

Article

Investigating the Effects of Conventional and No-Tillage Cultivation Methods on Plant Physiological Processes Using Genome-Wide Transcriptomic Analysis

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Abstract

Despite the growing demand for sustainable agricultural systems, the long-term effects of tillage practices remain controversial. No-tillage (NT) systems offer several potential benefits, including improved soil structure, enhanced soil biological activity, and reduced environmental stress, but their application can also be associated with challenges such as difficulty in weed control or variable crop yield. Although previous studies have extensively investigated the effects of NT systems on soil and crop, limited knowledge is available about the cellular adaptation mechanisms of plants, especially gene expression and biochemical responses. The aim of this study was to compare the effects of conventional tillage (CT) and NT systems in sunflower plants using an integrated transcriptomic and biochemical approach. We performed genome-wide transcriptomic analysis based on next-generation sequencing on leaf samples from three different field sites, supplemented by measurements of biochemical parameters related to selected metabolic processes. Exploratory transcriptomic analysis indicated that several gene expression changes related to primary metabolic processes occurred in plants grown in the NT system compared to the CT system. These included processes related to photosynthesis, cellular respiration, carbohydrate metabolism and the biosynthesis of some amino acids. In parallel, we observed transcriptional patterns indicating increased activity of several secondary metabolic pathways, which may be related to adaptation mechanisms to environmental stress. Determination of total soluble sugar, crude protein, total phenolics and total flavonoids provided independent biochemical support for the changes indicated by the transcriptomic results. Our results suggest that the tillage system affects the cellular regulatory processes of sunflower. During adaptation to a no-tillage environment, plants can simultaneously maintain basic metabolic processes and activate defense mechanisms that may contribute to adaptation to changed growing conditions. Our study contributes to a better understanding of the molecular and physiological consequences of tillage systems in plants.

Keywords: no-tillage; conventional tillage; next-generation sequencing (NGS); genome-wide transcriptional analysis; stress



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1. Introduction

Global climate change, increasing food demand and increasing pressure on agricultural resources pose increasing challenges in developing sustainable crop production systems. Traditional intensive cultivation practices can contribute to soil structure degradation, organic matter loss and reduced soil biological activity in the long term, and therefore there is increasing attention worldwide to the application of soil-conserving technologies [1–3].

No-tillage (NT) systems are one of the most important alternatives in sustainable agriculture. According to several studies, NT can improve soil structure, increase water retention capacity, promote soil microbial activity and contribute to carbon sequestration [4–8]. However, the agronomic benefits of NT systems cannot be considered universal, as their effects are significantly influenced by climate, soil type, crop species and applied cultivation technology [9–15]. Several global meta-analyses and papers have suggested that NT can have a positive impact on environmental sustainability under certain conditions [16–25], but its impact on crop yield is often variable and in many cases does not differ significantly from conventional cropping systems [10,13,20,23].

Previous research has focused primarily on soil physical, chemical and microbiological properties, as well as crop performance [5,11,12,26]. These results have shown that the effects of NT systems are highly site-specific, and the interaction between the growing environment and the cropping system fundamentally determines their success [23,27]. However, less is known about how the altered soil environment directly affects plant cellular regulatory processes, especially gene expression patterns and related biochemical responses.

Plant adaptation to environmental changes occurs through complex molecular regulatory processes that include the activation of stress response genes, the rearrangement of metabolic pathways, and the modification of antioxidant defense mechanisms. Although several studies have investigated the effects of tillage systems on soil and crop yield, integrated analysis of plant transcriptomic and biochemical responses remains limited. In particular, little information is available on the molecular mechanisms through which the NT system influences plant adaptability under long-term field conditions.

We hypothesize that long-term changes in tillage systems impose environmental selection pressures on plants that modify gene expression processes related to stress response and metabolism, and these changes are also reflected in measurable biochemical differences. To investigate this, in the present study, we performed genome-wide transcriptomic analysis on leaf samples of sunflowers grown under conventional tillage and no-tillage systems at three different field sites. The transcriptomic results were complemented with biochemical parameters to reveal plant adaptation mechanisms related to the two cropping systems.

2. Results

2.1. Genome-Wide Transcriptomic Analyses

A de novo transcriptome was reconstructed using the pooled reads of 6 libraries from quality-controlled, filtered, and trimmed samples (67,840,034 short reads). The de novo transcriptome assembly yielded 66,372 transcripts, from which 43,749 genes were identified, and their functions delineated. The average gene length was 995 bp. The super transcriptome (Transcriptome Shotgun Assembly—TSA) is available in Supplementary File S2. After software blasting, mapping, and annotation of the TSA, the expression levels of the genes were determined individually per site and cultivation method.

The results were obtained in a so-called count table, which is placed in Supplementary File S3_Worksheet_1. The count table serves as a basis for determining the expression of individual contigs, which is essential for further transcript-level quantification. The overall alignment rate ranged between 53.4% and 58.9%, which is within the expected range for

de novo transcriptome-based mapping in a non-reference-guided system. The unmapped fraction likely reflects novel transcripts, assembly fragmentation, and species-specific transcript diversity.

The number of multiple aligned reads used for the count table was 294,740 in the sunflower Kiszombor conventional tillage sample, 258,245 in the sunflower Kiszombor no-tillage sample, 274,409 in the sunflower Ecseg conventional tillage sample, 323,489 in the sunflower Ecseg no-tillage sample, 161,714 in the sunflower Vityapuszta conventional tillage sample, and 337,254 in the sunflower Vityapuszta no-tillage sample (Supplementary File S7). Then, pairwise differential expression analysis was performed.

The analyses were performed in 3 combinations: between Ecseg conventional tillage and Ecseg no-tillage plant datasets, between Kiszombor conventional tillage and Kiszombor no-tillage plant datasets, and between Vityapuszta conventional tillage and Vityapuszta no-tillage plant datasets. Conventional tillage was used as the reference condition for differential expression analysis, as it represents the prevailing and long-established soil management practice in the studied agricultural systems. This approach allowed the transcriptional and biochemical effects of the transition to no-tillage management to be assessed relative to the current standard agricultural practice. The aim was to determine the effects of the two treatments on plant populations at the gene level.

From the analysis of the Ecseg conventional tillage and Ecseg no-tillage datasets, 1098 of the 4393 differentially expressed sequences were up-regulated, and 3295 were down-regulated. The analysis of the datasets from the comparison of Kiszombor conventional tillage and Kiszombor no-tillage resulted in 822 differentially expressed sequences (likelihood > 0.9), of which 314 were up-regulated, and 508 were down-regulated (Supplementary File S3_Worksheet_2). From the analysis of the Vityapuszta conventional tillage and Vityapuszta no-tillage datasets, 1299 of the 3928 differentially expressed sequences were up-regulated, and 2629 were down-regulated.

Gene enrichment analyses were performed to determine how many of each gene were over- or under-represented in each sample, i.e., how many genes were present in each sample that occurred more or less frequently than expected by chance. 809 over- and 1770 under-represented genes were identified in the comparative analysis between the Ecseg conventional tillage and Ecseg no-tillage datasets. 207 over- and 427 under-represented genes were identified between Kiszombor conventional tillage and Kiszombor no-tillage. 935 over- and 1912 underrepresented sequences were discovered in the Vityapuszta conventional tillage and Vityapuszta no-tillage datasets.

In all three comparisons, individual genes were identified, their GO terms were determined, and their functional annotations were performed. By extracting the information obtained after identification from all three comparisons, we can conclude that, across all three experimental areas, biochemical processes (BPs) related to metabolism and anatomical structure development were activated in plants growing in the no-tillage area compared to conventional tillage (Figure 1A–C).

The obtained datasets were further used to determine which up- and down-regulated genes can be linked to biochemical processes. After performing combined pathway analyses, it was determined that some biochemical pathways were up- or down-regulated in plants grown using the no-tillage cultivation method in each experimental area compared to those grown using the conventional plowed cultivation method.

Based on combined biochemical pathway analyses of the area of Ecseg, 117 upregulated genes were linked to 98 pathways in the KEGG database, while 420 genes were linked to 364 pathways in the Plant Reactome database. In the Ecseg area, 369 genes are downregulated and participate in 148 biochemical processes according to the KEGG database, while 976 genes are linked to 723 pathways in the Plant Reactome database.

