Enhancing lodging resistance in two *Vicia* species: unveiling the morphological and stem anatomy transformations induced by Moddus

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Abstract

To assess the potential for enhancing lodging resistance in legumes through the application of plant growth regulators (PGR) and changes in stem structure, the stem morphological and anatomical characteristics, as well as the chemical composition, of *Vicia sativa* and *Vicia pannonica* were analysed before and after treatment with different doses of PGR trinexapac-ethyl. The aim was to identify stem morpho-anatomical components that impact lodging resistance, quantify the dose-dependent effect of the chosen PGR on the *Vicia* stem, and examine if stem lodging resistance

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could be improved through PGR application, as well as determine if some of the stem characteristics could be used as markers for lodging resistance prediction.

Although in both species lodging index increased (14−126%), suggesting improved resistance to lodging, and stem height decreased (12−38%) upon PGR application, the impact on *V. sativa* was more pronounced. The findings indicate that, apart from stem height, none of the examined morpho-anatomical characteristics showed a high and significant correlation with the lodging index. Therefore, none of these characteristics can be used as a marker for predicting lodging resistance. Increased proportion of cortex, cylinder parenchyma and collenchyma, along with reduced central cavity, might contribute to a greater lodging resistance in *V. sativa*. Plant growth regulator decreased the amount of lignin, cellulose and hemicellulose. These results encourage the use of PGR for lodging resistance improvement in vetches, through the reduction of stem height, since this modification did not adversely affect the stem structure or grain yield.

Keywords: forage, plant growth regulator, stem tissues, stem morphology, vetch

Introduction

Lodging is defined as persistent deviation of plant shoots from their vertical orientation due to different external (environmental) factors or internal plant characteristics (Ball *et al.*, 2006; Bourmaud *et al.*, 2015; Gomez *et al.*, 2017). It is dependent on the crop genotype, density and developmental stage, since these variables affect the stem mechanical and structural characteristics (Hall *et al.*, 2010). Lodging negatively affects yield, grain yield and quality, and harvest management and leads to economic losses, especially when it occurs during the grain-filling period (Kelbert *et al.*, 2004; Ball *et al.*, 2006; Kong *et al.*, 2013; Marcelo *et al.*, 2017). Therefore, many

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research programs are directed towards an improvement of lodging resistance and identifying plant characteristics that correlate with lodging and could be good indicators of lodging tolerance in early screening.

Stem morpho-anatomy, strength and weight distribution along the shoot exert a pivotal influence on lodging resistance (Hall *et al.*, 2010; Bourmaud *et al.*, 2015; Zhang *et al.*, 2016; Mangieri *et al.*, 2016; Gui *et al.*, 2018). Stem height is positively correlated with increased lodging in many plant species, and height reduction is thus the main target of their breeding programs (Zhang *et al.*, 2016; Gomez *et al.*, 2017, Gui *et al.*, 2018). Lodging occurs in the basal stem region, which makes lodging susceptibility of the basal stem internodes especially important. Stem strength is determined by its morphology, anatomy and chemical composition. (Banniza *et al.*, 2005; Bourmaud *et al.*, 2015). Shorter and thicker basal internodes, as well as greater stem thickness measured without the central cavity, lead to higher lodging resistance (Kelbert *et al.*, 2004; Beeck *et al.*, 2006; Mangieri *et al.*, 2016; Zhang *et al.*, 2016; Muhammad *et al.*, 2020). In red fescue, an increase in internode length was found by Szczepanek *et al.* (2021) to increase lodging, which decreased with an increase in the lower internode diameter. Conversely, Gomez *et al.* (2017) noted that internode strength was negatively correlated with diameter in sorghum, prompting them to posit that increased stem diameter reduces the stresses that are distributed across the stem. In wheat genotypes, stem thickness without central cavity, rather than total stem diameter, was found to be negatively correlated with lodging (Kelbert *et al.*, 2004; Kong *et al.*, 2013). Yet, Singh and Srivastava (2015) failed to establish significant correlation between field pea stem diameter and lodging, whereas according to Beeck *et al.* (2006) compressed stem thickness proved to be a better predictor of stem strength, compared to stem diameter.

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The cell wall chemical composition, and the proportions of cellulose, hemicellulose and lignin in particular, determines the stem mechanical properties to a significant extent (Mengistie and McDonald, 2023). In legume stems, lignified tissues (e.g. xylem vessels, phloem and xylem fibres, sclerenchymatous parenchyma) contribute to the stem strength, since lignin provides strength to the secondary thickened cell walls (Engels and Jung, 2005; Jung and Lamb, 2006; Zoric *et al.*, 2014). Epidermal and collenchyma cells have thick, but not lignified primary cell walls, and therefore contribute less to the overall stem stiffness (Jung and Engels, 2002). However, a positive effect of higher lignin content on stem lodging resistance in wheat is not always observed (Banniza *et al.*, 2005; Ball *et al,* 2006; Kong *et al.*, 2013; Muhammad *et al.*, 2020).

Considering stem anatomy, enhanced lodging resistance is particularly associated with stems characterized by the presence of peripherally situated sclerenchyma tissue and vascular bundles, especially in grasses (Kaack *et al.*, 2003; Kelbert *et al.*, 2004; Kong *et al.*, 2013; Zhang *et al.*, 2016; Marcelo *et al.,* 2017; Gui *et al.,* 2018; Muhammad *et al.*, 2020). Anatomical trait-associated factors influencing stem lodging in cereals are cell wall thickness, number and diameter of vascular bundles, and sclerenchyma thickness (Mengistie and McDonald, 2023). Moreover, stalk strength in cereal crops is closely associated with stalk geometry, structure, and cell wall composition.

Sclerenchyma fibre bundles, their proportion, number, diameter and distribution of individual fibres, reinforce flax stem and contribute to lodging resistance (Bourmaud *et al.*, 2015). A higher proportion of secondary xylem and sclerenchyma has also been reported to hinder sunflower stem lodging (Mangieri *et al.*, 2016). Banniza *et al.* (2005) revealed a negative correlation between lodging and the proportion of supportive tissues, xylem, lignin, cellulose and fibre content in field pea stems. Ball *et al.* (2006) posited that increased lodging associated with

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greater xylem proportion of lentil stems was probably due to increased stem brittleness. On the other hand, proportion of the pith cavity was not correlated with lodging. Despite the thin-walled nature of parenchyma cells, this tissue plays a crucial role in stabilizing the stem by facilitating energy transfer during bending, thus reducing the risk of lodging and stem breakdown (Kong *et al.,* 2013). As parenchyma takes up most of the pea stem, variation in its proportion, number and size of the cells, rather than the total lignin content, may explain the differences in stem strength among pea genotypes (Beeck *et al.*, 2006).

Selection of semi-dwarf plant varieties and use of plant growth regulators (PGRs), most of which are growth retardants, can minimize the effects of lodging on crop production (Miziniak *et al.*, 2017). Moddus is very popular PGR characterized by rapid foliar absorption. It is mostly used on grass grain crops, with trinexapac-ethyl (TE) being its main component (Anderson *et al.*, 2011; Subedi *et al.*, 2021). Trinexapac-ethyl belongs to the cyclohexanone group, which stimulates the substrate of dioxygenases and inhibits biosynthesis of gibberellins and cell elongation. In the absence of gibberellins, the growth of internodes is retarded (Subedi *et al.* 2021). The effectiveness of growth retardants depends on several factors, weather conditions and precipitation in particular (Harasim *et al.*, 2016; Miziniak *et al.*, 2017). In grasses, Moddus shortens internodes, which results in lodging reduction without significant change in yield performances, like in barley (Miziniak *et al.,* 2017) and wheat (Harasim *et al.*, 2016). Again, internode diameters varied between years, but radial stem thickness, without central cavity, significantly increased at the 4th internode. These experiments also demonstrated that plant susceptibility to lodging is mostly dependent on the meteorological conditions and the retardant dose. More recently, Szczepanek *et al.* (2021) reported 3rd internode shortening and decreased lodging following the TE application on red fescue. However, TE is not commonly used in legume species. Strydhorst *et al.* (2019) applied different

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PGRs to improve field pea standability. Plant growth regulator treatment had a negligible impact on standability, as plant height was reduced by 8-13% at only 66% of the sites/years, while the effects on seed yield, weight and protein content were inconsistent. Based on these responses, the authors could not recommend the use of growth regulators for the purpose of pea plant height reduction. They further noted that PGRs should be applied with caution, to avoid any potential negative impacts on the plants, as their effects can vary depending on the plant species, growing conditions, as well as application rates and dosage.

Vicia sativa L. and *Vicia pannonica* Crantz. are annual forage species that are widely used as grain crops or as a green forage (Naydenova & Aleksieva, 2014). As they are also affected by the stem lodging problem, in the present study, comparative and correlative analysis of stem morpho-anatomical characteristics and chemical composition of these two *Vicia* species was performed, before and after the treatment with different concentrations of the exogenous growth regulator TE. The hypotheses were that some of the vetch stem anatomical and morphological characteristics could contribute to lodging resistance and, therefore, could be used as markers for lodging resistance prediction; that higher lodging resistance could be expected from plants having higher proportions of lignin, ADF, NDF and tissues composed of thick-walled cells, as well as that the application of PGR (TE) could reduce lodging by reducing stem growth or affecting its anatomy and morphology. Therefore, the aim of this research was to (a) improve the understanding of the mechanisms of lodging in vetches by identifying stem anatomical and morphological components of lodging resistance; (b) determine and quantify the effect of different doses of Moddus growth retardant on *Vicia* stem morphology, anatomy and chemical composition, and their relationship to lodging resistance; (c) examine if stem mechanical properties and lodging resistance could be improved by the application of TE, without negatively affecting biomass and

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grain yield; and (d) establish if some of the anatomical or morphological characteristics could be used as markers for lodging resistance prediction.

Materials and methods

The plant species used in the current investigation, the widely cultivated forage crops *Vicia sativa* (common vetch) and *Vicia pannonica* (Hungarian vetch), were grown in 2020 and 2021 at the experimental field of the Institute of Field and Vegetable Crops at Rimski Šančevi, Northern Serbia (45°20′ N, 19°51′ E, 84 m a.s.l.). The trial was established as a randomized block design, with three replications. The plot size was 10 m², and the seed sowing rate was 120 kg/ha, in accordance with the local practice. Sowing dates were 10 October 2020 and 15 October 2021. The following mechanical operations were conducted: stubble cultivation, ploughing to a depth of 25 cm, harrowing, seedbed preparation, sowing, and rolling after sowing. Sowing was carried out with an inter-row spacing of 12.5 cm and at a depth of 4 to 5 cm with grain drill. No inoculation was performed. Sampling dates were 25 May 2020 and 21 May 2021. The soil was classified as slightly carbonated loamic chernozem, specifically Calcic Gleyic Chernozem (CH-cc.gl-lo), according to the WRB (2014). It had a pH value of 7.2 in KCl, with 2.2 % of organic matter, 6.3% of $CaCO_3$, 0.2% of nitrogen, and contained 22.7 mg / 100 g of K_2O and P_2O_5. Bulk density was 1.35 g/cm^3. No fertilizers were used.

There were six treatments with different TE concentrations, along with an untreated control. Plots C1, C2 and C3 were treated once with the following amounts of TE (trade name Moddus, Syngenta, 250g/l of the active ingredient TE): 1.6 l/ha, 2.4 l/ha and 3.2 l/ha, respectively. Plots C4, C5 and C6 received two treatments, with the second treatment applied two weeks after

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the first. The first treatment involved the same TE amounts as the single-treatment plots: 1.6 l/ha, 2.4 l/ha and 3.2 l/ha, respectively. The second treatment applied 1.6 l/ha TE to all three plots.

The BBCH scale (Biologische Bundesanstalt, Bundessortenamt, and CHemical industry scale) is a standardized coding system that describes the developmental stages of plants. It was developed to provide a universal framework for identifying and recording growth stages in various plant species, which is crucial for agricultural practices, research, and crop management. The first application was carried out at BBCH 3. According to the UPOV (International Union for the Protection of New Varieties of Plants) Guidelines (International Union for the Protection of New Varieties of Plants, 2013), the plants were at the principal growth stage 3: Stem elongation, stage 30 - beginning of stem elongation. The second application took place two weeks later, when the internodes were visibly extended (principal growth stage 3: Stem elongation, stage 39 - nine or more visibly extended internodes).

