Suppl. 1. Correlation matrices between rooting percentage and growth parameters, pigments, and soluble protein content for *Rosa* ‘Hurdal’ cuttings prepared from shoots with flower buds closed (H1) rooted in peat-perlite substrate and treated with rooting enhancers.

Variable	Rooting percentage													
	Control <sup>1</sup>	IBA	NAA	RH 0.4x1	RH 0.6x1	RH 0.4x2	RH 0.6x2	RH 0.4x3	RH 0.6x3	RJ 0.1	RJ 0.2	RJ 0.4	BR 0.012	BR 0.02
Rooting degree	-0.816****	0.964****	0.900****	0.981****	0.966****	0.221	0.688****	0.507***	0.807****	0.876****	0.640****	0.640****	-0.816****	0.758****
Weight of cuttings	-0.213	0.932****	0.916****	-0.698****	0.064	-0.460**	0.032	-0.087	-0.303*	0.366**	0.708****	-0.655****	-0.963****	0.674****
Percentage of cuttings with retained stock plant leaf	nd	0.775****	nd	0.831****	-0.258	nd	0.668****	nd	0.001	-0.269*	0.518****	0.730****	nd	0.927****
Percentage of cuttings with young shoot	nd	0.258*	-0.098	0.878****	0.775****	0.515****	0.001	0.333	-0.471**	-0.741****	-0.18332	0.088	-0.174	0.228
Total leaf area	0.046	-0.658****	-0.124	-0.960****	-0.326*	-0.001	0.310**	0.117	-0.216	0.209	0.230	0.973****	-0.357**	-0.709****
Chlorophyll a	0.494**	0.368**	-0.226	-0.657****	0.961****	-0.858****	-0.570****	-0.704****	0.113	0.418**	-0.120	-0.094	-0.198	0.374**
Chlorophyll b	0.573***	0.720****	-0.348*	-0.699****	0.935****	-0.783****	-0.577****	-0.719****	0.122	0.377**	-0.124	-0.030	-0.207	0.374**
Carotenoids	0.570***	-0.217	-0.296*	-0.714****	0.911****	-0.865****	-0.565****	-0.636****	0.205	0.446**	-0.102	-0.102	-0.119	0.374**
Soluble proteins	-0.849****	-0.278*	0.510***	0.478**	0.810****	0.891****	0.557****	-0.817****	0.273*	-0.851****	-0.143	0.591***	-0.129	0.371**
Chlorophyll a before rooting	-0.850****	0.594***	0.435**	0.204	-0.957****	-0.791****	0.029	0.791****	0.297*	-0.851****	-0.209	0.190	0.514***	-0.019
Chlorophyll b before rooting	-0.841****	0.007	0.576***	-0.528****	-0.919****	-0.615****	-0.070	0.615****	0.266*	0.195	-0.229	0.789****	0.460**	-0.853****
Carotenoids before rooting	-0.853****	0.676****	0.493**	0.346*	-0.945****	-0.769****	-0.010	0.770****	0.286*	-0.918*	-0.217	0.129	0.495**	0.138
Proteins before rooting	-0.619****	-0.043	-0.222	-0.300*	-0.814****	-0.269****	0.397**	0.269*	0.324*	-0.619****	-0.070	-0.281*	0.561***	-0.257

<sup>1</sup>Control – the control cuttings; IBA – 0.4% IBA (Ukorzeniacz A<sub>aqua</sub>); NAA – 0.2% NAA (Ukorzeniacz B<sub>aqua</sub>); RH – Algae extract: watering with 0.4% or 0.6% Bio Rhizotonic (10 ml) 1, 2 or 3-time (after planting and 10 and 20 days later); RJ – Organic Preparation – watering with 0.1%, 0.2% or 0.4% Root Juice™ (10 ml) after planting; BR – Plant Extract – watering with 0.012% or 0.02% Bio Roots (10 ml) after planting.

\*Marked correlations are significant at p<0.05. Correlation significance: \*0.265–0.349 – very low; \*\*0.350-0.499 – low;\*\*\*0.500-0.599 – restrained; \*\*\*\*0.600-0.899 – high; \*\*\*\*\*>0.900 – very high.