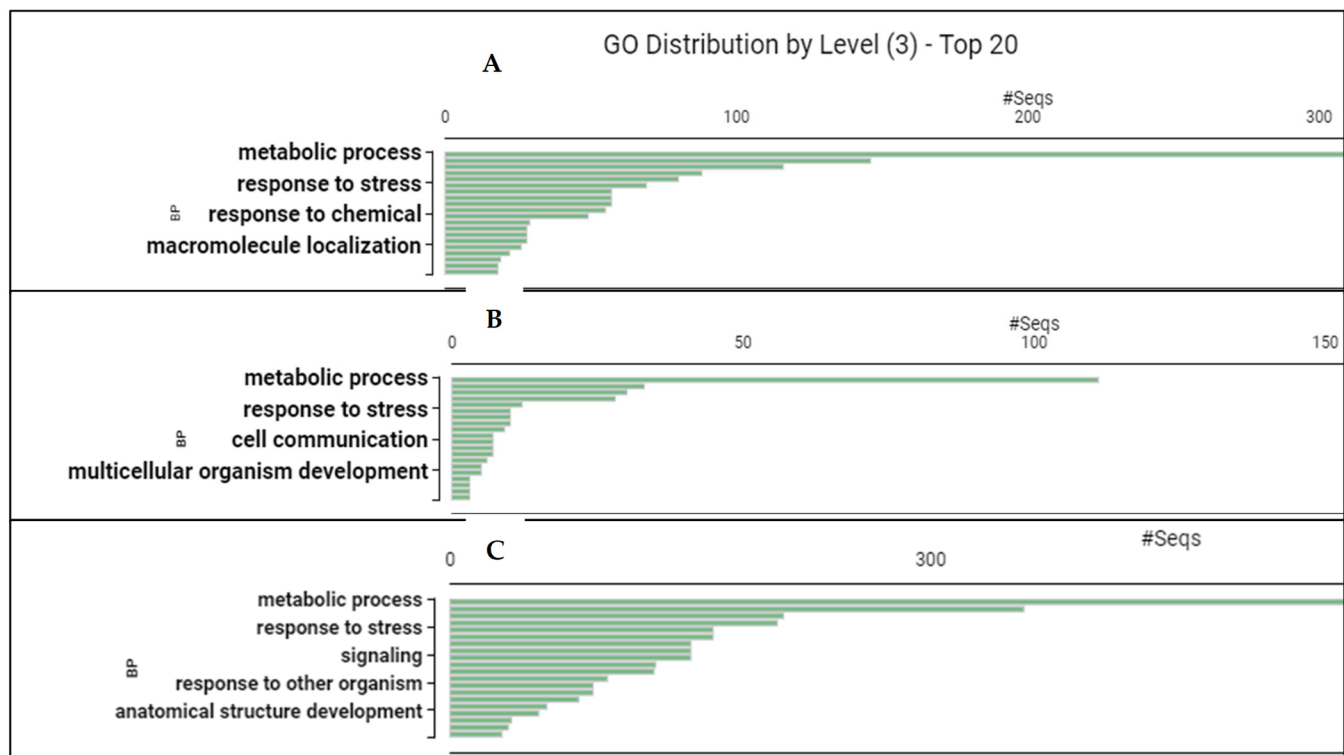


Figure 1. Upregulated biochemical processes in the plants grown in no-tillage areas ((A): Ecseg; (B): Kiszombor; (C): Vityapuszta). The image is the original output file of the OmicsBox Biobam 3.4. software, which is protected by copyright and cannot be edited or modified.

According to the combined biochemical pathway analyses in Kiszombor, 40 up-regulated genes can be linked to 36 pathways in the KEGG database, while 94 genes can be linked to 98 pathways in the Plant Reactome database using the cultivation method. In the same area, among the downregulated sequences, 111 genes are linked to 73 biochemical processes in the KEGG database, while 215 sequences are linked to 244 pathways in the Plant Reactome database.

They also examined how many genes were up- and down-regulated in all no-tillage treatments, regardless of the environment. This showed that the majority of gene expression changes were due to tillage methods, while a smaller proportion was due to environmental factors (Figure 2).

In Vityapuszta area, the same data were as follows: the number of upregulated, overexpressed genes based on KEGG is 159, which can be linked to 104 biochemical processes, while 441 genes can be linked to 393 pathways based on the Plant Reactome analysis. In the case of downregulated, overexpressed genes, 369 genes can be linked to 144 pathways based on KEGG, and 1137 genes to 773 pathways based on the Plant Reactome database.

In Ecseg, the strongest upregulation was observed in arginine and proline metabolism, phenylalanine metabolism, glycerolipid metabolism, beta-alanine metabolism, and phenylalanine, tyrosine, and tryptophan biosynthesis (Figure 3). In the no-tillage cultivation, the strongest downregulation was observed in cysteine and methionine metabolism; carbon fixation by the Calvin cycle; glycine, serine, and threonine metabolism; galactose metabolism; pentose and glucuronate interconversions; glycerophospholipid metabolism; purine metabolism; porphyrin metabolism; pyruvate metabolism; starch and sugar metabolism; terpenoid skeleton construction; and glycolysis-gluconeogenesis processes.

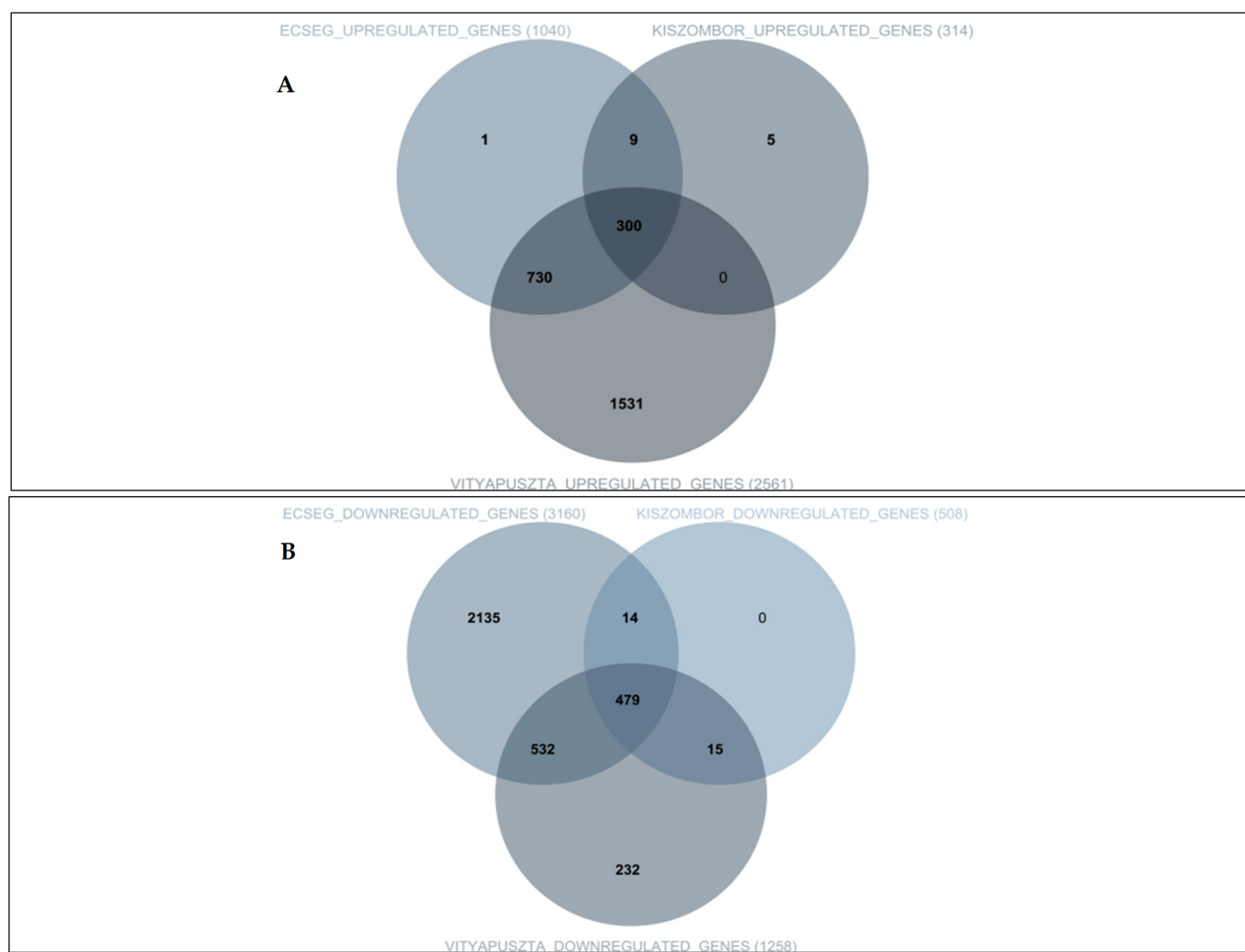


Figure 2. Venn diagrams of genes up- (A) and down-regulated (B) in the experimental areas.