Lodging index was determined as a decrease in canopy height (LI = canopy height/plant height), with greater values signifying higher lodging resistance (Mikić *et al.*, 2015; Luo *et al.*, 2022).

For morphological analysis, ten randomly selected plants were cut at the full flowering stage, coinciding with the formation of the first visible pods (second half of May). For anatomical analyses, five plants from each group were cut in full bloom and were fixed in 50% ethanol (Ruzin, 1999). For stereological investigations, cross-sections were made along the stem axis, according to the methodology developed by Kubinova (1991), as explained in Zoric *et al.* (2014). Stem cross-sections were cut in positions sampled according to the principle of systematic uniform random sampling, along the stem axis, resulting in five segments per stem. The segments were numbered incrementally, starting from the top (1st) to the bottom (5th) part of the stem. All sections were

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obtained at −20°C using a cryostat Leica CM 1850 (Leica, Germany), at 40 μm cutting intervals. Light microscopy observations and measurements were performed using the image analysing system Motic Images Plus (Motic, Germany). The proportion of tissues was estimated by point-counting method, using the point grid test system of 391 test points. Approximately 1500–2000 points per stem were counted. Volume densities of tissues (Vv, in %) were calculated using the equation given by Kubinova (1993):

$$Vv(x) = \frac{\sum_{j=1}^{n} Pj(x)}{\sum_{j=1}^{n} Pj(y)}$$

where *n* is the number of examined sections, *Pj*(x) (j = 1, ..., n) – denoted the number of test points hitting the tissue (epidermis, collenchyma, cortex parenchyma, sclerenchyma, phloem, xylem, sclerenchymatous parenchyma, cylinder parenchyma and central cavity) within *j*-number of sections and *Pj*(y) (j = 1, ... , n) – represents the number of test points hitting the entire stem cross-section area within *j*-number of sections.

Collected samples were also subjected to chemical analyses to determine the neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) content. These analyses were performed using an Ankom 2000 Fibre Analyser (Ankom Technology Corp., NY, USA) in the Laboratory for Soil and Agroecology at the Institute of Field and Vegetable Crops in Novi Sad.

Data were statistically processed, and the means, standard errors, coefficients of variation and correlation coefficients were calculated using STATISTICA software (TIBCO Software Inc. 2020). The significance of differences in the measured characteristics between treatment and

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control plants was determined using t-test ($P \leq 0.050$). In graphs shown in Figure 1, 3, and 4, only data for stem characteristics with significant differences compared to controls were presented.

Results

The values obtained for the analysed morphological and anatomical traits of the *V. sativa* and *V. pannonica* before and after the treatment are given in Tables 1-4. It is evident that, in the second experimental year, control plants of both *Vicia* species formed more intensively branched stems, with a significantly greater number of pods and seeds. Stem height and cross-section area did not differ significantly between the years for the *V.* sativa control plants, whereas *V. pannonica* stems were significantly taller in the second year and their thickness was significantly less in the third segment ($P< 0.05$). Significant phenotypic variation in the stem-related traits was recorded before, as well as after the treatment. Plant growth regulator significantly reduced the stem height of both *Vicia* species in all applied concentrations (Figure 1, Tables 1 and 2). Under the effect of PGR, stem and stem cross-section area without central cavity in the middle and bottom part was significantly reduced in *V. sativa*, especially in the second experimental year, while these changes were not observed in *V. pannonica* ($P< 0.05$). Plant growth regulator application positively affected branching in the first, but not in the second experimental year. When applied in C4 concentration, it had positive effect on the number of pods and seeds and seed weight in *V. sativa*. However, in *V. pannonica*, PGR stimulated formation of pods and seeds in the first experimental year, especially when applied in higher concentrations, while having the opposite effect in the second year.

As shown in Figure 2, the stem of both *Vicia* species is rounded in cross-section, winged and has primary anatomical structure. Epidermis is present at the surface, whereas primary cortex

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is composed of cortex parenchyma and peripherally positioned collenchyma. Central cylinder contains vascular bundles arranged in a circle, with sclerenchyma groups adjacent to the phloem and sclerenchymatous parenchyma located at the inner side of the xylem and between the bundles. Cylinder parenchyma cells separate to form a cavity in the central part of the stem. All Vv values exhibited relatively high variability, as indicated by the coefficients of variation reported in Tables 3 and 4.

After PGR application in different doses, rather inconsistent changes in the Vv of stem tissues were recorded, especially in *V. sativa* (Figure 3a). Thus, only significant differences in the Vv of stem tissues between control and treated plants, which were confirmed in both experimental years, were considered as important. In *V. sativa*, in the first year, the Vv of cortex parenchyma significantly increased under C3 and C4 treatments, while in the second year all treatments, except C4, yielded this result ($P< 0.05$). The Vv of cylinder parenchyma increased under C2−C5 treatments in the first year and under C2 in the second. At the same time, the xylem proportion decreased significantly under treatments C3 and C4 in the first year, and under C5 treatment in the second year ($P< 0.05$). Proportions of collenchyma were significantly greater, and those of central cavity significantly less in most of the treatments, especially when higher PGR concentrations were applied, but only in the first experimental year ($P< 0.05$). Other recorded changes in Vv were variable and inconsistent in both years and were therefore not deemed significant. In general, the strongest effect was observed with the PGR application in C3 concentration.

Plant growth regulator application induced fewer significant changes in the Vv of stem tissues of *V. pannonica* (Figure 3b). Proportion of sclerenchyma was significantly reduced in the second experimental year, and that of phloem significantly increased in the first experimental year, compared to control ($P< 0.05$).

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Stem segments closer to the ground are most responsible for lodging, since that is the point at which the stem bends. Accordingly, the effect of PGR on their structure was examined in more detail (Figure 4, Tables S1-S6). In *V. sativa* PGR application induced a decrease in the Vv of sclerenchyma and increase in the Vv of cortex parenchyma in some doses on segment 4, and only in the second year on segment 5, compared to control plants. The C3, C5 and C6 concentrations were most effective. However, in most of the cases, the results were not consistent in both study years, so the changes in tissue proportions could not be assigned to the PGR effect only. In *V. pannonica* the changes in tissue proportions along the stem maturity gradient were not confirmed in both study years. In bottom segments, decreased proportion of sclerenchyma was recorded, under C1−C3 treatments, but only in the second year.

In both examined species lodging index increased with the application of PGR, which pointed to the higher lodging resistance of treated plants (Figure 5). In *V. sativa*, the index values were the highest after PGR application in medium concentrations (C2, C5 and C6 in the first, and C2, C4 and C6 in the second year). In *V. pannonica*, the higher concentrations (C4−C6) were most effective in both years. Lodging index was significantly negatively correlated with stem height (r_{VS} = *-0.77,* r_{VP} = *-0.71*) and positively correlated with the number of branches (r_{VS} = *0.31,* r_{VP} = *0.16*) and seed weight (r_{VS} = *0.46,* r_{VP} = *0.20*) in both species. In *V. sativa*, lodging index was positively correlated with the number of pods (r = *0.42*) and seeds (r = *0.51*), whereas in *V pannonica* these correlations were negative and low. Unexpectedly, correlations of lodging index with stem cross-section area and stem cross-section area without central cavity were low, and were even negative in *V. sativa* (r_{VS} = *-0.14, -0.14*; r_{VP} = *0.16, 0.18*).

Considering anatomical characteristics, it seems that increased lodging resistance did not arise from the changes in stem tissue proportions, as even the correlations which appeared

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statistically significant were low (Figure 6 and 7). These results indicate that the origin of increased lodging resistance may lie among the changes in characteristics that are not of anatomical nature.

As the highest NDF, ADF and ADL values were obtained for the control plants of both species, despite increasing the lodging resistance, Moddus application did not increase lignification of treated plants (Table 5). The obtained results point to lower lignin, cellulose and hemicellulose proportions in the PGR-treated *V. sativa* plants. The corresponding values were rather similar across all of the *V. pannonica* treatments and control plants. The percentage of ash increased in both species with the application of PGR. Increased protein and N content were recorded in *V. sativa* during the first year, and in *V. pannonica* during the second year only, compared to control plants.

Discussion

Understanding the mechanisms of lodging and stem strength improvement should be one of the major strategies in the future vetch breeding programs aimed at achieving lodging resistance. Since breeding for lodging resistance takes many years, finding alternative ways to enhance lodging resistance in a short time is challenging. One of the methods tested in this work was an application of the growth retardant Moddus on vetches, as this PGR gave good results in cereal crops and grasses (Harasim *et al.*, 2016; Miziniak *et al.*, 2017; Szczepanek *et al.*, 2021). The results varied between the two experimental years, which could be attributed to the environmental influences, as lodging susceptibility may change depending on the year and weather (Ball *et al.*, 2006). Moreover, growth retardant effectiveness is highly dependent on the weather conditions (Harasim *et al.*, 2016; Miziniak *et al.*, 2017). Under conditions that promote high vegetative development in *Vicia*, plants become highly susceptible to lodging. Yield loss in lodged crops occurs due to poor

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grain filling and pod loss. Lodged plants are also more susceptible to stem rotting and diseases, which can affect both forage and seed quality. Lodging at the later stages of crop development can compromise grain yield and quality, which is crucial for seed production. Souza et al. (2022) found similar results, noting that TE reduced plant height and increased productivity in legumes. The application of TE has been studied extensively in small grains, with some research also conducted on beans, wheat and rapeseed (de Faria et al., 2022; Gomes et al. 2018; Ijaz et al., 2015). In this context, our research represents pioneering work on the application of TE in vetches.

In both studied species, the lodging index increased upon the application of PGR, indicating an enhanced lodging resistance in the treated plants (Figure 5). In *V. sativa*, Moddus doses C2, C5 and C6 were the most effective in the first year, and C2, C4 and C6 in the second year, whereas in *V. pannonica* higher doses (C4−C6) were the most effective in both years. It was important to discover the changes in which of the examined morpho-anatomical characteristics explained this increased lodging resistance.

In both examined species, in almost all applied doses, PGR significantly reduced stem height, which correlated with a decline in lodging. In *V. sativa*, a reduction of the stem height from 11.8% to 36.9% (23.7% on average) was achieved, whereas 12.1% to 40.1% (30.4% on average) decrease was noted in *V. pannonica*, depending on the applied dose (Figure 1). These values were greater than those recorded by Strydhorst *et al.* (2019) for field pea (8−13%), Miziniak *et al.* (2017) for barley (5.6%−16.5%) and Harasim *et al.* (2016), but in all these studies lower TE doses were used. The same was true for lentil stems, where according to Ball *et al.* (2006) plant height appeared to be the most influential on lodging, whereas stem diameter had an opposite effect. In extant literature stem diameter and stem thickness without central cavity are much more frequently recognized as reliable characteristics for the prediction of lodging resistance, emphasizing their

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positive correlation (Beeck *et al.*, 2006; Muhammad *et al.*, 2020). Surprisingly, in the current study, the stem cross-section area and stem cross-section area without central cavity of *V. sativa* were significantly reduced following PGR application, and correlated negatively with lodging resistance (Figure 1). Plant growth regulator did not affect the stem cross-section area with or without central cavity, of *V. pannonica.* The increased lodging resistance could not be attributed to increased stem thickness, but to reduced stem height, only.

In findings reported by Ball *et al.* (2006) for lentil stems, greater aboveground biomass was found to induce greater lodging. While these authors reported that the number of branches of lentil stems positively correlated with lodging, we recorded the opposite for the *Vicia* species. Positive correlation of lodging index with the number of branches and seed weight in *Vicia* species might be the result of proper weight distribution along the shoot, which stabilizes the stem. A lower sowing rate in vetch provides each plant more space, promoting stronger branching. In contrast, a higher sowing rate can reduce branching due to increased competition for resources. Although denser sowing may limit branching, its impact on lodging depends on factors like plant architecture, environmental conditions, and specific management practices. In addition, strong, upright stems enable more efficient transport of water, nutrients and assimilates, which consequently leads to a higher grain yield. This assertion is supported by Singh and Srivastava's (2015) finding that decreased seed yield and weight loss in pea plants are correlated with lodging. It is important to note that, in *V. sativa*, Moddus had a positive effect on the seed weight and the number of seeds and pods when applied in C4 dose, and that increase correlated positively with the lodging index (Figure 1). In *V. pannonica*, the effects were contradictory, since the PGR stimulated the formation of pods and seeds in the first experimental year, but inhibited it in the second year, especially when applied in higher doses, and the correlations with lodging index were

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negative and low. We thus concur with the conclusion reached by Strydhorst *et al.* (2019) that plant growth regulators do not work well on the stability improvement of field pea, since the obtained results were inconsistent and differed slightly from those related to the control plants. These observations led Strydhorst and colleagues to assert that plant growth regulators should not be used in field pea production.