Suppl. 2. Correlation matrices between rooting percentage and growth parameters, pigments, and soluble proteins content for *Rosa* ‘Hurdal’ cuttings prepared from shoots with flowers opened (H2) rooted in peat-perlite substrate and treated with rooting enhancers.

Variable	Rooting percentage													
	Control <sup>1</sup>	IBA	NAA	RH 0.4x1	RH 0.6x1	RH 0.4x2	RH 0.6x2	RH 0.4x3	RH 0.6x3	RJ 0.1	RJ 0.2	RJ 0.4	BR 0.012	BR 0.02
Rooting degree	0.539***	0.767***	0.957***	0.568***	0.914***	0.657***	0.207	0.590***	0.556***	0.379**	0.697***	0.110	0.676***	0.855***
Weight of cuttings	0.265*	0.858***	0.001	0.569***	-0.670***	0.131	0.120	-0.330*	0.379**	-0.057	-0.258	-0.220	0.982***	-0.819***
Percentage of cuttings with retained stock plant leaf	-0.647***	-0.011	0.912***	0.620***	0.859***	-0.794***	-0.516***	0.837***	-0.820***	-0.973***	0.280*	0.522***	0.870***	-0.555***
Percentage of cuttings with young shoot	nd	-0.522***	-0.986***	0.258	nd	0.707***	nd	-0.582***	0.651***	0.997***	0.556***	nd	0.301*	-0.778***
Total leaf area	-0.037	-0.495**	-0.861***	0.962***	-0.710***	0.091	0.853***	-0.786***	-0.935***	0.608***	0.313*	-0.327*	0.836***	-0.771***
Chlorophyll a	-0.267*	-0.843***	0.931***	0.366**	-0.851***	0.246	0.460**	0.738***	-0.184	-0.735***	0.314*	-0.132	-0.254	0.505***
Chlorophyll b	0.001	-0.806***	0.833***	0.501***	-0.929***	0.001	0.372**	0.853***	-0.526***	-0.786***	0.381*	0.001	0.090	0.473**
Carotenoids	0.688***	-0.761***	0.833***	0.478**	-0.943***	0.554***	0.399**	0.084***	-0.184	-0.745***	0.419**	0.878***	-0.498**	0.562***
Soluble proteins	0.470**	0.357**	0.166	-0.279*	0.357**	-0.288*	-0.210	-0.806***	-0.212	-0.224	-0.529***	-0.933***	0.890***	-0.627***
Chlorophyll a before rooting	-0.241	0.617***	-0.281*	-0.454**	0.921***	-0.570***	-0.150	-0.199	-0.216	-0.564***	-0.437**	0.270*	-0.287*	-0.437**
Chlorophyll b before rooting	-0.318*	0.735***	-0.400**	-0.569***	0.918***	-0.525***	-0.159	-0.031	-0.151	-0.424**	-0.306*	0.428**	-0.415**	-0.306*
Carotenoids before rooting	-0.293*	0.663***	-0.346**	-0.492**	0.931***	-0.570***	-0.176	-0.132	-0.210	-0.513***	-0.395**	0.339*	-0.354*	-0.395***
Proteins before rooting	0.349**	0.069	0.411**	-0.051	0.537***	-0.329**	0.210	-0.701***	-0.121	-0.814***	-0.627***	-0.403**	0.421**	-0.627***

<sup>1</sup>Control – the control cuttings; IBA – 0.4% IBA (Ukorzeniacz A<sub>aqua</sub>); NAA – 0.2% NAA (Ukorzeniacz B<sub>aqua</sub>); RH – Algae extract: watering with 0.4% or 0.6% Bio Rhizotonic (10 ml) 1, 2 or 3-time (after planting and 10 and 20 days later); RJ – Organic Preparation – watering with 0.1%, 0.2% or 0.4% Root Juice™ (10 ml) after planting; BR – Plant Extract – watering with 0.012% or 0.02% Bio Roots (10 ml) after planting.

\*Marked correlations are significant at p<0.05. Correlation significance: \*0.265–0.349 – very low; \*\*0.350–0.499 – low; \*\*\*0.500–0.599 – restrained; \*\*\*\*0.600–0.899 – high; \*\*\*\*\*>0.900 – very high.