In the Kiszombor area, the most upregulated processes were purine metabolism; oxidative phosphorylation; terpenoid skeleton biosynthesis; riboflavin metabolism; pyrimidine metabolism; biosynthesis of diverse plant secondary metabolites; thiamine metabolism. Within the same experimental area, the most strongly downregulated processes in plants grown under no-tillage cultivation were starch and sugar metabolism (Figure 4), glycerophospholipid metabolism, pentose and glucuronate interconversions, and galactose metabolism.

In Vityapuszta, the most important upregulated processes in the no-tillage area were glycolysis/gluconeogenesis, purine metabolism, pyruvate metabolism, cysteine and methionine metabolism, starch and sugar metabolism, glycerophospholipid metabolism, terpenoid skeleton biosynthesis, glycine, serine, threonine metabolism, carbon fixation by the Calvin cycle, and phenylpropanoid biosynthesis. The main downregulated processes were aminosugar and nucleotide sugar metabolism, porphyrin metabolism, and sphingolipid metabolism. Based on results from all three areas, a strong downregulation is observed in no-tillage areas compared to conventional tillage. However, the picture is much more nuanced, as the up- and downregulated biochemical processes taking place in the plant can only be interpreted together.

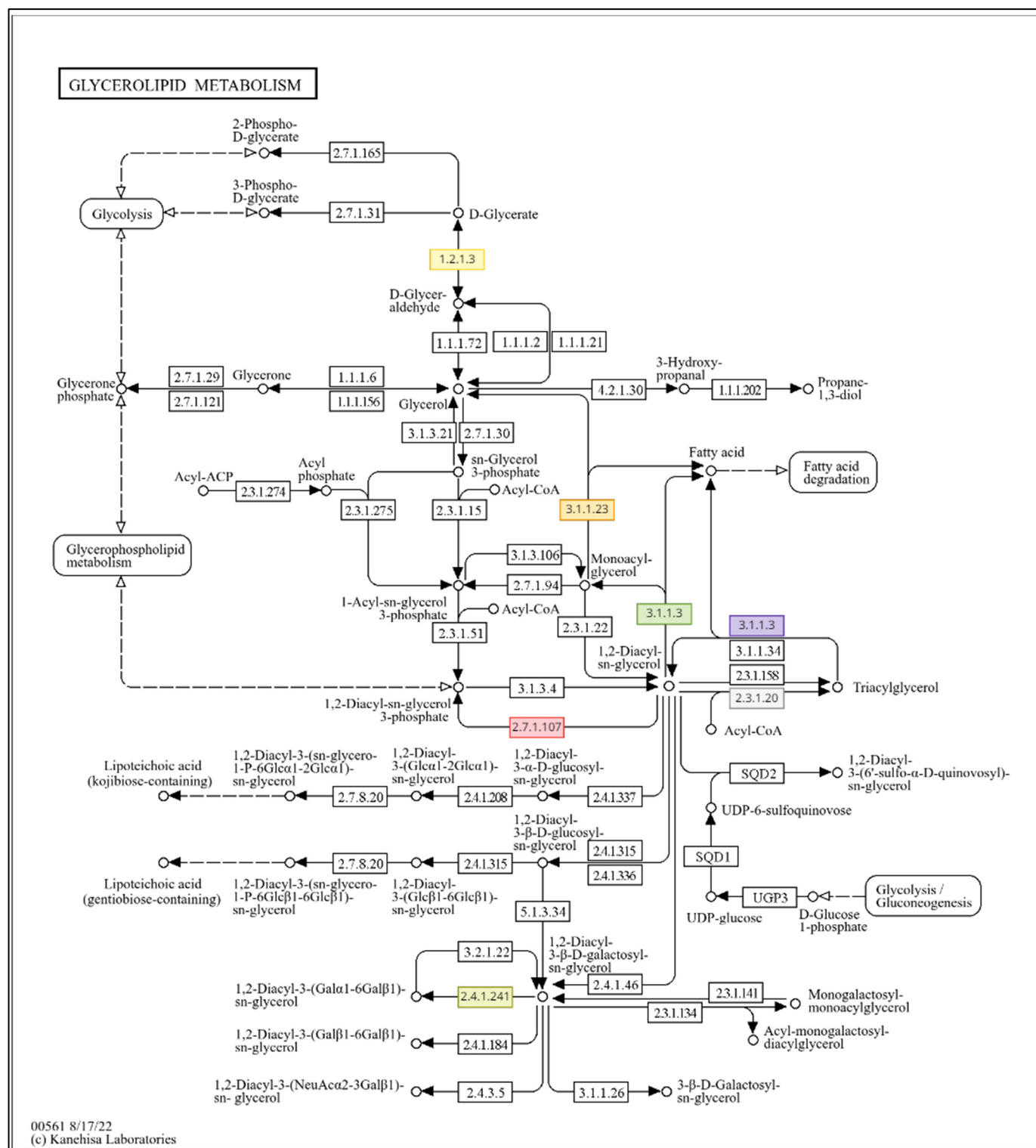
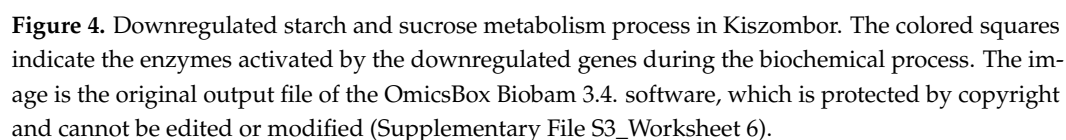


Figure 3. Glycerolipid metabolism process in the Ecseg area. The colored squares indicate the enzymes activated by the upregulated genes during the biochemical process. The image is the original output file of the OmicsBox Biobam 3.4. software, which is protected by copyright and cannot be edited or modified (Supplementary File S3_Worksheet 5).



In order to support the results of the transcriptomic studies, we performed laboratory studies (Figure 5A–D), which can be interpreted in comparison with the results obtained during the genome-wide transcriptomic analyses and confirm the gene expression results. In the Ecseg area, laboratory measurements showed an increase in soluble sugar content

in the uncultivated area, which enhances the tolerance of the plant. In the same area, the relative crude protein concentration in leaf tissues also increased, showing a synergistic effect with the previous ones. In addition to these primary metabolic processes, the production of secondary metabolites started, and the levels of phenolic compounds and flavonoids increased in plants grown in uncultivated areas, indicating the beginning of acclimation to environmental challenges. The results of laboratory measurements of content indicators showed an increase in the levels of the above compounds, which further supports the transcriptomic data.