The results obtained in the present study for the effect of PGR on the anatomical characteristics were highly variable both within and across the two study years. As no significant anatomical changes in *V. pannonica* with control plants were recorded in both experimental years, it is not plausible to attribute these outcomes solely to the application of Moddus, which exerted a more pronounced influence on *V. sativa* (Figure 3).

Greater stem stability was expected to be positively correlated with the amount of strength-determining tissues, such as lignified sclerenchyma, xylem or sclerenchymatous parenchyma (Engels and Jung, 2005; Jung and Lamb, 2006). However, all correlations between lodging index and volume densities of stem tissues were very low, including those few that were statistically significant (Figure 6 and 7). The obtained results did not validate the proposed hypothesis.

The key characteristics of most lodging-resistant stems are peripherally located, well-developed mechanical and vascular tissue and a greater number of vascular bundles (Kaack *et al.*, 2003; Zhang *et al.*, 2016). Sclerenchyma fibre bundles, their number, distribution and diameter of individual fibres were found by Bourmaud *et al.* (2015) to positively affect the lodging resistance of flax stems. According to Mangieri *et al.* (2016), well-developed secondary xylem and sclerenchyma decrease lodging of sunflower stems. Likewise, rice stems with better developed mechanical tissue, and higher lignin content, stem thickness without central cavity and proportion of vascular bundles are more resistant to lodging (Zhang *et al.*, 2016; Gui *et al.*, 2018). Lodging-

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tolerant wheat genotypes examined by Kelbert *et al.* (2004) had thicker culms, measured without central cavity, greater number of vascular bundles and thicker sclerenchyma. This trend was not obvious in the vetch species in the present study. However, the proportion of subepidermal collenchyma significantly increased in *V. sativa* treated with Moddus, but only in the first study year (Figure 3). The number of vascular bundles was not affected and the proportion of xylem with sclerenchymatic parenchyma even decreased significantly under the C3 and C4 doses in the first year and under C5 in the second year. Therefore, increased lodging resistance, which was evident after PGR application, could not be related to the proportions of mechanical or vascular tissue. The hypothesis about possible positive effect of the presence of tissues composed of thick-walled cells on lodging resistance was not supported.

A thick parenchyma layer increases lodging resistance, since it contributes to the stem stabilization and increases its thickness (Kaack *et al.*, 2003). Parenchyma can absorb the energy imparted by external forces without mechanical damage to the plant. Moddus induced an increase in the Vv of cortex parenchyma (at C3 and C4 doses in the first year, and under all treatments except C4 in the second year) and cylinder parenchyma (under C2−C5 in the first year and C2 in the second year) in *V. sativa* (Figure 3). These results are in line with those reported by Hall *et al.* (2010) who found that the epidermis and cortex thickness in sunflower stems contributed to higher resistance to failure. The size of the central cavity of hollow stems does matter, since compressed stem thickness was found by Beeck at al. (2006) to be more strongly related to stem strength than total stem diameter in field pea. In rice, a smaller inner stem diameter was shown by Zhang *et al.* (2016) to be helpful in enhancing mechanical strength. However, in the present study, PGR induced a significant decrease in the central cavity size in *V. sativa* only in the first experimental year (Figure 3). Synergistic effect of an increased proportion of cortex and cylinder parenchyma

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and collenchyma, together with a reduced central cavity, might thus be a path to follow towards an increased lodging resistance in this species. While these results were not expected, increased lodging resistance without increased lignifications means that forage nutritive value was maintained in treated plants.

No significant correlations were found between lodging and anatomical traits. However, traits such as proportions of cortex and cylinder parenchyma, collenchyma and central cavity might still be useful in the selection of parent plants for breeding and crosses. The same lack of general association of stem morpho-anatomical characteristics, other than stem height, with lodging was previously reported for wheat genotypes (Kelbert *et al.*, 2004).

Plant growth regulator, in all applied concentrations, decreased the amount of lignin, cellulose and hemicellulose in *V. sativa*, but had a lesser effect on these components in *V. pannonica* (Table 5). It is evident that, although the extent of lodging decreased after PGR application, this effect was not due to the additional stem lignification and our hypothesis was not supported. According to the meta-analysis conducted by Mengistie and McDonald (2023), lignin content of the cereal stalks was positively correlated with lodging resistance in 63.9% of the reviewed studies, cellulose content in 53.9%, and hemicellulose indicated no significant correlation with lodging in 52.9% of the cases. On the other hand, Muhammad *et al.* (2020) found that lignin content was not associated with the wheat stem breaking force. In lentils, Ball *et al.* (2006) noted that lodging increased with increased fibre content (NDF and ADF), whereas in field pea, Banizza *et al.* (2005) established a negative correlation between fibre, lignin and cellulose content and lodging. Such contradictory data concerning lignin effect on lodging might suggest that the distribution of lignified tissues in the stem is more important for stem strength than total lignin or cellulose content. Actually, lignin may induce two opposite effects: higher amounts of

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lignin might give the stem additional strength and prevent lodging, or it might contribute to excessive weight of the aboveground part which would increase lodging.

We believe that two years of field experiments are sufficient to obtain valid results under real field conditions. However, one limiting factor is the variability in weather conditions, which can affect the timing of TE applications and the lodging index. Despite these challenges, our study simulates production conditions better than controlled laboratory environments. We conducted this study to evaluate the impact of TE on winter vetches, aiming to assess the feasibility and risks of using TE to mitigate lodging. Future research should focus on determining the effects of varying rates and application timings of TE on nodulation, stem structure, including the distribution of lignified tissues, plant height, grain yield, and quality, as well as sowing density, and testing different varieties to ensure stable *Vicia* seed production.

Lodging resistance could be enhanced by selecting vetch species or genotypes with shorter stems. Application of PGR could contribute to the formation of such a phenotype without adversely affecting stem structure or grain yield. In the present study, lodging resistance enhancement observed in the PGR-treated plants did not arise due to stem structural changes, which were minimal and inconsistent, but was mostly attributable to the stem growth reduction. None of the examined morphological or anatomical characteristics, except stem height, could be used as a marker for lodging resistance prediction. Nonetheless, the obtained results encourage the use of Moddus growth regulator for the study of lodging resistance improvement in vetch species.

Conclusion

In both *Vicia* species, the lodging index increased following the application of Moddus, suggesting improved resistance to lodging in the treated plants. The correlations between lodging index and

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volume densities of stem tissues were very low and mostly insignificant. None of the investigated morpho-anatomical features, apart from stem height, proved viable for predicting lodging resistance. These findings suggest that employing Moddus to enhance lodging resistance in vetches by lowering stem height is a promising strategy, as it does not seem to adversely affect the stem structure or grain yield.

Supplementary material. The supplementary material for this article can be found at DOI

Author contributions. LZ, ĐK and JL designed the study. LZ, DK and DŽ conducted morphological and anatomical measurements. DŽ and ĐK conducted chemical analyses. All authors discussed the results. LZ and DŽ wrote the article.

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Ethical standards. Not applicable

References

Anderson NP, Chastain TG, Garbacik CJ and Silberstein TB (2011) Effect of foliar applications of trinexapac-ethyl plant growth regulator on red clover seed crops. In: Young

https://doi.org/10.1017/S0021859624000704 Published online by Cambridge University Press

WC (ed). *Seed Production Research at Oregon State University USDA-ARS Cooperating*, pp. 10-12. Oregon State University, USA.

Ball RA, Hanlan TG and Vandenberg A (2006) Stem and canopy attributes that affect lodging resistance in lentil. *Canadian Journal of Plant Science* **86**, 71-81.

Banniza S, Hashemi P, Warkentin TD, Vandenberg A and Davis AR (2005) The relationships among lodging, stem anatomy, degree of lignification and resistance to mycosphaerella blight in field pea (*Pisum sativum*). *Canadian Journal of Botany* **83,** 954-967.

Beeck CP, Wroth J and Cowling WA (2006) Genetic variation in stem strength in field pea (*Pisum sativum* L.) and its association with compressed stem thickness. *Australian Journal of Agricultural Research* **57,** 193-199.

Bourmaud A, Gibaud M, Lefeuver A, Morvan C and Baley C (2015) Influence of the morphology characters of the stem on the lodging resistance of Marylin flax. *Industrial Crops and Products* **66**, 27-37.

de Faria LP, Silva SR and Lollato RP (2022) Nitrogen and trinexapac-ethyl effects on wheat grain yield, lodging and seed physiological quality in southern Brazil. *Experimental Agriculture* **58**, e21.

Engels FM and Jung HJG (2005) Alfalfa stem tissues: impact of lignification and cell length on ruminal degradation of large particles. *Animal Feed Science and Techology* **120**, 309– 321.

Gomes GR, Freiria GH, Barbosa AP and Takahashi LSA (2018) Glyphosate, trinexapac-ethyl, and mepiquat chloride on the productive performance of bush snap beans. *Semina: Ciências Agrárias*, **39,** 2313-2324.

https://doi.org/10.1017/S0021859624000704 Published online by Cambridge University Press

Gomez FE, Muliana AH, Niklas KJ and Rooney WL (2017) Identifying morphological and mechanical traits associated with stem lodging in bioenergy sorghum (*Sorghum bicolor*). *BioEnergy Research* **10**, 635-647.

Gui MY, Wang D, Xiao HH, Tu M, Li FL, Li WC, Ji SD, Wang TX and Li JY (2018) Studies of the relationship between rice stem composition and lodging resistance. *The Journal of Agricultural Science* **156**, 387-395.

Hall AJ, Sposaro MM and Chimenti CA (2010) Stem lodging in sunflower: variations in stem failure moment of force and structure across crop population densities and post-anthesis developmental stages in two genotypes of contrasting susceptibility to lodging. *Field Crops Research* **116**, 46-51.

Harasim E, Wesolowski M, Kwiatkowski C, Rozylo K and Maziarz P (2016) Effect of retardants on the biometric characteristics of winter wheat (*Triticum aestivum* L.) stem. *Romanian Agricultural Research* **33**, 205-215.

Ijaz M, Mahmood K and Honermeier B (2015) Interactive role of fungicides and plant growth regulator (Trinexapac) on seed yield and oil quality of winter rapeseed. *Agronomy* **5**, 435-446.

International Union for the Protection of New Varieties of Plants (2013) Common vetch, Guidelines for the conduct of tests for distinctness, uniformity and stability. International Union for the Protection of New Varieties of Plants, Geneva. Available online from: https://www.upov.int/edocs/tgdocs/en/tg032.pdf (Accessed 29 October 2024)

Jung HG and Engels FM (2002) Alfalfa stem tissues: cell wall deposition, composition and degradability. *Crop Science* **42**, 524–534.

Jung HG and Lamb JFS (2006) Stem morphological and cell wall traits associated with divergent in vitro neutral detergent fibre digestibility in alfalfa clones. *Crop Science* **46**, 2054– 2061.

https://doi.org/10.1017/S0021859624000704 Published online by Cambridge University Press

Kaack K, Schwarz KU and Brander PE (2003) Variation in morphology, anatomy and chemistry of stem of *Miscanthus* genotypes differing in mechanical properties. *Industrial Crops and products* **17**, 131-142.

Kelbert AJ, Spaner D, Briggs KG and King JR (2004) The association of culm anatomy with lodging susceptibility in modern spring wheat genotypes. *Euphytica* **136**, 211-221.

Kong E, Liu D, Guo X, Yang W, Sun J, Li X, Zhan K, Cui D, Lin J and Zhang A (2013) Anatomical and chemical characteristics associated with lodging resistance in wheat. *The Crop Journal* **1**, 43-49.