Suppl. 3. Correlation matrices between rooting percentage and growth parameters, pigments, and soluble protein content for *Rosa* ‘Hurdal’ cuttings prepared from shoots immediately after flower petals shedding (H3) rooted in peat-perlite substrate and treated with rooting enhancers.

Variable	Rooting percentage													
	Control <sup>1</sup>	IBA	NAA	RH 0.4x1	RH 0.6x1	RH 0.4x2	RH 0.6x2	RH 0.4x3	RH 0.6x3	RJ 0.1	RJ 0.2	RJ 0.4	BR 0.012	BR 0.02
Rooting degree	0.680****	0.918*****	0.469**	0.381**	-0.417**	0.017	-0.594***	0.998*****	-0.471**	0.663****	0.259	-0.867****	0.723****	0.102
Weight of cuttings	-0.783****	0.778****	-0.172	-0.085	0.921*****	-0.119	0.068	0.623****	0.577***	0.904*****	0.621****	-0.572***	-0.491**	0.857****
Percentage of cuttings with retained stock plant leaf	-0.796****	0.778****	0.447**	-0.090	0.623****	-0.134	0.422**	0.623****	0.577***	0.912*****	0.428**	-0.575****	-0.483**	0.859****
Percentage of cuttings with young shoot	-0.716****	nd	nd	0.037	0.999*****	nd	-0.715****	nd	-0.537***	0.294*	-0.513**	0.643****	-0.721****	0.895****
Total leaf area	0.086	-0.690****	0.806****	0.647****	0.112	0.488**	0.208	0.409***	0.336*	-0.721****	-0.494**	0.333*	0.538***	0.860****
Chlorophyll a	-0.145	0.397**	0.923*****	0.483**	0.080	0.323*	0.585***	-0.444***	-0.316*	-0.100	-0.560***	0.785****	-0.026	-0.781****
Chlorophyll b	-0.273*	0.429**	0.926*****	0.428**	-0.087	0.488**	0.473***	-0.349*	0.194	-0.076	-0.170	0.849****	0.626****	-0.653****
Carotenoids	-0.662****	0.413**	0.943*****	-0.509***	-0.009	0.257	0.549***	-0.597***	-0.354*	0.048	-0.236	0.685****	0.994*****	-0.753****
Soluble proteins	-0.588***	0.389**	0.409**	0.921*****	0.413**	-0.191	0.830****	-0.510***	0.471**	0.484**	-0.861****	0.757****	-0.819****	0.815****
Chlorophyll a before rooting	0.226	0.444**	-0.261	-0.654****	-0.478**	0.341*	-0.819****	0.715****	0.654****	-0.560***	0.811****	0.915****	-0.967****	-0.697****
Chlorophyll b before rooting	0.229	0.444**	-0.265	-0.651****	-0.478**	0.340*	-0.818****	0.715****	0.651****	-0.561***	0.812****	0.916****	-0.968****	-0.699****
Carotenoids before rooting	0.239	0.446**	-0.276*	-0.643****	-0.480**	0.336*	-0.815****	0.717****	0.642****	-0.563***	0.819****	0.919****	-0.971****	-0.705****
Proteins before rooting	-0.481	0.184	0.556****	-0.937****	-0.198	0.489**	-0.688****	0.297*	0.937****	-0.233	0.079412	0.381**	-0.402**	-0.068

<sup>1</sup>Control – the control cuttings; IBA – 0.4% IBA (Ukorzeniacz A<sub>aqua</sub>); NAA – 0.2% NAA (Ukorzeniacz B<sub>aqua</sub>); RH – Algae extract: watering with 0.4% or 0.6% Bio Rhizotonic (10 ml) 1, 2 or 3-time (after planting and 10 and 20 days later); RJ – Organic Preparation – watering with 0.1%, 0.2% or 0.4% Root Juice™ (10 ml) after planting; BR – Plant Extract – watering with 0.012% or 0.02% Bio Roots (10 ml) after planting.

\*Marked correlations are significant at p<0.05. Correlation significance: \*0.265–0.349 – very low; \*\*0.350-0.499 – low; \*\*\*0.500-0.599 – restrained; \*\*\*\*0.600-0.899 – high; \*\*\*\*\*>0.900 – very high.