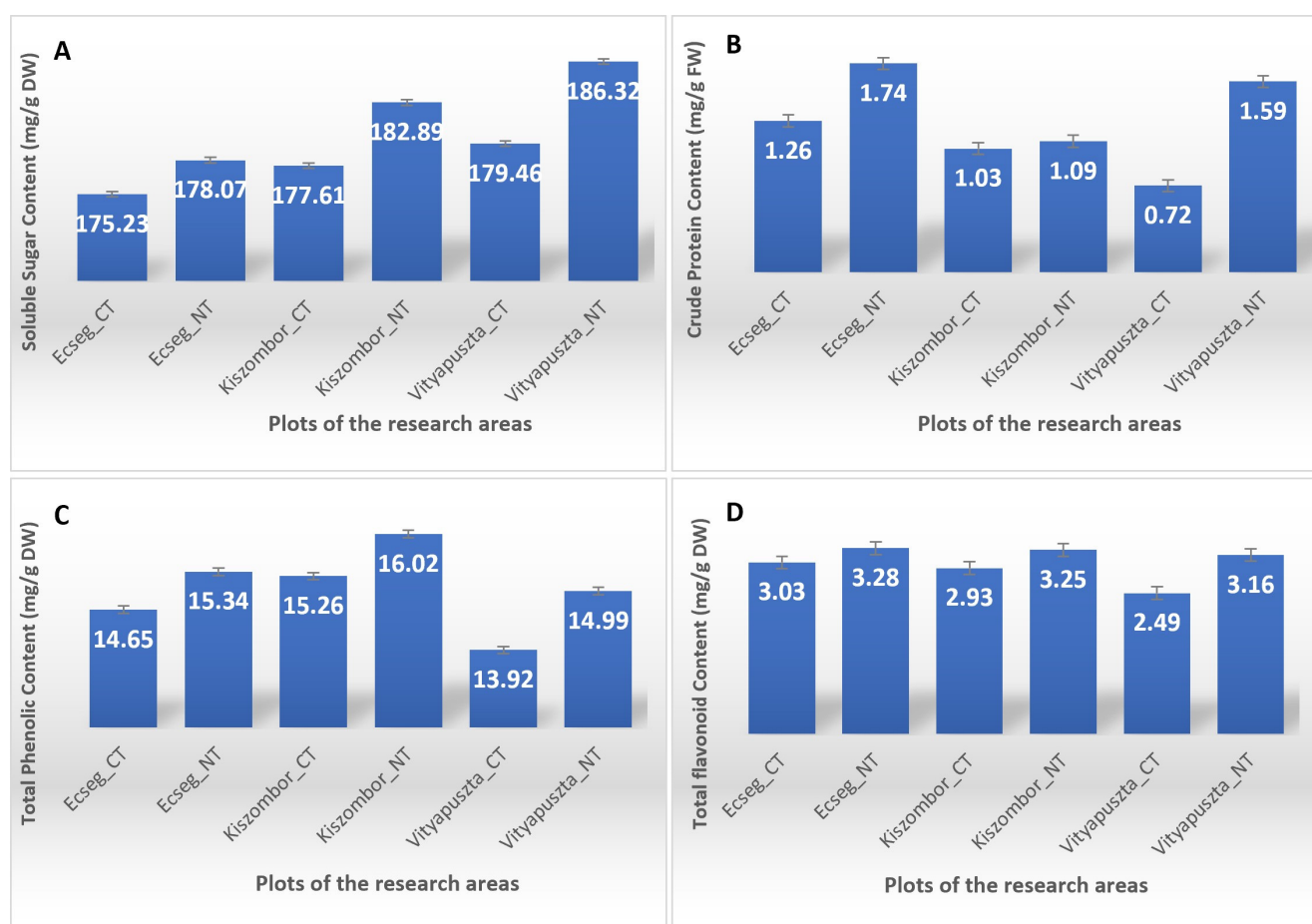


Figure 5. Laboratory test results. The results of the statistical analyses for the raw data of Figure 5 (A) (soluble sugar content), (B) (crude protein), (C) (total phenolic content), and (D) (total flavonoid content) are included in the Supplementary File S3_Worksheet 3.

In Kiszombor, the laboratory parameters are consistent with the results measured in the Ecseg areas, but the differences between the two treatments are less pronounced. In addition to the intensive metabolism, signs of acclimation also appear, although to a lesser extent than in the Ecseg case. In laboratory measurements, increasing trends can be observed in the soluble sugar, protein, phenol and flavonoid content. A rearrangement of metabolic processes can be observed, and as the plants grow intensively, they start to focus their resources on acclimation and defensive responses. Of the three experimental areas, the appearance of processes characteristic of continuous change and the onset of acclimatization mechanisms is most pronounced in this area.

In the case of Vityapuszta, the laboratory measurements showed a similar trend as the Ecseg and Kiszombor cultivated data sets, but in this area there were not such large differences between the direct cultivation and conventional cultivation data sets. Consequently,

the highest yield averages were obtained in the Vityapuszta area (Figure 6). Overall, based on the observations, the largest metabolic changes were observed in plants growing in the Ecseg region. In Kiszombor, we observed a strong activation of acclimatization mechanisms in order to adapt to environmental changes. In the case of Vityapuszta, we found the most balanced production conditions during the experiments.

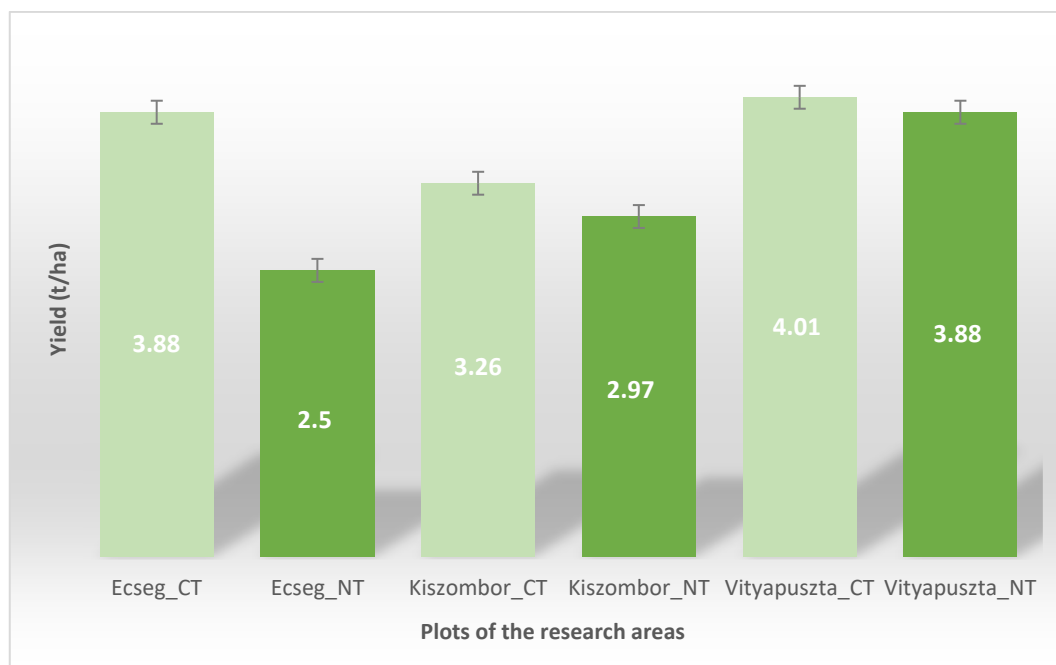


Figure 6. Grain yield development in the plots of the three research areas.

Two-way analysis of variance was used to evaluate the laboratory parameters (total soluble sugar content—TSS, crude protein content—CPC, total phenolic compounds—TPC, and total flavonoid content—TFC), where site and tillage method (no-tillage vs. conventional cultivation) were fixed factors, as well as their interaction, in the model (Supplementary File S8). Based on the results, both site and treatment, and in several cases their interaction, had a significant effect on each of the parameters examined. In the case of total soluble sugar content (TSS), the effect of treatment proved to be significant, and differences between sites also contributed to the variability. Similarly, for crude protein content (CPC), the tillage method had a strong, significant effect, while site and interaction effects were variable.

For total phenolic compounds (TPC) and total flavonoid content (TFC), both treatment and site had significant effects, and the interaction suggests that the effect of tillage is partly environmentally dependent. This is consistent with the observation that different sites responded differently to the no-tillage treatment. Overall, the effect of the no-tillage system on the examined biochemical parameters is not uniform but develops in interaction with site conditions. However, a general trend was observed across several parameters, indicating that the no-tillage treatment modifies plant metabolic processes, with their expression varying depending on environmental conditions.

2.3. Post-Harvest Studies

The post-harvest yield results (Figure 6) showed a similar trend to the gene-level profiles. While in Ecseg, the plants in the no-tillage areas may have perceived the environmental conditions resulting from the cultivation method as an inhibiting factor (which may have resulted from the more compacted state of the soil, competition with weeds, etc.), the yields still remained at the yield levels expected from sunflowers. In Kiszombor, the yield of the

no-tillage areas was only slightly lower than the yield averages of conventional cultivation. However, in Vityapuszta, we obtained yield averages similar to the conditions provided by conventional cultivation, which can be considered optimal, on the no-tillage plots. A statistically significant difference can be demonstrated in the case of the Ecseg (P1% significant) and Kiszombor (P10% significant) samples (Supplementary File S3_Worksheet_4).

2.4. Combined Effects of Tillage and Site Conditions on Plant Traits and Yield Parameters

The two-way ANOVA showed that both site and tillage system significantly affected the measured biochemical parameters, although the magnitude and consistency of these effects differed among traits. For total soluble sugar content (TSS), the treatment effect was significant, and differences among sites also contributed to the observed variability. Similarly, crude protein content (CPC) was strongly influenced by the tillage system, whereas the effects of site and the site $\times$ treatment interaction varied among locations.

For total phenolic compounds (TPC) and total flavonoid content (TFC), both treatment and site had significant effects, and the interaction indicated that the effect of tillage was partly dependent on environmental conditions. This is consistent with the observation that the three experimental sites responded differently to the no-tillage treatment. Overall, the effect of the no-tillage system on the examined biochemical parameters was not uniform but depended on site-specific environmental conditions. Nevertheless, a consistent overall trend was observed across several parameters, indicating that no-tillage altered plant metabolic processes, although the magnitude of these responses varied among sites.

The strongest treatment and environmental effects were observed in the case of crude protein content (CPC). Based on the two-way ANOVA, the main effects of site ($F_{2,12} = 202.68$; $p < 0.001$), tillage system ($F_{1,12} = 631.62$; $p < 0.001$), and their interaction were all significant ($F_{2,12} = 158.68$; $p < 0.001$). This shows that the change in protein content cannot be considered solely as a general consequence of the tillage system, but largely depended on the environmental conditions of the given site. Based on the comparisons made by site, the no-tillage treatment resulted in a significant increase in protein content in Ecseg and especially in Vityapuszta, while the change was significantly smaller in Kiszombor.