Kubinova L (1991) Stomata and mesophyll characteristics of barley leaf as affected by light: stereological analysis. *Journal of Experimental Botany* **42**, 995–1001.

Kubinova L (1993) Recent stereological methods for the measurement of leaf anatomical characteristics: estimation of volume density, volume and surface area. *Journal of Experimental Botany* **44**, 165–173.

Luo X, Wu Z, Fu L, Dan Z, Yuan Z, Liang T, Zhu R, Hu Z and Wu X (2022) Evaluation of lodging resistance in rice based on an optimized parameter from lodging index. *Crop Science* **62,** 1318-1332.

Mangieri MA, Mantese AI and Schurmann AA (2016) Effects of ethephon on anatomical changes in sunflower (*Helianthus annuus* L.) stems associated with lodging. *Crop & Pasture Science* **67**, 988-999.

Marcelo PNM, Tapic RT and Manangkil OE (2017) Relationship of culm anatomy and lodging resistance in rice (*Oryza sativa* L.) genotypes under direct-seeded system. *International Journal of Agricultural Technology* **13**, 2367-2385.

https://doi.org/10.1017/S0021859624000704 Published online by Cambridge University Press

Mengistie E and McDonald AG (2023) Effect of cell wall compositions on lodging resistance of cereal crops: review. *The Journal of Agricultural Science* **161**, 794–807.

Mikić A, Ćupina B, Rubiales D, Mihailović V, Šarūnaitė L, Fustec J, Antanasović S, Krstić Đ, Bedoussac L, Zorić L, Đorđević V, Perić V and Srebrić M. (2015) Models, developments, and perspectives of mutual legume intercropping. *Advances in Agronomy* **130:** 337–419

Miziniak W, Matysiak K and Kaczmarek S (2017) Studies on trinexapac-ethyl dose reduction by combined application with adjuvants in spring barley. *Journal of Plant Protection Research* **57**, 36-42.

Muhammad A, Hao H, Xue Y, Alam A, Bai S, Hu W, Sajid M, Hu Z, Samad RA, Li Z, Liu P, Gong Z and Wang L (2020) Survey of wheat straw stem characteristics for enhanced resistance to lodging. *Cellulose* **27**, 2469-2484.

Naydenova GK and Aleksieva AK (2014) Phenotypic performance and genotypic variance for reproductive characteristics in Hungarian vetch (*Vicia pannonica* Crantz). *Romanian Agricultural Research* **31**, 69-74.

Ruzin ES (1999) Plant Microtechnique and Microscopy. Oxford University Press, London.

Singh AK and Srivastava CP (2015) Effect of plant types on grain yield and lodging resistance in pea (*Pisum sativum* L.). *Indian Journal of Genetics and Plant Breeding* **75**, 69-74.

Souza IL, da SILVA TRB and Albrecht LP (2022) Management of trinexapac-ethyl application in soybean. *Nativa* **10,** 16-21.

Strydhorst SM, Yang RC, Gill KS and Bowness R (2019): Inter-row stubble seeding and plant growth regulators to improve field pea standability and production. *Canadian Journal of Plant Sciences* **99**, 184-198.

https://doi.org/10.1017/S0021859624000704 Published online by Cambridge University Press

Subedi M, Karimi R, Wang Z, Graf RJ, Mohr RM, O'Donovan JT, Brandt S and Beres BL (2021) Winter cereal responses to dose and application timing of trinexapac-ethyl. *Crop Science* **61**, 2722-2732.

Szczepanek M, Baczynski M and Graczyk R (2021) Effects of plant growth regulators and N rate on the morphological traits of generative tillers and seed yields of chewings and strong creeping red fescue. *Agronomy* **11**, 65.

TIBCO Software Inc. (2020) Data Science Workbench, version 14. http://tibco.com.

WRB (2014) World reference base for soil resources. World Soil Resources Reports No. 106. FAO, Rome.

Zhang W, Wu L, Wu X, Ding Y, Li G, Li J, Weng F, Lu Z, Tang S, Ding C and Wang S (2016) Lodging resistance of japonica rice (*Oryza sativa* L.): morphological and anatomical traits due to top-dressing nitrogen application rates. *Rice* **9**, 31-41.

Zoric L, Mikic A, Cupina B, Lukovic J, Krstic Dj and Antanasovic S (2014) Digestibility-related histological attributes of vegetative organs of barrel medic (*Medicago truncatula* Gaertn.) cultivars. *Zemdirbyste-Agriculture* **101**, 257-264.

https://doi.org/10.1017/S0021859624000704 Published online by Cambridge University Press

Table 1. Stem morphological parameters of *V. sativa* control plants and changes following PGR application (mean ± standard error, coefficient of variation, D.F. = 48)

	Stem height (cm)	Number of branches	Number of pods per plant	Number of seeds per plant	Seed weight per plant (g)	Stem c-s area (seg. 3) (mm^2)	Stem c-s area (seg. 4) (mm^2)	Stem c-s area (seg. 5) (mm^2)	Stem c-s area without cavity (seg. 3) (mm^2)	Stem c-s area without cavity (seg. 4) (mm^2)	Stem c-s area without cavity (seg. 5) (mm^2)
Control	163.0 ± 4.42 (8.6)	1.1 ± 0.23 (67.1)	10.6 ± 1.17 (34.8)	59.4 ± 7.72 (41.1)	3.3 ± 0.43 (41.1)	13.5 ± 0.71 (11.7)	11.1 ± 0.78 (15.6)	9.1 ± 0.65 (15.8)	9.4 ± 0.29 (6.9)	8.2 ± 0.56 (15.4)	7.4 ± 0.50 (15.1)
C1[1]	140.4 ± 9.88 (22.3)	2.0 ± 0.26 (40.8)*	14.6 ± 2.57 (55.6)	55.0 ± 10.02 (57.6)	2.7 ± 0.47 (55.0)	10.4 ± 0.61 (13.1)*	9.8 ± 0.67 (15.2)	8.1 ± 0.71 (19.6)	7.6 ± 0.39 (11.5)*	7.2 ± 0.53 (16.5)	6.3 ± 0.51 (18.1)
C2	113.5 ± 5.04 (14.0)*	1.5 ± 0.27 (56.7)	10.6 ± 1.32 (39.3)	47.4 ± 6.57 (43.9)	2.3 ± 0.36 (50.0)	8.9 ± 1.08 (27.3)*	10.8 ± 1.43 (29.5)	8.7 ± 0.92 (23.6)	8.0 ± 0.95 (26.5)	8.0 ± 1.01 (28.0)	6.9 ± 0.59 (19.1)
C3	151.9 ± 7.49 (15.6)	2.2 ± 0.33 (46.9)*	14.6 ± 2.56 (55.4)	49.3 ± 9.99 (64.1)	2.4 ± 0.49 (65.6)	8.5 ± 0.84 (22.1)*	8.2 ± 1.02 (27.7)	6.7 ± 0.83 (27.8)*	6.5 ± 0.49 (17.1)*	6.3 ± 0.76 (26.8)	5.6 ± 0.57 (23.0)*
C4	143.8 ±5.76 (12.7)*	1.7 ±0.33 (62.3)	16.8 ± 2.19 (41.3)*	89.7 ± 8.93 (31.5)*	4.8 ± 0.50 (32.7)*	10.3 ± 1.09 (23.7)*	9.1 ± 1.22 (29.8)	7.4 ± 1.01 (30.5)	8.1 ± 0.87 (24.0)	7.0 ± 0.99 (31.4)	6.2 ± 0.89 (31.9)
C5	102.8 ± 7.10 (21.8)*	1.5 ± 0.40 (84.6)	10.2 ± 2.52 (78.1)	51.5 ± 10.81 (66.4)	2.9 ± 0.66 (70.7)	12.3 ± 1.56 (28.5)	11.5 ± 1.33 (25.9)	9.5 ± 0.80 (18.8)	9.5 ± 1.12 (26.3)	8.7 ± 0.97 (25.0)	7.8 ± 0.77 (22.2)
C6	138.7 ± 7.30 (16.6)*	1.9 ± 0.38 (63.0)	16.0 ± 2.23 (44.0)*	75.1 ± 12.79 (53.9)	4.4 ± 0.80 (57.1)	10.2 ± 1.06 (23.3)*	9.1 ± 0.61 (15.0)	7.3 ± 0.90 (27.4)	7.8 ± 0.67 (19.2)	6.8 ± 0.44 (14.3)	6.0 ± 0.59 (22.1)
Control-2	172.6 ± 5.55 (10.1)	3.9 ± 0.28 (22.5)	20.7 ± 2.53 (38.7)	119.6 ± 16.56 (43.8)	4.9 ± 0.73 (47.2)	18.9 ± 2.44 (28.9)	14.4 ± 1.59 (24.6)	10.8 ± 1.06 (22.1)	12.8 ± 1.57 (27.4)	10.3 ± 1.09 (23.6)	9.2 ± 1.04 (25.4)
C1-2	134.4 ± 7.00 (16.5)*	3.3 ± 0.26 (24.9)	28.7 ± 2.04 (22.5)*	166.2 ± 14.28 (27.2)*	7.7 ± 0.65 (26.4)*	9.2 ± 1.32 (32.2)*	8.7 ± 0.77 (19.8)*	6.4 ± 0.75 (26.2)*	6.8 ± 0.98 (32.1)*	6.3 ± 0.53 (19.0)*	5.1 ± 0.41 (18.1)*
C2-2	139.1 ± 6.35 (14.4)*	2.9 ± 0.23 (25.4)*	18.6 ± 2.12 (36.1)	125.0 ± 12.82 (32.4)	4.7 ± 0.52 (35.0)	8.5 ± 1.04 (27.6)*	8.6 ± 0.48 (12.5)*	6.7 ± 0.25 (8.2)*	6.5 ± 0.83 (28.4)*	6.1 ± 0.39 (14.3)*	5.4 ± 0.22 (9.0)*
C3-2	128.1 ± 4.22 (10.4)*	3.4 ± 0.27 (24.8)	26.0 ± 3.34 (40.6)	154.2 ± 28.08 (57.6)	6.1 ± 0.96 (50.0)	8.5 ± 1.18 (31.3)*	7.3 ± 0.97 (29.6)*	5.7 ± 0.75 (29.5)*	6.1 ± 0.64 (23.2)*	5.4 ± 0.60 (24.9)*	4.8 ± 0.69 (32.3)*
C4-2	123.3 ± 3.33 (8.5)*	3.0 ± 0.33 (35.1)	28.1 ± 1.91 (21.5)*	191.0 ± 20.16 (33.4)*	8.6 ± 0.96 (35.5)*	10.9 ± 1.14 (23.3)*	10.5 ± 1.35 (28.7)	8.0 ± 0.86 (24.0)	7.4 ± 0.80 (24.0)*	7.1 ± 1.01 (31.7)	5.7 ± 0.49 (19.3)*
C5-2	152.2 ± 5.90 (12.3)*	3.6 ± 0.22 (19.4)	22.4 ± 2.05 (28.9)	121.1 ± 9.92 (25.9)	4.4 ± 0.72 (51.8)	9.2 ± 0.69 (16.7)*	8.4 ± 0.75 (19.9)*	6.5 ± 0.64 (21.9)*	6.3 ± 0.44 (15.9)*	5.7 ± 0.37 (14.5)*	4.9 ± 0.42 (19.4)*

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C6-2	111.0 ± 4.33 (12.3)*	3.3 ± 0.21 (20.5)	25.8 ± 1.60 (19.7)	140.3 ± 10.69 (24.1)	5.7 ± 0.53 (29.3)	10.9 ± 1.27 (26.1)*	11.5 ± 1.36 (26.3)	8.8 ± 0.54 (13.8)	7.7 ± 0.80 (23.3)	7.9 ± 0.81 (22.8)	7.1 ± 0.42 (13.1)

* - indicates that differences within one species were significant between the years, according to t-test, $P < 0.05$

[1]- C1-C6, first year and C1-2-C6-2, second year. Plots C1, C2 and C3 treated once with TE: 1.6 l/ha, 2.4 l/ha and 3.2 l/ha, respectively. Plots C4, C5 and C6 treated twice - the first treatment the same as previous, the second 1.6 l/ha TE to all three plots.