Suppl. 4. Correlation matrices between rooting percentage and growth parameters, pigments, and soluble protein content for *Rosa* ‘Hurdal’ cuttings prepared from shoots with flower buds closed (H1) rooted in peat-sand substrate and treated with rooting enhancers.

Variable	Rooting percentage													
	Control <sup>1</sup>	IBA	NAA	RH 0.4x1	RH 0.6x1	RH 0.4x2	RH 0.6x2	RH 0.4x3	RH 0.6x3	RJ 0.1	RJ 0.2	RJ 0.4	BR 0.012	BR 0.02
Rooting degree	0.541***	0.861****	0.999*****	0.792****	nd	-0.106	-0.07559	0.723****	-0.380**	0.791****	-0.102	-0.754****	0.648****	0.950****
Weight of cuttings	0.541***	0.701****	0.666****	0.128	nd	-0.284*	-0.605****	0.151	-0.474**	0.932****	-0.353**	0.723****	-0.775****	0.640****
Percentage of cuttings with retained stock plant leaf	nd	0.404**	-0.904****	nd	nd	0	-0.255	nd	nd	-0.333*	nd	0.817****	-0.904****	nd
Percentage of cuttings with young shoot	nd	0.566***	nd	0.839****	nd	-0.123	0.258	nd	-0.142	0.778****	0.683****	-0.344*	0.599***	0.401**
Total leaf area	-0.541***	-0.053	-0.631****	0.853****	nd	-0.229	0.152	0.991****	-0.605****	-0.20066	0.484**	-0.723****	0.300*	-0.145
Chlorophyll a	-0.638****	-0.34	-0.523***	-0.352****	nd	-0.401**	0.425**	-0.001	-0.744****	0.654****	-0.128	-0.916****	-0.837****	0.977****
Chlorophyll b	-0.295*	-0.409**	-0.593***	0.125236	nd	-0.319*	0.309**	-0.001	-0.723****	0.628****	-0.282*	-0.905****	-0.859****	0.916****
Carotenoids	-0.268*	-0.530****	-0.469**	0.828****	nd	-0.346*	0.378**	-0.001	-0.723****	0.506****	0.042	-0.897****	-0.838****	0.923****
Soluble proteins	0.868****	-0.530***	0.107	0.297****	nd	-0.568***	0.584***	-0.001	-0.577***	0.157135	-0.229	0.778****	-0.962****	-0.980****
Chlorophyll a before rooting	0.870****	0.803****	0.388**	0.388**	nd	-0.485**	0.957****	-0.001	-0.245	0.312*	0.435**	-0.455**	-0.050	-0.943****
Chlorophyll b before rooting	0.843****	0.794****	0.514***	0.668****	nd	-0.950****	0.919****	-0.937****	-0.389**	-0.460**	0.576***	-0.209	0.120	-0.423**
Carotenoids before rooting	0.863****	0.700****	0.440**	0.361**	nd	-0.384**	0.945****	0.141	-0.304*	0.334*	0.493**	-0.511***	0.018	-0.892****
Proteins before rooting	0.714****	0.732****	-0.198	-0.198	nd	-0.164	0.814****	0.051	0.363**	0.738****	-0.222	0.232	-0.677****	-0.917****

<sup>1</sup>Control – the control cuttings; IBA – 0.4% IBA (Ukorzeniacz A<sub>aqua</sub>); NAA – 0.2% NAA (Ukorzeniacz B<sub>aqua</sub>); RH – Algae extract: watering with 0.4% or 0.6% Bio Rhizotonic (10 ml) 1, 2 or 3-time (after planting and 10 and 20 days later); RJ – Organic Preparation – watering with 0.1%, 0.2% or 0.4% Root Juice™ (10 ml) after planting; BR – Plant Extract – watering with 0.012% or 0.02% Bio Roots (10 ml) after planting.

\*Marked correlations are significant at p<0.05. Correlation significance: \*0.265–0.349 – very low; \*\*0.350–0.499 – low; \*\*\*0.500–0.599 – restrained; \*\*\*\*0.600–0.899 – high; \*\*\*\*\*>0.900 – very high.