In the case of total phenolic content (TPC), the two-way ANOVA showed a significant site and tillage effect. The effect of site was significant ($F_{2,12} = 31.63$; $p < 0.001$), and tillage system also significantly influenced the amount of phenolic compounds ($F_{1,12} = 46.87$; $p < 0.001$). However, the tillage $\times$ site interaction was not significant ($F_{2,12} = 0.93$; $p = 0.421$), indicating that the effect of the no-tillage system on phenolic content was similar in all three studied areas. One-way analyses confirmed this trend: although the degree of increase differed at each site, the no-tillage treatment was associated with higher phenolic content at all sites.

For total flavonoid content (TFC), both site and tillage system also had significant effects. Based on the two-way ANOVA, the effect of site was significant ($F_{2,12} = 90.20$; $p < 0.001$), as was the effect of tillage system ($F_{1,12} = 369.43$; $p < 0.001$). In addition, the tillage $\times$ site interaction was also significant ($F_{2,12} = 35.91$; $p < 0.001$), indicating that the response of flavonoid accumulation was significantly dependent on the test environment. Although the no-tillage system increased flavonoid content in all areas, the extent of the change was particularly pronounced in the case of Vityapuszta.

Overall, the results of the two-way ANOVA indicate that the effect of the no-tillage system cannot be considered a uniform response that occurs in the same way in all environments. Based on the biochemical parameters examined, some traits, such as phenolic content, responded relatively consistently to changes in tillage system, while other parameters, especially protein and flavonoid content, showed strong site-dependent responses.

This suggests that plants manage changes in tillage system through dynamically regulated biochemical responses in accordance with local environmental conditions.

Based on the results of the one-way ANOVA, the effect of the no-tillage system on yield was highly site-specific. In Ecseg, the cultivation system caused a significant yield reduction, while in Kiszombor, only a moderate, statistically weak trend was observed. In Vityapuszta, the difference between the two systems was minimal and did not prove to be significant. Therefore, based on the yield results, it cannot be generally stated that the no-tillage system causes yield reduction in all environments, but the effect depended to a large extent on the growing site conditions. The different responses of the three study areas indicate that the soil cultivation system and the environmental background together determine the productivity of the plant.

3. Discussion

3.1. Ecseg

Based on the transcriptomic analysis, no-tillage cultivation in the Ecseg experimental area was associated with a coordinated reprogramming of processes related to growth, metabolism and stress response compared to conventional cultivation. The combined interpretation of the up- and down-regulated pathways suggests that the plant simultaneously activates mechanisms that support structural organization and environmental adaptation, while the transcriptional activity of several energy-intensive primary metabolic processes is reduced. This gene expression pattern can be associated with a transient physiological adaptation state rather than a single dominant metabolic response.

Among the up-regulated pathways, glycerolipid metabolism, β -alanine metabolism, phenylalanine, tyrosine and tryptophan biosynthesis, and arginine and proline metabolism represent complementary functions. The increased activity of glycerolipid metabolism may be consistent with the increased synthesis of membrane lipids, which is a fundamental condition for cell growth and structural maintenance of cells [28,29]. Activation of β -alanine metabolism may promote the formation of pantothenate and, through it, coenzyme A, which is an essential cofactor for many biosynthetic and energy conversion processes [30]. In parallel, the enhancement of phenylalanine, tyrosine and tryptophan biosynthesis may not only increase the availability of precursors for protein synthesis [31], but also provide starting compounds for secondary metabolism [32]. In particular, the role of phenylalanine can be highlighted, which can contribute to lignin and flavonoid biosynthesis as a precursor of the phenylpropanoid pathway [33–35]. Similarly, increased activity in arginine and proline metabolism may be consistent with activation of nitrogen metabolism and osmoprotective mechanisms [36,37]. Together, these changes suggest that the plant is activating biosynthetic and defense processes that may facilitate adaptation to environmental conditions.

However, at the same time, downregulation of several pathways related to primary carbon and energy metabolism was also observed. Reduced gene expression of Calvin cycle carbon fixation, starch and sugar metabolism, glycolysis/gluconeogenesis, pyruvate metabolism and galactose metabolism [38–43] may indicate that transcriptional regulation of primary carbon turnover is altered in the no-tillage system.

Reduced transcriptional activity also affected processes related to nucleotide and membrane biosynthesis. Downregulation of purine metabolism and glycerophospholipid metabolism [44–46] may be consistent with reduced nucleotide synthesis and membrane biogenesis. In addition, reduced activity of terpenoid scaffold biosynthesis and glycine, serine and threonine, and cysteine and methionine metabolism [47–50] may indicate that transcriptional regulation of processes related to protein synthesis, hormone production, and the production of sulfur-containing protective molecules is also altered. Downregula-

tion of porphyrin metabolism and pentose and glucuronate conversions [39,51–53] may also be associated with reprogramming of processes related to chlorophyll biosynthesis and cell wall metabolism.

Overall, the transcriptomic pattern observed in the Ecseg area does not reflect a dominant change in a single metabolic process, but rather the simultaneous presence of growth and adaptation processes. The up-regulated anabolic and defense pathways, as well as the down-regulation of some elements of energy-intensive primary metabolism, together suggest that no-tillage cultivation in this environment may induce a physiological state in which the plant divides the available resources between growth and adaptation to stress.

3.2. Kiszombor

In the Kiszombor area, the transcriptomic changes observed in the no-tillage system indicate a complex metabolic transformation, in which processes related to increased energy production and biosynthetic activity are present. Overall, the upregulated pathways suggest that the plant has developed a metabolic state in which energy supply, cellular construction processes and mechanisms related to environmental adaptation operate in a coordinated manner.

Among the most significant changes is the increased activity of oxidative phosphorylation, which is a central process of mitochondrial ATP production. The upregulation of this process may be consistent with the increased energy demand that may be required by the parallel activated biosynthetic pathways and cellular maintenance processes [54]. In addition to energy supply, increased activity of pathways related to nucleotide and cofactor formation was also observed. Purine and pyrimidine metabolism provides nucleotides for DNA and RNA synthesis, which play essential roles in cell division and gene expression [55,56]. In line with this, increased riboflavin and thiamine metabolism may support the production of cofactors (e.g., FAD, FMN and TPP) that are involved in oxidative phosphorylation and other central metabolic processes, such as the pyruvate dehydrogenase complex [57–61]. In addition to the activation of primary metabolism, increased activity of secondary metabolic pathways has also been observed. The biosynthesis of various plant secondary metabolites and the increase in the formation of the terpenoid skeleton may indicate that the plant allocates metabolic resources not only for growth but also to support environmental response mechanisms [62–66]. These compounds, such as flavonoids, phytonutrients and essential oil components, may be involved in a variety of functions, including defense against pathogens, defense against herbivores, protection against UV radiation and hormonal signaling [67–69]. The significance of the terpenoid pathway is particularly complex, as the compounds produced through it may play a role in plant hormonal regulation and stress responses [62–69]. At the same time, however, downregulation of several processes related to growth and structural organization was also observed in the Kiszombor area. The reduced activity of starch and sugar metabolism may indicate that the regulation of carbohydrate metabolism has been altered under no-tillage conditions [70,71]. Since sugars serve not only as an energy source but also as carbon skeletons for several biosynthetic processes [72], transcriptional changes in these processes may influence the resource allocation of the plant. This may be consistent with the proposed alterations in photosynthesis-related processes [73,74], but without direct quantification of photosynthetic performance, this can only be interpreted as a transcriptomic inference.

The alterations in pathways involved in the formation of structural components are also noteworthy. The reduced activity of glycerophospholipid metabolism may be related to the transcriptional regulation of membrane lipid formation [75]. As membrane formation is closely linked to cell growth and the formation of new cell structures, alterations in this

process may indicate a reallocation of growth resources. Similarly, the reduction in pentose and glucuronate conversions and galactose metabolism may be related to alterations in the biosynthesis of cell wall polysaccharides and membrane-forming galactolipids [76–79]. Together, these changes may indicate that the plant is downregulating processes related to structural growth while maintaining other energy-intensive response mechanisms.

Overall, the transcriptomic pattern observed in the Kiszombor area indicates a complex, dual regulatory state. On the one hand, the increase in oxidative phosphorylation, nucleotide and cofactor formation, and secondary metabolism may indicate active energy supply and adaptive capacity. On the other hand, the reduced activity of some pathways related to carbohydrate metabolism and cell structure construction indicates that resource allocation has been modified. Overall, these results suggest that the plant responds to no-tillage conditions with increased metabolic activity, while limiting certain growth processes. This response may reflect a transitional state in which the plant tries to establish a balance between maintaining growth and adapting to environmental challenges.