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Table 2. Stem morphological parameters of *V. pannonica* control plants and changes following PGR application (mean ± standard error, coefficient of variation, D.F. = 48)

	Stem height (cm)	Number of branches	Number of pods per plant	Number of seeds per plant	Seed weight per plant (g)	Stem c-s area (seg. 3) (mm^2)	Stem c-s area (seg. 4) (mm^2)	Stem c-s area (seg. 5) (mm^2)	Stem c-s area without cavity (seg. 3) (mm^2)	Stem c-s area without cavity (seg. 4) (mm^2)	Stem c-s area without cavity (seg. 5) (mm^2)
Control	94.7 ± 2.18 (7.3)	1.0 ± 0.00 (0.0)	8.2 ± 0.53 (20.6)	26.6 ± 1.73 (20.6)	1.2 ± 0.09 (25.1)	9.2 ± 0.49 (11.69)	7.9 ± 0.46 (13.0)	6.4 ± 0.78 (27.3)	9.0 ± 0.41 (10.1)	7.6 ± 0.42 (12.3)	6.0 ± 0.71 (26.3)
C1[1]	83.2 ± 0.48 (9.4)*	1.6 ± 0.16 (32.3)*	8.4 ± 0.92 (34.7)	27.1 ± 2.78 (32.5)	1.3 ± 0.14 (34.6)	8.1 ± 0.72 (19.9)	7.2 ± 0.64 (19.8)	6.4 ± 0.50 (17.3)	7.9 ± 0.71 (20.0)	6.8 ± 0.67 (22.1)	6.2 ± 0.56 (20.2)
C2	71.5 ± 2.14 (9.5)*	2.4 ± 0.27 (35.1)*	17.6 ± 2.97 (53.4)*	37.0 ± 8.65 (73.9)	2.1 ± 0.47 (72.1)	8.9 ± 0.52 (13.2)	7.8 ± 0.79 (22.8)	5.4 ± 0.34 (14.8)	8.6 ± 0.53 (13.8)	7.3 ± 0.78 (23.8)	5.1 ± 0.34 (14.9)
C3	72.0 ± 2.21 (9.7)*	1.8 ± 0.33 (57.4)*	12.1 ± 2.06 (53.8)	37.3 ± 6.66 (56.4)	1.7 ± 0.28 (52.6)	8.2 ± 0.73 (19.8)	8.6 ± 0.84 (21.9)	6.1 ± 0.55 (20.2)	7.9 ± 0.76 (21.5)	8.2 ± 0.83 (22.8)	5.7 ± 0.54 (21.0)
C4	64.6 ± 2.71 (13.3)*	3.0 ± 0.58 (60.9)*	23.7 ± 7.82 (104.4)	84.6 ± 27.44 (102.6)*	3.8 ± 1.20 (99.6)*	10.3 ± 0.84 (18.2)	9.8 ± 0.87 (19.8)	8.7 ± 0.94 (24.3)	9.9 ± 0.80 (18.0)	9.4 ± 0.83 (19.7)	8.2 ± 0.90 (24.6)
C5	60.4 ± 2.91 (14.4)*	4.1 ± 0.99 (72.40)*	23.2 ± 4.62 (59.7)*	106.7 ± 24.99 (70.3)*	3.7 ± 0.83 (66.8)*	8.5 ± 0.45 (11.8)	8.1 ± 0.45 (12.5)	7.1 ± 0.43 (13.5)	8.3 ± 0.43 (11.7)	7.8 ± 0.53 (15.2)	6.8 ± 0.47 (15.6)
C6	60.0 ± 7.30 (29.8)*	10.1 ± 1.25 (30.1)*	33.7 ± 8.82 (64.1)*	83.0 ± 20.64 (60.9) *	2.8 ± 0.73 (63.6)*	9.4 ± 0.78 (18.5)	8.7 ± 0.63 (16.2)	7.0 ± 0.41 (12.9)	9.3 ± 0.80 (19.1)	8.4 ± 0.62 (16.7)	6.9 ± 0.41 (13.4)
Control -2	124.6 ± 2.60 (6.6)	5.4 ± 0.37 (21.7)	55.4 ± 3.06 (17.5)	254.7 ± 16.36 (20.3)	8.8 ± 0.56 (20.1)	6.9 ± 0.44 (14.5)	6.6 ± 0.69 (23.1)	5.0 ± 0.41 (18.1)	6.0 ± 0.41 (15.3)	5.8 ± 0.43 (16.5)	4.6 ± 0.26 (12.4)
C1-2	82.0 ± 2.36 (9.1)*	6.4 ± 0.60 (29.6)	57.3 ± 6.27 (34.6)	209.6 ± 25.66 (38.7)	6.5 ± 1.01 (49.5)	6.2 ± 0.68 (24.7)	5.3 ± 0.56 (23.4)	4.5 ± 0.66 (32.6)	5.6 ± 0.74 (29.3)	4.8 ± 0.45 (21.2)	4.0 ± 0.49 (27.4)
C2-2	74.6 ± 3.03 (12.8)*	5.0 ± 0.26 (16.3)	53.5 ± 1.24 (7.3)	189.4 ± 4.35 (7.3)*	7.2 ± 0.29 (12.6)*	6.8 ± 0.56 (18.3)	6.2 ± 0.51 (18.3)	6.1 ± 0.45 (16.8)	6.0 ± 0.27 (10.1)	5.2 ± 0.26 (11.2)	5.2 ± 0.33 (14.2)
C3-2	90.0 ± 2.85 (10.0)*	5.3 ± 0.26 (15.5)	70.4 ± 2.11 (9.5)*	302.9 ± 15.86 (16.6)*	9.4 ± 0.59 (20.1)	6.9 ± 0.85 (27.5)	6.8 ± 0.55 (18.2)	5.7 ± 0.48 (18.9)	5.9 ± 0.57 (21.5)	6.0 ± 0.44 (16.4)	4.9 ± 0.41 (18.7)
C4-2	81.6 ± 2.68 (10.4)*	4.6 ± 0.31 (21.0)	45.5 ± 6.95 (48.3)	157.1 ± 26.90 (54.1)*	6.1 ± 0.89 (46.2)*	5.0 ± 0.20 (8.9)*	5.3 ± 0.35 (14.7)	5.4 ± 0.88 (36.5)	4.5 ± 0.20 (9.7)*	4.8 ± 0.27 (12.6)	4.7 ± 0.57 (26.9)
C5-2	82.7 ± 2.34 (8.9)*	4.4 ± 0.16 (11.7)*	39.5 ± 3.05 (24.4)*	142.4 ± 9.78 (21.7)*	4.1 ± 0.34 (26.3)*	6.8 ± 0.87 (28.6)	6.5 ± 0.86 (29.4)	5.6 ± 0.71 (28.3)	6.0 ± 0.64 (24.0)	5.7 ± 0.68 (26.7)	4.9 ± 0.62 (28.7)
C6-2	77.2 ± 3.64 (14.9)*	3.6 ± 0.27 (23.4)*	38.0 ± 2.64 (22.0)*	145.3 ± 13.60 (29.6)*	4.4 ± 0.37 (26.8)*	6.8 ± 0.77 (25.4)	6.1 ± 0.61 (22.1)	4.6 ± 0.37 (18.0)	5.6 ± 0.61 (26.3)	5.1 ± 0.38 (16.7)	4.2 ± 0.33 (17.7)

* - indicates that differences within one species were significant between the years, according to t-test, $P < 0.05$

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[1]- C1-C6, first year and C1-2-C6-2, second year. Plots C1, C2 and C3 treated once with TE: 1.6 l/ha, 2.4 l/ha and 3.2 l/ha, respectively. Plots C4, C5 and C6 treated twice - the first treatment the same as previous, the second 1.6 l/ha TE to all three plots.

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Table 3. Vv of stem tissues and the number of vascular bundles of *V. sativa* control plants and changes following PGR application (mean ± standard error, coefficient of variation, D.F. = 48)

	Vv[1] epidermis	Vv collenchyma	Vv cortex parenchyma	Vv sclerenchyma	Vv phloem	Vv xylem+sclpar	Vv cyl parenchyma	Vv central cavity	Number of vasc bundles
Control	3.3 ± 0.20 (29.8)	1.9 ± 0.11 (29.5)	19.8 ± 1.12 (28.1)	4.1 ± 0.16 (19.4)	10.0 ± 0.37 (18.8)	17.9 ± 0.91 (25.6)	18.1 ± 1.50 (41.4)	24.9 ± 1.69 (33.9)	18.9 ± 0.40 (10.5)
C1[2]	3.6 ± 0.22 (30.5)	2.6 ± 0.21 (41.5)*	19.9 ± 0.78 (19.5)	4.0 ± 0.22 (27.2)	10.8 ± 0.42 (19.3)	15.4 ± 0.87 (28.1)	21.6 ± 1.40 (32.5)	22.2 ± 1.24 (27.9)	18.2 ± 0.43 (11.8)
C2	3.9 ± 0.18 (22.5)*	2.2 ± 0.21 (48.4)	21.8 ± 0.61 (14.1)	3.8 ± 0.18 (23.7)	10.6 ± 0.48 (22.6)	16.7 ± 1.05 (31.3)	25.1 ± 2.32 (46.2)*	15.9 ± 1.62 (51.1)*	18.1 ± 0.41 (11.1)
C3	4.1 ± 0.32 (38.4) *	2.6 ± 0.24 (45.9)*	23.7 ± 0.84 (17.8)*	3.7 ± 0.20 (27.7)	11.5 ± 0.35 (15.1)*	13.8 ± 0.88 (32.1) *	23.5 ± 2.10 (44.6)*	17.1 ± 1.50 (43.8)*	17.0 ± 0.52 (15.3)*
C4	3.6 ± 0.22 (31.1)	2.5 ± 0.18 (35.7)*	22.8 ± 0.92 (20.1)*	4.0 ± 0.15 (18.3)	11.1 ± 0.56 (25.0)	15.1 ± 1.03 (34.2)*	24.1 ± 2.12 (44.0)*	16.8 ± 1.62 (48.3)*	18.3 ± 0.45 (12.4)
C5	3.7 ± 0.25 (32.8)	2.6 ± 0.19 (37.3)*	19.9 ± 0.75 (18.9)	4.5 ± 0.23 (26.1)	10.8 ± 0.35 (16.0)	16.3 ± 0.84 (25.8)	25.2 ± 2.07 (41.1)*	17.0 ± 1.75 (51.4)*	18.0 ± 0.45 (12.5)
C6	3.3 ± 0.24 (35.9)	3.0 ± 0.22 (35.5)*	21.0 ± 0.96 (23.0)	3.9 ± 0.18 (23.6)	11.8 ± 0.51 (21.8)*	16.4 ± 0.66 (20.2)	22.3 ± 1.99 (44.7)	18.5 ± 1.68 (45.5)*	18.5 ± 0.43 (11.8)
Control-2	3.0 ± 0.13 (22.8)	2.3 ± 0.10 (20.6)	16.4 ± 0.39 (11.8)	4.4 ± 0.14 (15.7)	9.6 ± 0.42 (22.2)	16.7 ± 0.91 (27.3)	22.6 ± 1.29 (28.6)	25.1 ± 1.50 (30.0)	19.7 ± 0.45 (11.4)
C1-2	2.9 ± 0.16 (27.7)	2.2 ± 0.16 (38.2)	18.5 ± 0.62 (16.7)*	4.1 ± 0.16 (19.6)	9.3 ± 0.40 (21.3)	16.6 ± 1.02 (30.8)	22.9 ± 1.38 (30.0)	23.5 ± 1.68 (35.7)	18.4 ± 0.51 (13.7)
C2-2	2.9 ± 0.19 (32.7)	2.6 ± 0.15 (29.3)	18.4 ± 0.68 (18.3)*	4.3 ± 0.23 (27.0)	8.6 ± 0.39 (23.0)	14.8 ± 0.82 (27.6)	27.1 ± 1.56 (28.8)*	21.2 ± 1.59 (37.5)	19.4 ± 0.30 (7.9)
C3-2	3.4 ± 0.24 (34.8)	2.3 ± 0.21 (44.2)	18.2 ± 0.57 (15.6)*	5.0 ± 0.24 (23.6)*	9.7 ± 0.47 (24.3)	18.9 ± 0.90 (23.8)	21.7 ± 2.06 (47.2)	20.7 ± 1.64 (39.7)	19.2 ± 0.31 (8.1)
C4-2	2.7 ± 0.22 (41.4)	2.2 ± 0.19 (41.3)	16.4 ± 0.53 (16.0)	3.9 ± 0.17 (21.4)*	9.2 ± 0.39 (21.4)	15.3 ± 0.62 (20.2)	22.8 ± 1.71 (37.5)	27.5 ± 1.64 (30.0)	20.8 ± 0.49 (11.7)
C5-2	2.9 ± 0.17 (30.5)	2.5 ± 0.21 (41.4)	19.9 ± 0.72 (18.0)*	3.3 ± 0.14 (21.2)*	9.9 ± 0.41 (20.5)	11.1 ± 0.42 (18.9)*	21.7 ± 1.28 (29.5)	28.7 ± 1.27 (22.1)	18.8 ± 0.50 (13.3)
C6-2	2.6 ± 0.16 (30.3)	2.5 ± 0.17 (33.3)	19.3 ± 0.75 (19.3)*	3.5 ± 0.11 (15.8)*	10.7 ± 0.54 (25.5)	15.0 ± 1.09 (36.4)	22.2 ± 2.09 (47.0)	24.2 ± 1.60 (33.0)	20.2 ± 0.50 (12.3)

* - indicates that differences within one species were significant between the years, according to t-test, $P < 0.05$

[1] - Vv – Volume density of the tissue (%)

[2] - C1-C6, first year and C1-2-C6-2, second year. Plots C1, C2 and C3 treated once with TE: 1.6 l/ha, 2.4 l/ha and 3.2 l/ha, respectively. Plots C4, C5 and C6 treated twice - the first treatment the same as previous, the second 1.6 l/ha TE to all three plots.