Suppl. 5. Correlation matrices between rooting percentage and growth parameters, pigments, and soluble protein content for *Rosa* ‘Hurdal’ cuttings prepared from shoots with flowers opened (H2) rooted in peat-sand substrate and treated with rooting enhancers.

Variable	Rooting percentage													
	Control <sup>1</sup>	IBA	NAA	RH 0.4x1	RH 0.6x1	RH 0.4x2	RH 0.6x2	RH 0.4x3	RH 0.6x3	RJ 0.1	RJ 0.2	RJ 0.4	BR 0.012	BR 0.02
Rooting degree	0.795****	-0.203	0.873****	0.307*	0.610****	0.993****	0.923****	-0.943****	0.830****	0.973****	0.991****	0.080	0.991****	0.979****
Weight of cuttings	0.302*	0.259*	0.187	0.083	0.208	0.173	0.726****	0.962****	0.640****	0.283*	0.971****	-0.447**	0.601****	0.820****
Percentage of cuttings with retained stock plant leaf	0.801****	0.394*	-0.109	-0.382**	0.775****	0.688****	0.585***	-0.269*	0.989****	-0.130	-0.873****	0	0.917****	nd
Percentage of cuttings with young shoot	0.778****	nd	nd	0.687****	0.381**	0.228	0.211	nd	0.837****	0.510***	0.439**	-0.163	0.774****	-0.831****
Total leaf area	0.182	0.450**	-0.118	0.104	0.916****	0.925****	0.972****	0.933****	-0.205	0.537***	-0.635****	-0.359**	-0.738****	-0.632****
Chlorophyll a	0.475**	0.279*	0.701****	0.285*	0.151	0.213	-0.155	0.943****	-0.786****	0.376**	-0.221	-0.155	0.345*	-0.400**
Chlorophyll b	0.544***	0.408**	0.621****	0.234	0.478**	0.226	-0.012	0.971****	-0.845****	-0.130	-0.249	0.128	-0.471**	0.316****
Carotenoids	0.097	0.254	0.538**	0.249	-0.068	0.116	-0.184	0.943****	-0.513***	-0.225	-0.134	0.369**	0.577***	0.075
Soluble proteins	-0.563****	-0.770****	0.740****	0.395**	0.602****	0.519***	0.441**	-0.172	0.679****	0.237	-0.188	0.376**	-0.818****	-0.632****
Chlorophyll a before rooting	0.437**	-0.790****	-0.670****	0.189	0.454**	0.736****	-0.468**	-0.817****	-0.564****	-0.237	-0.370**	0.159	0.104	0.454**
Chlorophyll b before rooting	0.306*	-0.848****	-0.547***	0.224	0.569***	0.705****	-0.566***	-0.708****	-0.424**	-0.096	-0.283*	0.300**	-0.064	0.569***
Carotenoids before rooting	0.395**	-0.806****	-0.628****	0.225	0.492**	0.740****	-0.496**	-0.774****	-0.513**	-0.190	-0.353**	0.207	0.035	0.492**
Proteins before rooting	0.627****	-0.464**	-0.817****	-0.268**	0.051	0.427**	-0.150	-0.959****	-0.814****	-0.515***	-0.342*	-0.247	0.654****	0.051

<sup>1</sup>Control – the control cuttings; IBA – 0.4% IBA (Ukorzeniacz A<sub>aqua</sub>); NAA – 0.2% NAA (Ukorzeniacz B<sub>aqua</sub>); RH – Algae extract: watering with 0.4% or 0.6% Bio Rhizotonic (10 ml) 1, 2 or 3-time (after planting and 10 and 20 days later); RJ – Organic Preparation – watering with 0.1%, 0.2% or 0.4% Root Juice™ (10 ml) after planting; BR – Plant Extract – watering with 0.012% or 0.02% Bio Roots (10 ml) after planting.

\*Marked correlations are significant at p<0.05. Correlation significance: \*0.265–0.349 – very low; \*\*0.350–0.499 – low; \*\*\*0.500–0.599 – restrained; \*\*\*\*0.600–0.899 – high; \*\*\*\*\*>0.900 – very high.