3.3. Vityapuszta

In the Vityapuszta experimental area, the transcriptomic changes observed in the no-tillage cultivation system indicate a metabolic state in which both growth-related biosynthetic processes and mechanisms supporting environmental adaptation are activated. Similar to the other areas studied, a rearrangement of plant resources was observed here, but the ratio of up- and down-regulated pathways suggests that growth-related processes may play a greater role in this environment.

Among the up-regulated pathways, the joint activation of processes related to carbon fixation, central energy metabolism and cellular architecture may be consistent with the development of an integrated anabolic state. Carbon fixation via the Calvin cycle is the fundamental process of photosynthesis, which ensures the production of glucose [80]. The carbon sources thus formed can be stored through starch and sugar metabolism, or, when broken down during glycolysis, can provide energy and carbon skeletons for further biosynthetic processes [81]. Increased activity of glycolysis and pyruvate metabolism may therefore contribute to ATP and NADH production and to the provision of metabolic intermediates required for growth [41].

In parallel with the activation of central energy and carbon metabolism, increased activity of several pathways related to cell structure was also observed. The increased activity of purine metabolism may be consistent with the increased formation of nucleotides, which are essential building blocks for DNA and RNA synthesis and cell division [44]. Similarly, the increased activity of glycerophospholipid metabolism may be related to the increased production of lipids required for membrane formation, which is particularly important for maintaining the volume and surface structures of growing cells [82].

Processes related to protein synthesis and the maintenance of cellular redox balance were also activated. The increased activity of glycine, serine and threonine metabolism, as well as cysteine and methionine metabolism, may indicate an increased demand for amino acids by the plant, which are necessary for protein synthesis and the formation of important sulfur-containing molecules, such as glutathione and S-adenosylmethionine [83]. These processes may play a role in growth processes as well as in adaptation to environmental stresses.

The activation of secondary metabolism was also an important element of the response observed in the Vityapuszta area. The increase in terpenoid skeleton biosynthesis may be related to the formation of compounds that are involved in the biosynthesis of plant hormones (such as gibberellins and abscisic acid) and in various defense mechanisms [84]. In parallel, activation of phenylpropanoid biosynthesis may contribute to the production

of lignin and other phenolic compounds, which play a role in cell wall reinforcement, stress tolerance, and signaling [85]. These results suggest that the plant, in addition to maintaining growth, also develops a metabolic capacity that supports adaptation to environmental changes.

However, in parallel with the upregulated processes, decreased activity of several pathways related to structural growth and cell wall metabolism was also observed. Down-regulation of amino-sugar and nucleotide-sugar metabolism may affect processes that provide precursors for the formation of cell wall polysaccharides, such as cellulose and hemicellulose [86]. This may indicate that the transcriptional regulation of certain cell wall-building processes has been altered, which may indicate a temporary reallocation of resources.

Alterations in processes related to photosynthetic activity may also be indicated by reduced activity of porphyrin metabolism [87]. The porphyrin pathway plays a fundamental role in the formation of chlorophyll and heme groups [88]; therefore, the modification of this process may be related to regulatory changes related to light utilization and chloroplast function. However, the reduction in porphyrin metabolism in this case cannot be interpreted directly as the cessation of photosynthesis, but rather as a regulatory rearrangement of the photosynthetic system [89]. Another notable change was the downregulation of sphingolipid metabolism. Sphingolipids play an important role in maintaining the structural stability of membranes and in signal transduction processes [90]. Alterations in their metabolism may affect membrane properties [91] and in some cases may be associated with the regulation of programmed cell death (PCD) [92]. However, based on the present data, this change may primarily indicate a possible reprogramming of membrane homeostasis and signaling processes.

Overall, the transcriptomic results obtained in the Vityapuszta area indicate a balanced metabolic state in which growth-supporting anabolic processes and adaptation mechanisms are simultaneously present. The increased pathways related to carbon assimilation, energy production, nucleotide, lipid and protein synthesis suggest that the plant maintains significant biosynthetic activity, while the activation of secondary metabolism may be part of the response to environmental challenges. However, some pathways with reduced activity observed in parallel suggest that the plant also finely regulates its resource use in this environment. Thus, the Vityapuszta area may represent a state among the studied systems where growth and adaptation processes operate in a relatively balanced manner in addition to no-tillage.

3.4. Integrated Interpretation of the Three Study Areas

Based on the combined evaluation of the transcriptomic results obtained in the three experimental areas, the no-tillage cultivation system in sunflower did not trigger a single, uniform metabolic response, but resulted in resource reallocation of varying intensity depending on the environmental conditions. A common feature of the studied areas was the simultaneous regulation of growth processes and adaptation mechanisms; however, their ratio and dominance differed significantly at the individual locations.

In the Ecseg area, based on the transcriptomic pattern, the response associated with no-tillage cultivation was more strongly adaptive. In addition to the activation of pathways related to cell structure maintenance, secondary metabolism and defense processes, reduced activity of several energy-intensive primary metabolic processes can be observed. This may indicate that the plant shared its resources more intensively in this environment in order to support maintenance and adaptation processes, while the regulation of certain processes related to growth was modified.

In the Kiszombor area, on the other hand, a more complex, transient metabolic state emerged. Increased activity of oxidative phosphorylation, nucleotide and cofactor formation, and secondary metabolite biosynthesis may indicate a significant metabolic capacity. At the same time, reduced activity of pathways related to carbohydrate metabolism and certain structural building processes suggests that the available resources were not exclusively devoted to growth processes. This pattern may indicate a regulated response in which the plant maintains energy supply and adaptive capacity while limiting some growth investments.

In contrast, the changes observed in the Vityapuszta area indicate a more balanced metabolic state. Activation of pathways related to photosynthetic carbon fixation, energy production, nucleotide, lipid, and protein synthesis may indicate that the plant was able to maintain the biosynthetic activity necessary for growth. In parallel, the increase in secondary metabolism indicates that in addition to growth processes, environmental adaptation mechanisms also remained active.

Based on the comparison of the three areas, a general model can be outlined, according to which no-tillage cultivation primarily causes a redistribution of resources in the plant, rather than activating a single specific stress response. The plant dynamically allocates the available carbon sources, energy and biosynthetic capacity between growth, cell structure maintenance and defense mechanisms.

It is important to emphasize that the present study is based on transcriptomic data; therefore, changes in the identified pathways indicate modifications in gene expression regulation and do not necessarily mean direct changes in the actual activity of the given metabolic processes. Accordingly, the results primarily reveal potential regulatory patterns that can be confirmed by further physiological, biochemical and functional studies.

4. Materials and Methods

4.1. Experimental Areas, Cultivation Methods, Plant Material, and Agrotechnical Parameters

The field experiments were conducted in three adjacent (named Ecseg, Kiszombor and Vityapuszta), identically owned agricultural plots in Hungary (46.60511° N, 17.72377° E; approximately 211 m above sea level). The investigated arable plots were located within an area of approximately 5 km² and therefore represented the same agroecological environment. The region has a temperate continental climate, with an annual mean temperature of approximately 10 °C and an annual precipitation of typically 600–650 mm.

At each of the three investigated sites, two sunflower fields were investigated, located close to each other (approximately 200 m apart). One field was managed under conventional tillage, while the other was managed under long-term no-tillage. The studied areas belonged to the same economic unit; therefore, the cultivation technology parameters, including crop rotation, plant protection treatments and agrotechnical interventions, were coordinated.

The no-tillage technology has been applied in all three areas for 10 years, while the conventional tillage system has been used for several decades. Since the aim of the present study was to explore the effect of the cultivation systems on plant gene expression and biochemical responses, rather than to characterize soil physical and soil chemical parameters in detail, the study focused on the molecular and biochemical analysis of plant samples.

As a result of the above, the three areas are suitable for comparative field studies. The plant samples from the areas included in the experiment were named as follows:

1. Sunflower Kiszombor conventional tillage (Sample name: Sunflower_KCT_1 (containing forward and reverse pairs)).

2. Sunflower Kiszombor no-tillage (Sample name: Sunflower_KNT_1 (containing forward and reverse pairs)).
3. Sunflower Ecseg conventional tillage (Sample name: Sunflower_ECT_1 (containing forward and reverse pairs)).
4. Sunflower Ecseg no-tillage (Sample name: Sunflower_ENT_1 (containing forward and reverse pairs)).
5. Sunflower Vityapuszta conventional tillage (Sample name: Sunflower_VCT_1 (containing forward and reverse pairs)).
6. Sunflower Vityapuszta no-tillage (Sample name: Sunflower_VNT_1 (containing forward and reverse pairs)).