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Table 4. Vv of stem tissues and the number of vascular bundles of *V. pannonica* control plants and changes following PGR application (mean ± standard error, coefficient of variation, D.F. = 48)

	Vv[1] epidermis	Vv collenchyma	Vv cortex parenchyma	Vv sclerenchyma	Vv phloem	Vv xylem+sclpar	Vv cyl parenchyma	Vv central cavity	Number of vasc bundles
Control	4.1 ± 0.22 (27.3)	3.5 ± 0.24 (34.8)	23.3 ± 0.94 (20.2)	4.3 ± 0.27 (31.9)	11.1 ± 0.29 (13.2)	17.9 ± 0.59 (16.6)	33.1 ± 1.54 (23.3)	2.7 ± 0.44 (80.8)	13.3 ± 0.41 (15.3)
C1[2]	4.6 ± 0.30 (32.6)	3.3 ± 0.29 (43.9)	22.1 ± 1.07 (24.3)	4.3 ± 0.23 (26.6)	11.3 ± 0.36 (15.8)	22.2 ± 0.55 (12.5)*	29.2 ± 1.50 (25.7)	2.9 ± 0.61 (104.4)	13.3 ± 0.33 (12.4)
C2	4.6 ± 0.28 (30.1)	3.9 ± 0.37 (47.9)	25.3 ± 0.95 (18.8)	4.1 ± 0.18 (21.8)	12.2 ± 0.45 (18.2)*	19.5 ± 0.62 (15.9)	27.5 ± 1.33 (24.1)*	2.9 ± 0.60 (104.7)	13.2 ± 0.35 (13.5)
C3	4.1 ± 0.33 (40.8)	3.5 ± 0.37 (53.0)	23.3 ± 0.92 (19.7)	3.8 ± 0.23 (30.1)	12.3 ± 0.35 (14.1)*	17.9 ± 0.58 (16.3)	32.3 ± 1.58 (24.4)	3.2 ± 0.72 (111.3)	13.2 ±0.31 (11.7)
C4	3.4 ± 0.21 (31.5)*	4.1 ± 0.49 (59.8)	22.1 ± 1.09 (24.7)	3.9 ± 0.24 (30.4)	12.9 ± 0.34 (13.1)*	17.6 ± 0.58 (16.6)	32.6 ± 1.31 (20.1)	3.4 ± 0.55 (81.5)	13.2 ± 0.41 (15.3)
C5	4.2 ± 0.26 (31.5)	3.7 ± 0.41 (55.6)	23.5 ± 0.94 (20.1)	4.3 ± 0.17 (20.0)	12.9 ± 0.32 (12.6)*	19.7 ± 0.64 (16.3)	29.7 ± 1.39 (23.5)	2.1 ± 0.52 (124.6)	13.1 ± 0.31 (11.7)
C6	4.2 ± 0.27 (32.0)	4.1 ± 0.38 (46.4)	23.4 ± 0.80 (17.2)	4.2 ± 0.24 (28.8)	11.9 ± 0.28 (11.6)*	19.0 ± 0.82 (21.6)	31.5 ± 1.21 (19.2)	1.7 ± 0.40 (118.7)	13.4 ± 0.37 (14.0)
Control-2	4.3 ± 0.29 (33.3)	3.3 ± 0.30 (44.5)	19.6 ± 0.66 (16.9)	6.2 ± 0.26 (20.9)	9.6 ± 0.39 (20.1)	20.5 ± 1.11 (27.1)	26.6 ± 1.17 (22.1)	10.0 ± 1.39 (69.5)	14.5 ± 0.31 (10.7)
C1-2	4.3 ± 0.36 (42.0)	4.0 ± 0.35 (43.1)	19.3 ± 0.56 (14.6)	5.2 ± 0.28 (26.6)*	9.7 ± 0.37 (19.0)	18.5 ± 0.47 (12.6)	30.4 ± 0.90 (14.8)*	8.7 ± 1.19 (68.8)	14.1 ± 0.45 (15.9)
C2-2	4.1 ± 0.43 (52.2)	4.3 ± 0.45 (53.1)	20.4 ± 1.41 (34.4)	4.7 ± 0.17 (18.6)*	10.4 ± 0.33 (16.0)	19.4 ± 0.57 (14.6)	26.9 ± 1.22 (22.7)	10.2 ± 1.57 (75.1)	14.4 ± 0.37 (12.8)
C3-2	3.9 ± 0.26 (33.5)	3.9 ± 0.46 (58.7)	21.1 ± 0.66 (15.8)	4.6 ± 0.22 (24.3)*	9.6 ± 0.31 (15.9)	16.3 ± 0.49 (15.1)*	32.0 ± 1.22 (19.2)*	8.5 ± 1.29 (75.5)	13.8 ± 0.37 (13.6)
C4-2	4.6 ± 0.38 (41.8)	4.2 ± 0.37 (43.7)	20.5 ± 0.38 (9.3)	5.5 ± 0.24 (21.7)	10.1 ± 0.33 (16.5)	22.5 ± 0.78 (17.4)	25.1 ± 1.12 (22.4)	7.6 ± 1.30 (85.5)	14.0 ± 0.31 (11.2)
C5-2	3.7 ± 0.23 (30.5)	3.6 ± 0.36 (50.7)	20.8 ± 0.57 (13.8)	5.2 ± 0.23 (22.5)*	9.9 ± 0.30 (15.3)	18.9 ± 0.47 (12.4)	30.2 ± 1.31 (21.7)*	7.8 ± 1.29 (82.8)	14.1 ± 0.35 (12.5)
C6-2	4.3 ± 0.29 (34.1)	4.0 ± 0.34 (43.2)	21.4 ± 0.65 (15.1)	5.5 ± 0.19 (16.8)*	9.5 ± 0.28 (14.9)	19.2 ± 0.45 (11.4)	24.4 ± 1.03 (21.0)	11.7 ± 1.30 (55.8)	13.1 ± 0.33 (12.7)*

* - indicates that differences within one species were significant between the years, according to t-test, $P < 0.05$

[1] - Vv – Volume density of the tissue (%)

[2] - C1-C6, first year and C1-2-C6-2, second year. Plots C1, C2 and C3 treated once with TE: 1.6 l/ha, 2.4 l/ha and 3.2 l/ha, respectively. Plots C4, C5 and C6 treated twice - the first treatment the same as previous, the second 1.6 l/ha TE to all three plots.

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Table 5. Chemical composition of *Vicia* stems under different PGR treatments (%)

	N	Proteins	Ash	NDF	ADF	ADL		N	Proteins	Ash	NDF	ADF	ADL
							V. sativa						
Control	1.31	8.16	7.44	55.85	43.71	10.42	Control-2	1.63	10.21	7.42	68.88	50.47	12.02
C1	1.95	12.16	7.95	50.43	37.24	9.01	C1-2	1.16	7.23	8.53	55.67	44.18	10.08
C2	1.82	11.38	8.47	47.01	35.77	8.28	C2-2	1.44	9.01	8.42	55.36	45.35	10.34
C3	1.78	11.09	8.40	49.59	38.23	9.09	C3-2	1.81	11.28	10.13	51.71	42.41	10.01
C4	1.88	11.73	9.25	45.30	35.75	8.26	C4-2	0.88	5.49	8.80	56.69	47.77	10.58
C5	1.87	11.66	9.34	47.98	36.39	8.37	C5-2	2.01	12.58	8.65	52.15	43.54	9.93
C6	2.10	13.12	9.53	43.66	33.21	7.96	C6-2	1.76	11.02	8.70	50.18	40.55	9.05
							V. pannonica						
Control	1.35	8.41	7.41	61.47	48.03	11.96	Control-2	0.95	5.95	6.13	59.26	50.62	11.44
C1	1.37	8.53	8.70	54.45	43.51	10.15	C1-2	1.35	8.42	5.64	47.88	38.79	8.03
C2	1.70	10.61	9.14	51.20	38.65	9.21	C2-2	1.01	6.28	6.76	57.08	44.81	8.48
C3	1.24	7.73	8.72	54.137	42.39	10.34	C3-2	1.03	6.44	6.31	57.28	45.84	9.14
C4	1.14	7.14	8.24	56.37	45.27	10.129	C4-2	1.09	6.82	6.60	57.12	49.32	11.02
C5	1.11	6.93	9.46	55.25	45.52	10.13	C5-2	1.30	8.13	6.50	56.42	45.60	10.24
C6	1.39	8.70	10.48	53.13	44.11	9.57	C6-2	1.16	7.24	6.98	56.34	44.53	9.39

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Figure 1. Relative changes in morphological characteristics (%) in relation to control plants in *V. sativa* (a) and *V. pannonica* (b) as a results of PGR application (C1-C6, first year and C1-2-C6-2, second year; Plots C1, C2 and C3 treated once with TE: 1.6 l/ha, 2.4 l/ha and 3.2 l/ha, respectively. Plots C4, C5 and C6 treated twice - the first treatment the same as previous, the second 1.6 l/ha TE to all three plots. Only statistically significant changes are presented, compared to control plants, according to t-test

Figure 2. Cross-sections of middle stem part of (a) *V. sativa* and (b) *V. pannonica* control plants

Figure 3. Relative changes in volume density (Vv) of stem tissues (%) in *V. sativa* (a) and *V. pannonica* (b) as a results of PGR application (C1-C6, first year and C1-2-C6-2, second year; Plots C1, C2 and C3 treated once with TE: 1.6 l/ha, 2.4 l/ha and 3.2 l/ha, respectively. Plots C4, C5 and C6 treated twice - the first treatment the same as previous, the second 1.6 l/ha TE to all three plots). Only statistically significant changes were presented, compared to control plants, according to t-test. epid – epidermis, coll – collenchyma, cor par – cortex parenchyma, scl – sclerenchyma, phl – phloem, xyl+sclpar – xylem with sclerenchymatous parenchyma, cyl par – cylinder parenchyma, cav – central cavity, vasc bun – number of vascular bundles.