Suppl. 6. Correlation matrices between rooting percentage and growth parameters, pigments, and soluble protein content for cuttings prepared from shoots immediately after the shedding of flower petals (H3) of the *Rosa* ‘Hurdal’ rooted in peat-sand substrate and treated with rooting enhancers.

Variable	Rooting percentage													
	Control <sup>1</sup>	IBA	NAA	RH 0.4x1	RH 0.6x1	RH 0.4x2	RH 0.6x2	RH 0.4x3	RH 0.6x3	RJ 0.1	RJ 0.2	RJ 0.4	BR 0.012	BR 0.02
Rooting degree	0.762****	0.974*****	0.981*****	0.005	-0.205	0.966*****	0.954*****	0.903*****	0.592***	0.648*****	0.704*****	0.099	0.106	-0.151
Weight of cuttings	0.667****	0.910*****	0.907*****	0.718****	-0.132	0.967*****	-0.089	0.938*****	0.795****	0.168	-0.408**	0.925*****	0.540***	0.786****
Percentage of cuttings with retained stock plant leaf	0.668****	0.909*****	0.904*****	0.718****	-0.803****	0.968*****	-0.090	0.939*****	0.787****	0.706****	-0.410**	0.928*****	0.534***	0.788****
Percentage of cuttings with young shoot	0.904*****	0.922*****	0.636****	-0.814****	0.229	-0.097	-0.651****	0.973*****	0.577***	-0.258*	-0.236	-0.025	-0.373**	0.001
Total leaf area	0.708****	-0.713****	-0.587***	0.198	0.694****	-0.467**	0.068	0.438**	-0.484**	0.826****	0.071	0.728****	-0.159	-0.849****
Chlorophyll a	0.294*	-0.029	-0.527***	-0.919*****	0.184	0.206	-0.232	0.183	0.625****	0.851****	-0.298*	0.567***	0.184	0.114
Chlorophyll b	0.266*	0.404****	-0.613****	-0.946****	0.375**	0.191	-0.022	0.006	0.558****	-0.759****	-0.426**	0.975*****	0.119	0.143
Carotenoids	0.643****	0.495**	-0.576***	-0.956****	0.442****	0.188	-0.370**	0.086	0.596****	-0.431**	-0.742****	-0.978****	0.214	0.012
Soluble proteins	-0.685****	-0.728****	0.591***	-0.965****	0.677****	0.717****	0.594***	-0.790****	-0.196	0.316*	-0.141	-0.780****	-0.865****	-0.146
Chlorophyll a before rooting	0.204	0.950*****	0.614****	-0.405**	0.727****	-0.306*	-0.729****	-0.654****	0.792****	-0.810****	-0.416**	0.037	0.261	-0.261
Chlorophyll b before rooting	0.201	0.950*****	0.617****	-0.401**	0.728****	-0.303*	-0.731****	-0.651****	0.793****	-0.812****	-0.412**	0.041	0.265*	-0.265*
Carotenoids before rooting	0.191	0.951*****	0.624****	-0.390**	0.730****	-0.295*	-0.736****	-0.642****	0.796****	-0.819****	-0.401**	0.053	0.276*	-0.276*
Proteins before rooting	0.780****	0.516***	-0.091	-0.941****	0.302*	-0.712****	-0.142	-0.937****	0.330*	-0.079	-0.966****	-0.753****	-0.556***	0.556***

<sup>1</sup>Control – the control cuttings; IBA – 0.4% IBA (Ukorzeniacz A<sub>aqua</sub>); NAA – 0.2% NAA (Ukorzeniacz B<sub>aqua</sub>); RH – Algae extract: watering with 0.4% or 0.6% Bio Rhizotonic (10 ml) 1, 2 or 3-time (after planting and 10 and 20 days later); RJ – Organic Preparation – watering with 0.1%, 0.2% or 0.4% Root Juice™ (10 ml) after planting; BR – Plant Extract – watering with 0.012% or 0.02% Bio Roots (10 ml) after planting.

\*Marked correlations are significant at p<0.05. Correlation significance: \*0.265–0.349 – very low; \*\*0.350–0.499 – low; \*\*\*0.500–0.599 – restrained; \*\*\*\*0.600–0.899 – high; \*\*\*\*\*>0.900 – very high.