Since the country is small, the soil and climatic conditions of the fields involved in the three experiments are quite similar, so the test datasets of the three areas can be considered suitable for comparison. When designing the experiment, great emphasis was placed on including fields in the experiment where all soil factors were the same (soil type, previous crop (maize), nutrient supply, firmness, pH, cultivation methods, chemical treatments, etc.), thus eliminating any potentially distorting differences resulting from soil conditions. All three areas belonged to the chernozem soil type. As the basis of the no-tillage sowing system, the previous crop in all three areas was winter wheat, providing stable stalk residue for the sunflower. The stalk residue protects the soil, reduces weed growth, and improves water retention. In each of the experimental areas, the SY Excellio sunflower, a high-oleic hybrid of Syngenta Ltd., was sown, which is tolerant to the active ingredient imazamox. Its disease resistance is characterized by complete downy mildew resistance, making it resistant to all pathotypes known worldwide. It is an extremely homogeneous hybrid with a strong root system and broad adaptability. It is characterized by early maturity and a shorter-than-average growing season. Given its cultivation/agronomic characteristics (Table 1), the ideal sowing time was from 10 April to 20 May, with a recommended seed rate of 50,000–56,000 seeds/ha. The hybrid was sown on 19 April 2025, at a plant density of 55,000 plants/ha.

The product Pictor consists of two active ingredients, boscalid and dimoxystrobin. Boscalid is a nicotinamide fungicide that has a broad-spectrum bactericidal effect and a preventive effect [93]. Dimoxystrobin is a representative of strobilurins; its mode of action is based on the inhibition of mitochondrial respiration of fungi. By binding to mitochondrial enzyme systems, it inhibits the cellular respiration of pathogenic fungi, and spore germination and the development of germ tubes are eliminated [94].

One of the active ingredients of the Wing-P preparation is pendimethalin, which belongs to the dinitroaniline herbicide group. It enters the cells of the weed plant and inhibits RNA and DNA synthesis, and as a secondary effect, it disrupts the hormonal balance in the roots of young plants [95]. The preparation also contains dimethenamide, which is a widely used herbicide belonging to the class of chloroacetamides. It inhibits the synthesis of certain long-chain fatty acids, reducing the availability of lipids, proteins, and lignin, which are essential for plant growth [96].

In the case of pesticides based on the inhibition of the ALS (acetolactate synthase) enzyme (like Pulsar SL), cell division is reduced or absent due to the inhibition of protein synthesis. When these chemicals are used, protein cannot be synthesized because branched-chain amino acids (valine, leucine, and isoleucine) are not formed in the presence of these herbicides [97]. The insecticide Rafting contains acetamiprid, which strengthens the neonicotinoid group. The purpose of acetamiprid is to target the nicotinic acetylcholine receptors of insects [98]. The product Reglone Air is used for drying crops and contains diquat dibromide. Diquat dibromide is a herbicide, algicide, desiccant, and defoliant used on food crops [99].

Table 1. Agrotechnical operations performed on the tilling and no-tilling experimental areas.

Agrotechnical Operation	Treating Time	Chemical	Dosage
Stubble cultivation with an IH 6.2 tractor	October	-	-
Fertilizer spreading	October	7-20-14 NPK fertilizer	3 t/ha
Ploughing Lemken 6/6, 32 cm deep on the ploughed areas	November		
Fertilizer spreading	February	Pétisó fertilizer: 27% N, 5% Ca, 3% Mg	12 t/ha
Machining (gearing, finishing)	February	-	-
Seedbed preparation (NZ Väderstad combiner: An extreme pre-sowing cultivator)	April	-	-
Sowing and soil disinfection	April	Force 1.5 G SY Excellio	10 kg/ha 55.000 number of stems
Weed control (pre-emergence)	April	Wing P	3.5 L/ha
Weed control (post-emergence)	May	Pulsar SL	1.2 L/ha
Fungicide treatment	May	Pictor	0.5 L/ha
Insecticide treatment	June	Rafting	0.15 kg/ha
Desiccation	August	Reglone Air	1 L/ha
Harvesting	September	-	-

4.2. Genome-Wide Transcriptomic Analyses

For genome-wide transcriptomic studies, 20–40 mg of fresh leaf samples were collected (5 August 2025—BBCH 51—star bud stage) from each plant grown in both cultivation systems in all three areas in 4 biological replicates, which were preserved in 1.5 mL of RNALater (Invitrogen by Thermo Fisher Scientific Inc., Waltham, MA, USA) reagent until further use. The reagent rapidly enters the tissues and destroys RNase enzymes that would degrade RNA. Sample collection took place in the early morning hours, from the uppermost fully expanded, still photosynthetically active leaves of 4–4 plants per treatment in all three experimental areas.

Total RNA was extracted and purified from the stabilized samples, and the mRNA fraction was enriched using the NEXTFLEX® Poly(A) Beads 2.0 magnetic system (Revvity, Inc. Waltham, MA, USA). Only RNA samples with an RNA Integrity Number (RIN) of ≥ 7 , verified by repeated quality assessments, were used for subsequent analyses. RNA-seq libraries were prepared using the NEXTFLEX® Rapid Directional RNA-Seq 2.0 kit (Revvity, Inc. Waltham, MA, USA).

Equal amounts of RNA isolated from four independent biological samples within each treatment were pooled prior to library preparation. The resulting pooled RNA samples were sequenced using a low-depth strategy on the Illumina NovaSeq 6000 platform (Illumina Inc., San Diego, CA, USA). Consequently, six RNA-seq libraries were generated, each representing one of the two experimental conditions in each area (conventional tillage and no-tillage samples across the 3 sites).

Pooling biological samples prior to RNA sequencing has previously been described as a cost-effective strategy for exploratory transcriptomic studies [100]. However, this experimental design does not provide biological replication at the RNA-seq level and therefore does not allow direct estimation of biological variance. Each RNA-seq library represented the average transcriptomic profile of the corresponding treatment.

Accordingly, the transcriptomic analysis was considered exploratory and hypothesis-generating rather than statistically confirmatory. Differential transcript abundance was evaluated using the NOISeq-sim algorithm which generates simulated technical replicates to model technical variability in the absence of experimental replication. This approach does not replace biological replication and cannot estimate biological variance. Therefore, the identified differentially expressed genes were regarded as candidate genes rather than statistically confirmed differentially expressed genes, and this limitation was taken into account throughout the interpretation of the transcriptomic data.

To strengthen the biological relevance of the transcriptomic observations, the major pathways and selected candidate genes identified by RNA-seq were independently evaluated through biochemical analyses performed on biological samples. The observed changes in enzyme activities and metabolite accumulation were consistent with the transcriptomic patterns, providing independent experimental support for the biological processes suggested by the exploratory RNA-seq analysis.

Using FastQC version 0.12.0 software (https://timkahlke.github.io/LongRead_tutorials/QC_F.html (accessed: 22 January 2026)), regions with poor quality parameters were filtered from the raw reads, and then the reads were trimmed using Trimmomatic version 0.39 (<http://www.usadellab.org/cms/index.php?page=trimmomatic> (accessed: 22 January 2026)) [101]. The sequencing yielded a total of 210,311,447 raw reads across all libraries, with an average of approximately 30,000,000 reads per library after quality filtering (trimmed read counts are provided in the Supplementary File S7).

The average length of the raw paired-end forward and reverse reads obtained was 151 bp, with an average number of 30 million reads per sample [102] (Supplementary File S7).

A de novo transcriptome was constructed from the remaining, high-quality reads using Trinity software version 2.15.2 (<http://TrinityRNASeq.sourceforge.net> (accessed: 23 January 2026)) [103]. The aforementioned software can use sequenced data to create longer contigs by aligning short reads to one another, thereby reconstructing the entire transcriptome without a reference genome.

The identification of the longer unknown transcripts thus obtained was then carried out using the CloudBlast sequence alignment technique built into OmicsBox BioBam software version 3.4 [104]. The identified genes were then annotated with gene ontology (GO) terms using Blast2GO (a feature of OmicsBox BioBam software version 3.4) (accessed: 23 January 2026) [105]. In the next step, the discovery of the function (functional annotation) of the new sequences was performed using the EggNOG subprogram built into OmicsBox BioBam software version 3.4 [106].

To determine the individual gene expression levels of the identified contigs across two plant sample datasets, i.e., to map differences between the two plant populations at the gene level, differential expression analysis must be performed. The program used evaluates each transcript individually and then estimates the expression values of each read using the unique gene coordinates from the evaluation [107]. A count table is created from the program's calculations. This file is used to perform further pairwise comparisons, for which the authors used the NOISeq program (<https://bioconductor.org/packages/release/bioc/html/NOISeq.html>) (accessed: 23 January 2026).