Figure 4. Relative changes, compared to control plants, in volume density (Vv) of stem tissues (%) from the middle (segment 3) to the bottom (segment 5) part of the stem in *V. sativa* (a-c) and *V. pannonica* (d-f), as a result of PGR application (C1-C6, first year and C1-2-C6-2, second year;

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Plots C1, C2 and C3 treated once with TE: 1.6 l/ha, 2.4 l/ha and 3.2 l/ha, respectively. Plots C4, C5 and C6 treated twice - the first treatment the same as previous, the second 1.6 l/ha TE to all three plots). Only statistically significant changes were presented, compared to control plants, according to t-test. epid – epidermis, coll – collenchyma, cor par – cortex parenchyma, scl – sclerenchyma, phl – phloem, xyl+sclpar – xylem with sclerenchymatous parenchyma, cyl par – cylinder parenchyma, cav – central cavity, vasc bun – number of vascular bundles

Figure 5. Lodging index of *V. sativa* and *V. pannonica* before and after Moddus application (C-C6 - first year and C-2 - C6-2 - second year; C – control plants; Plots C1, C2 and C3 treated once with TE: 1.6 l/ha, 2.4 l/ha and 3.2 l/ha, respectively. Plots C4, C5 and C6 treated twice - the first treatment the same as previous, the second 1.6 l/ha TE to all three plots)

Figure 6. Correlations between lodging index and Vv of stem tissues in *V. sativa.* (marked *r* values are significant at $P < 0.050$)

Figure 7. Correlations between lodging index and Vv of stem tissues in *V. pannonica.* (marked *r* values are significant at $P < 0.050$)

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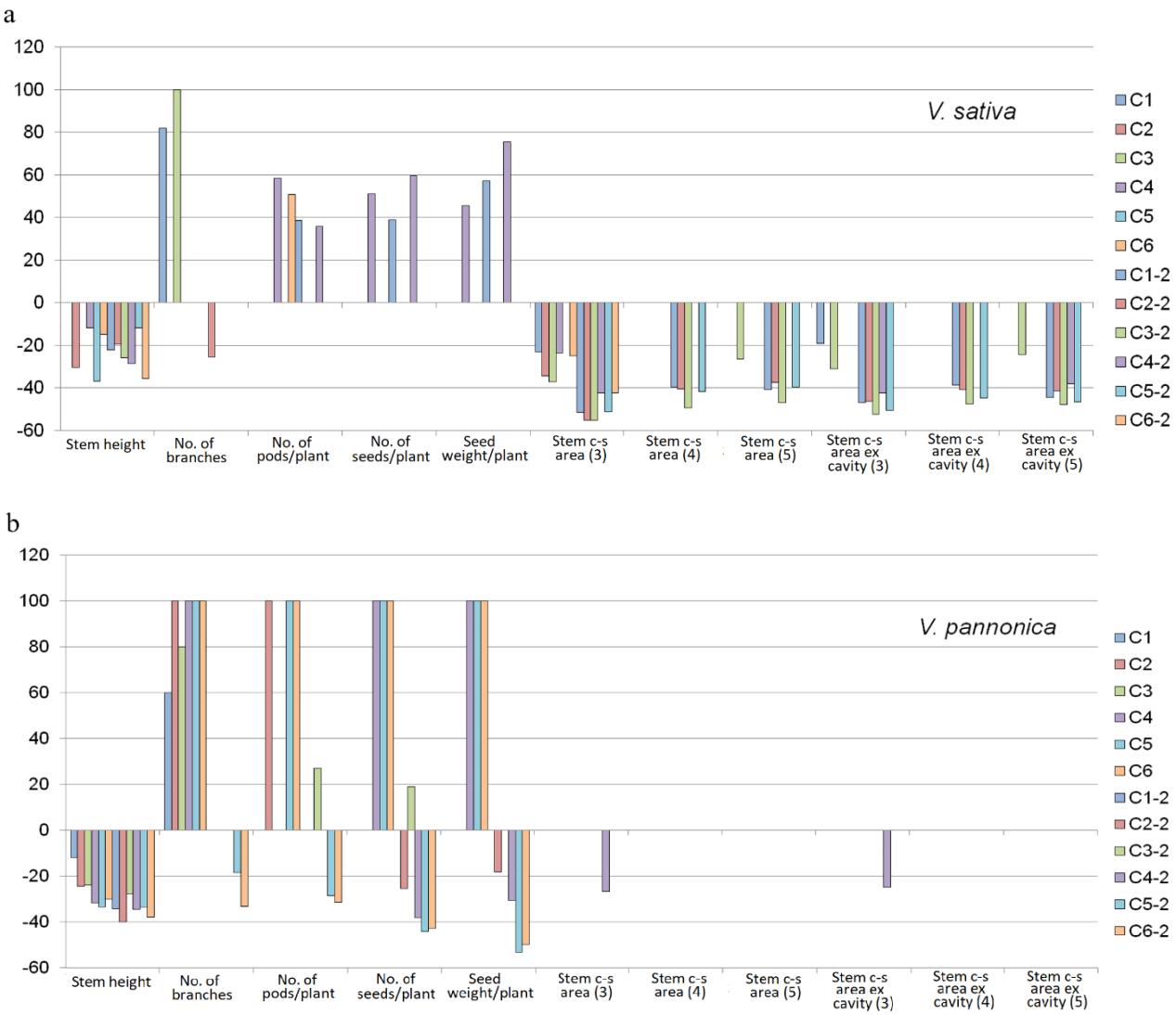

Figure 1.

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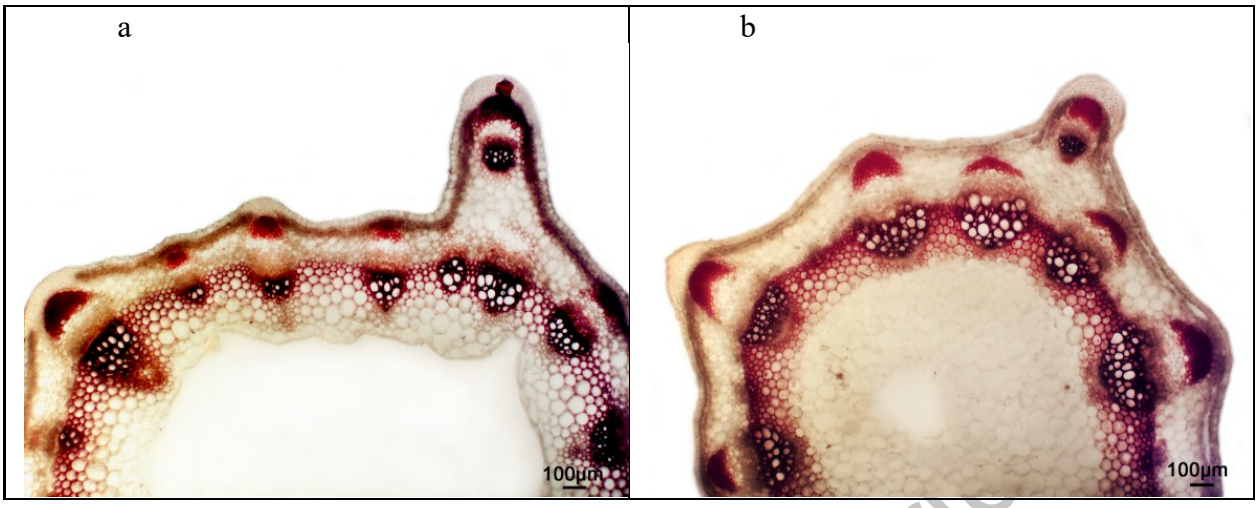

Figure 2.

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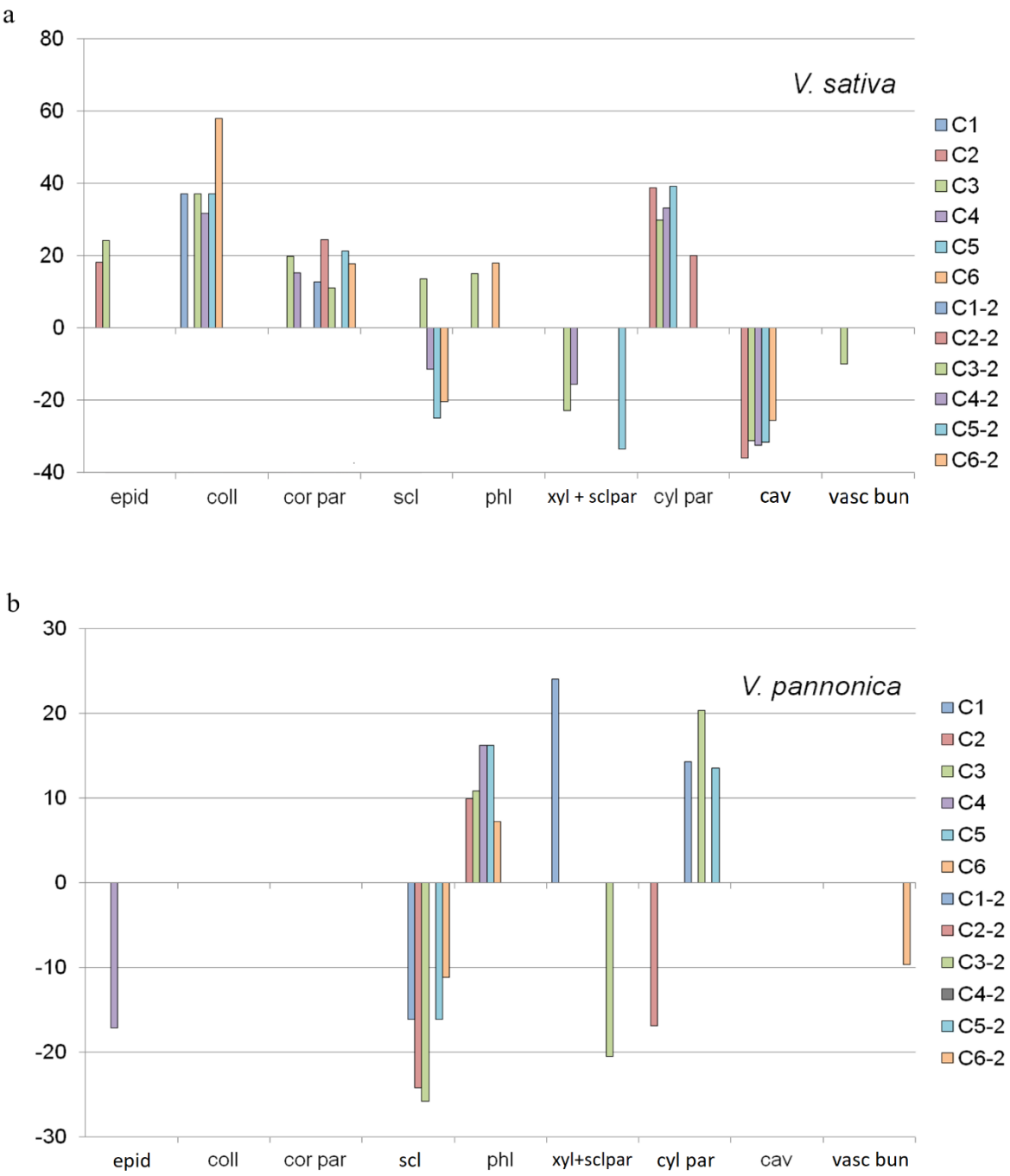

Figure 3.

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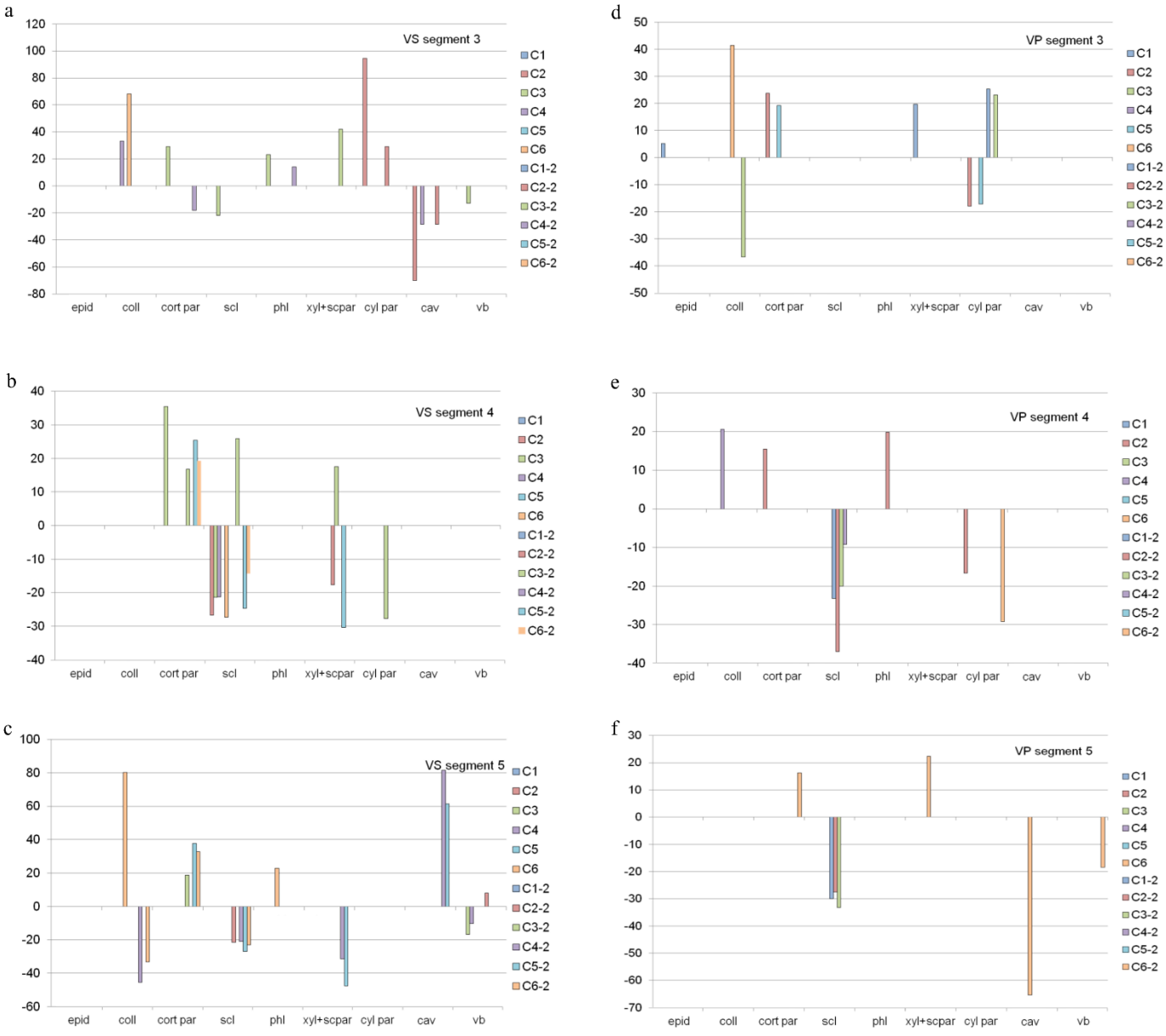

Figure 4.

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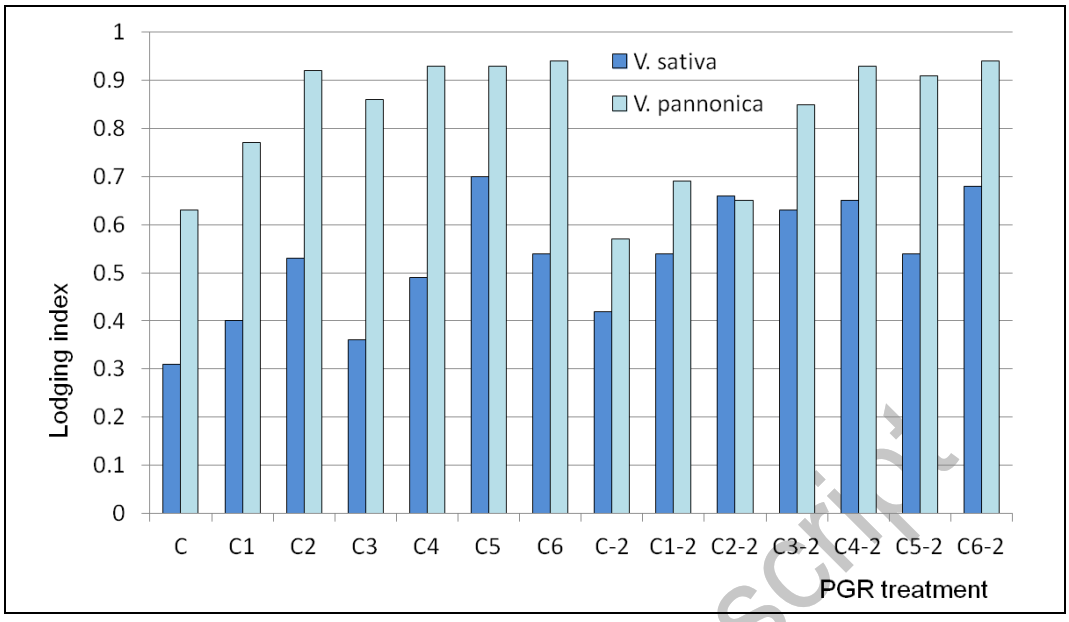

Figure 5.

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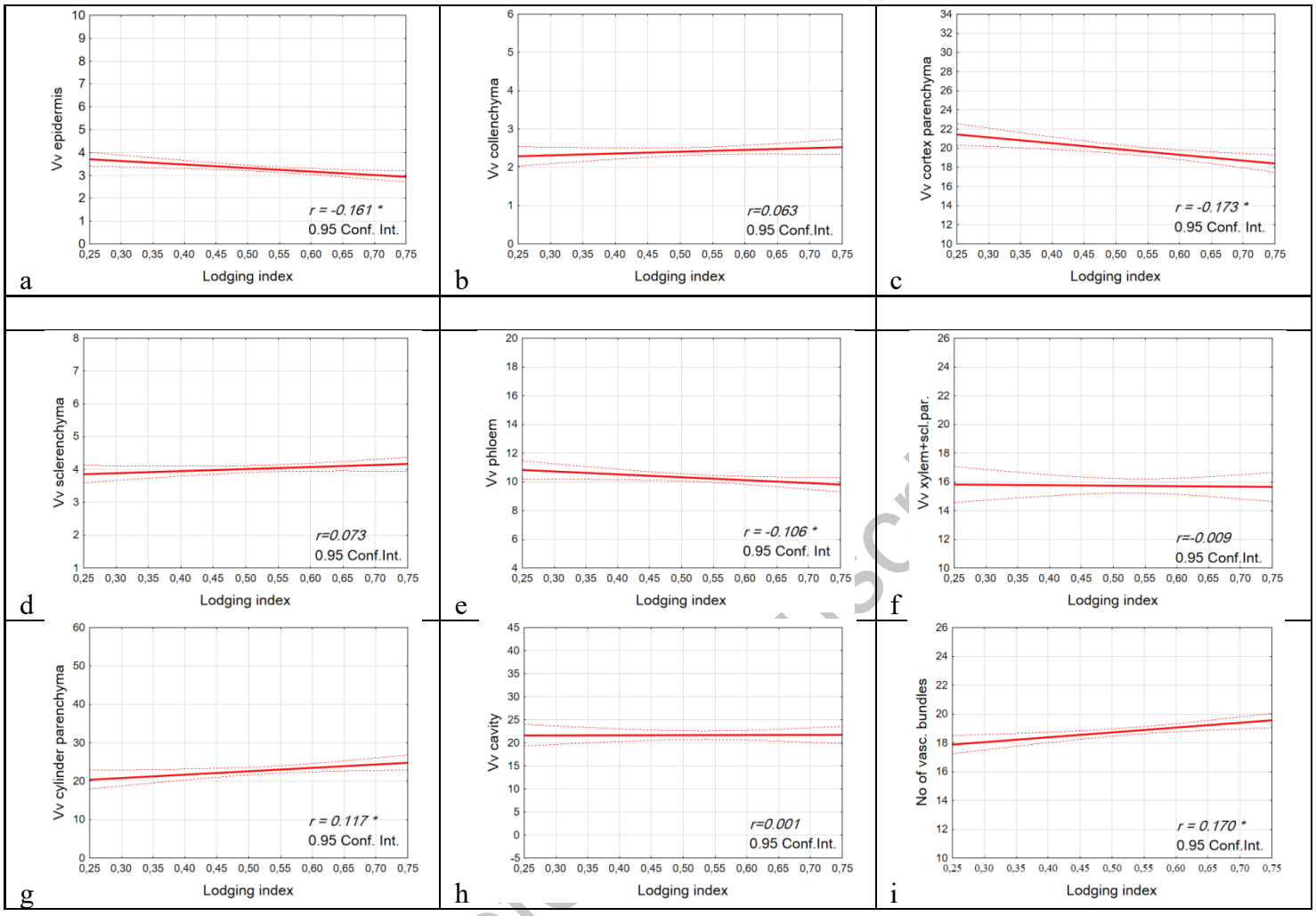

Figure 6.

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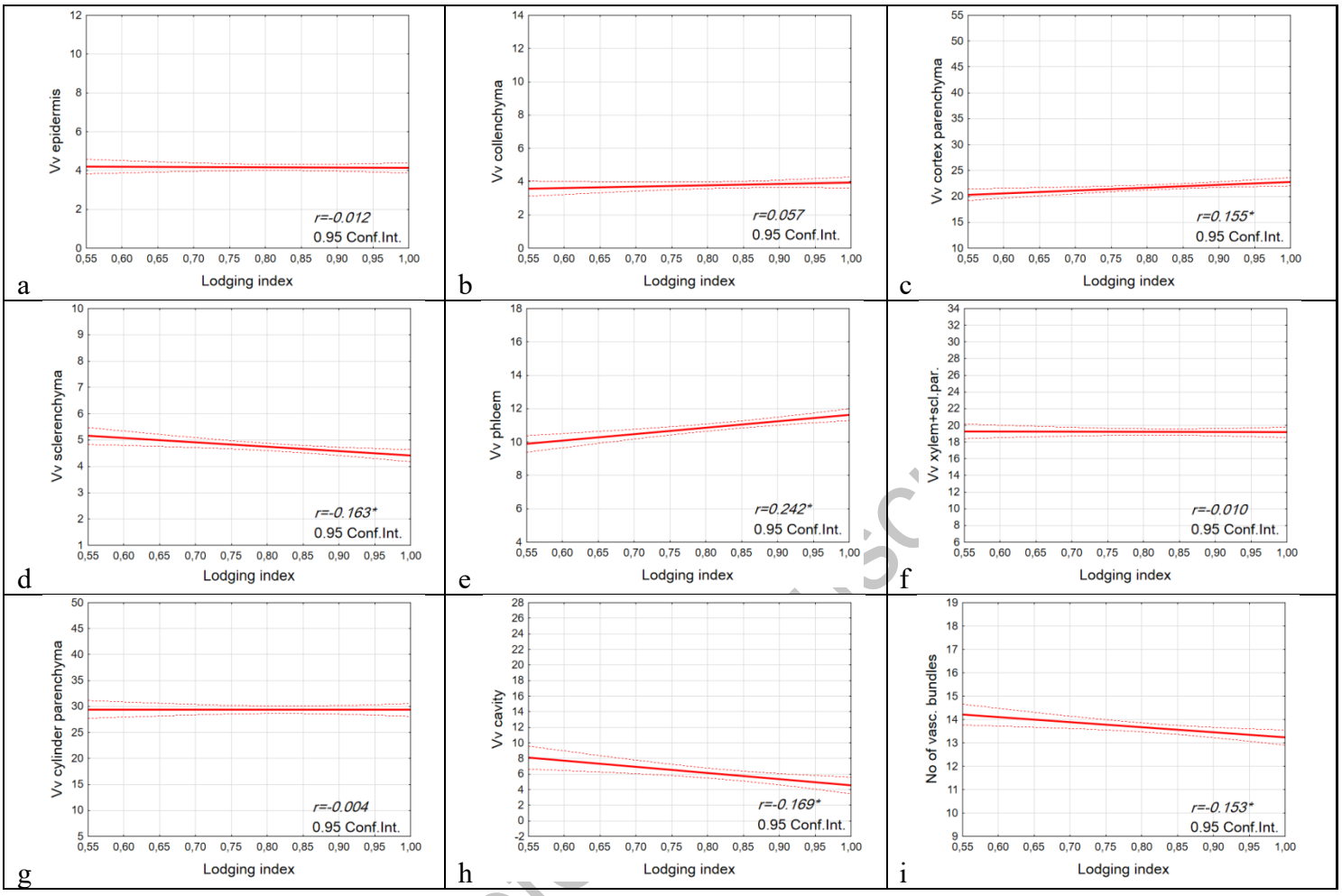

Figure 7.

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