The software can compare data sets from different sample locations pairwise and select genes with statistically significant expression differences [108]. Differential expression was initially defined using NOISeq probability > 0.9. To address the concern about biological effect size, we also applied a threshold of $|M| \geq 1$, approximately corresponding to a 2-fold expression change, which reduced the DEG set and improved biological

interpretability. M is a log-ratio-based effect size measure in NOISeq software (version 2.54.0 (Bioconductor package)).

The resulting genes were further examined, and those that were up- or down-regulated, over- or under-represented in each sample were determined [109]. They were individually verified, and their molecular and biochemical functions were determined [110]. All genes were then associated with the biochemical pathways in which they participate. For this, a combined pathway analysis was performed using the KEGG [111] and Plant Reactome [112] databases.

All relevant information required to ensure transparency and reproducibility of the RNA-seq workflow, including sample processing details, pooling strategy, sequencing quality parameters, transcriptome assembly statistics, transcript quantification data, normalization procedures, and differential expression analysis outputs, is provided in Supplementary File S7.

4.3. Laboratory Measurements of Total Phenolic Content, Total Flavonoid Content, Soluble Sugar Content, and Protein Content

The aggregated results from the transcriptomic analysis were compared with additional measurements of several typical laboratory parameters. Each of the parameters measured in the laboratory (total phenolic content, total flavonoid content, soluble sugar content, and protein content) is associated with some biochemical pathways that were significantly different in the individual data sets examined during the transcriptomic analyses. Consequently, the aforementioned laboratory test results are also suitable for validating the results obtained in the genome-wide analysis.

Simultaneously with the samples collected for transcriptomic analysis, whole leaves were detached and collected from the same sunflower plants at all three locations, both plowed and no-tillage, in 4 replicates each. The plant samples were stored in a -80°C refrigerator until further laboratory use. Total phenolic content and total flavonoid content were measured according to Ahmed et al. (2024) [113], but total soluble sugar content was estimated using the protocol of Prud'homme et al. (1992) [114], and crude protein content was measured according to Bradford (1976) [115]. The methodologies are described in detail in Supplementary File S1.

4.4. Post-Harvest Tests

After harvest, grain samples were taken from 4 to 4 plots per treatment, and their weights were measured to the nearest gram. The obtained quantities were then statistically analyzed and reported accordingly.

4.5. Statistical Analyses

Statistical analyses were performed to compare treatments for the measured laboratory parameters based on four biological replicates per sample. Mean $\pm$ standard deviation (SD) values were calculated, and one-way analysis of variance (ANOVA), followed by the least significant difference (LSD) test, was applied separately within each site to compare no-tillage and conventional tillage treatments under site-specific environmental conditions (Supplementary File S6_Worksheets 3–4). Statistical analyses were conducted using JASP software (version 0.19.3.0), and statistical significance was assessed at the 5% and 1% probability levels ($p < 0.05$ and $p < 0.01$).

To further evaluate the effects of site and tillage system on the measured biochemical parameters (total soluble sugar content, crude protein content, total phenolic content, and total flavonoid content), a two-way ANOVA was performed with site and tillage system as fixed factors, including their interaction (site $\times$ treatment) in the model (Supplementary File S8). The analysis was carried out using R software (version 4.5.3; R

Core Team) with the base *aov()* function within a linear model framework. The significance of the main effects and the interaction term was used to assess whether the effects of tillage were consistent across sites or depended on environmental conditions.

5. Conclusions

The combined interpretation of gene expression patterns from the Ecseg, Kiszombor, and Vityapuszta sites shows that sunflowers employ highly plastic metabolic strategies and strongly respond to different environmental conditions resulting from differences in cultivation methods. The three sites do not simply differ in the magnitude of metabolic activity but rather represent distinct physiological states that converge on a common adaptive survival strategy. In Ecseg, a metabolic polarization due to the different cultivations is the most typical. Upregulated amino acid, lipid, and aromatic metabolisms may indicate an intensive defense, stress-tolerant, and nitrogen-storing strategy (proline, arginine, phenylpropanoids).

In parallel, however, the strong downregulation of carbon fixation, central energy metabolism, nucleotide and porphyrin pathways clearly indicates the shutdown or stagnation of photosynthetic and growth functions. This combination suggests that in Ecseg, the sunflower does not want to develop, but to acclimate: it regroups its remaining resources for osmoprotective, antioxidant, and structural defense, while minimizing basic energy and biomass production.

In the case of Kiszombor, this is an energy-intensive but resource-deficient adaptive response. Upregulated energy and cofactor-producing processes (oxidative phosphorylation, purine/pyrimidine, riboflavin, and thiamine metabolism) indicate a state of high internal energy demand, while downregulation of carbohydrate and cell wall-building pathways suggests that the plant does not rely on fresh photosynthetic sources, but on internal reserves and efficient energy utilization. This duality describes a plant state that is active even under unfavorable conditions, but restrains its structural growth, where the emphasis is on maintaining the biochemical processes necessary for acclimation and on defensive readiness.

In Vityapuszta, a coherent, relatively active metabolic network emerges, but stress response activations also appear. Upregulated photosynthesis, glycolysis, purine, lipid, amino acid, and secondary metabolism indicate that the plant efficiently fixes carbon, actively produces energy, builds its cells (DNA, membranes, cell wall), and simultaneously strengthens its defense system (phenylpropanoids, terpenoids). Downregulated processes (aminosugar, porphyrin, sphingolipid metabolism) indicate fine-tuning rather than complete shutdown, indicating that the plant is trying to optimize its resources so that unfavourable environmental conditions do not force it to switch to survival mode.

To summarize, despite the pronounced site-specific differences in metabolic regulation, the three field sites revealed a common adaptive survival strategy in response to no-tillage. While Ecseg was characterized by a survival-oriented metabolic shutdown, Kiszombor by energy-demanding acclimation supported by internal reserves, and Vityapuszta by the maintenance of active growth together with enhanced defense, all three environments converged on the same fundamental adaptive strategy. No-tillage consistently promoted the reallocation of resources from primary growth processes toward secondary metabolism and defense-related pathways, indicating a conserved, location-independent survival response. These findings demonstrate that sunflowers do not passively suffer environmental impacts but dynamically reorganize their metabolism at the transcriptional level according to local conditions while maintaining a common adaptive framework. This metabolic flexibility likely contributes to the resilience and crop stability of sunflowers under sustainable agriculture.

Supplementary Materials: The following supporting information can be downloaded at: <https://data.mendeley.com/datasets/nskjs74r4/2>. Decsi, Kincső (2026), “Decsi, Kincső (2026), “Decsi et al._2026_No-tillage article data”, Mendeley Data, V3, doi: 10.17632/nskjs74r4.2”. The raw reads (SRA’s) were deposited in the National Center for Biotechnology Information (NCBI) database under the following accession numbers: Raw reads were deposited in the National Center for Biotechnology Information (NCBI) database under the following accessions: Submission ID: SUB15974028 and BioProject ID: PRJNA1420154, Sunflower under conventional tillage and no-tillage cultivation methods. (1) Repository name: PRJNA1420154 Sunflower under conventional tillage and no-tillage cultivation methods; Data identification number: PRJNA1420154 Direct URL to data: <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1420154> (accessed on 7 February 2026). (2) Repository name: Sunflower Ecseg conventional tillage_forward and reverse (Sunflower_ECT_1); Data identification number: SRR37169684; Direct URL to data: <https://www.ncbi.nlm.nih.gov/sra/?term=SRR37169684> (accessed on 7 February 2026). (3) Repository name: Sunflower Ecseg non tillage_forward and reverse (Sunflower_ENT_1); Data identification number: SRR37169683; Direct URL to data: <https://www.ncbi.nlm.nih.gov/sra/?term=SRR37169683> (accessed on 7 February 2026). (4) Repository name: Sunflower Kiszombor conventional tillage_forward and reverse (Sunflower_KCT_1); Data identification number: SRR37169650; Direct URL to data: <https://www.ncbi.nlm.nih.gov/sra/?term=SRR37169650> (accessed on 7 February 2026). (5) Repository name: Sunflower Kiszombor non tillage_forward and reverse (Sunflower_KNT_1); Data identification number: SRR37169649; Direct URL to data: <https://www.ncbi.nlm.nih.gov/sra/?term=SRR37169649> (accessed on 7 February 2026). (6) Repository name: Sunflower Vityapuszta conventional tillage_forward and reverse (Sunflower_VCT_1); Data identification number: SRR37169682; Direct URL to data: <https://www.ncbi.nlm.nih.gov/sra/?term=SRR37169682>; (accessed on 7 February 2026). (7) Repository name: Sunflower Vityapuszta non tillage_forward and reverse (Sunflower_VNT_1); Data identification number: SRR37169681; Direct URL to data: <https://www.ncbi.nlm.nih.gov/sra/?term=SRR37169681> (accessed on 7 February 2026).

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