Free Amino Acids and Methylglyoxal as Players in the Radiation Hormesis Effect after Low-Dose \( \gamma \)-Irradiation of Barley Seeds

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**Abstract:** Low-dose \( \gamma \)-irradiation can stimulate plant growth and development; however, the knowledge on the molecular mechanisms of such stimulation is yet fragmented. Irradiation of seeds leads to the mobilisation of endosperm resources and reallocation of available nitrogen to facilitate development. Based on the metabolomic analysis, several metabolites possibly involved in radiation stimulation were studied using the HPLC approach in barley cultivars after \( \gamma \)-irradiation of seeds. The comparison of changes in metabolite concentrations and changes in morphological traits after irradiation revealed seven metabolites that may be involved in the growth stimulation after \( \gamma \)-irradiation of barley seeds. Among them are free amino acids, such as \( \gamma \)-aminobutyric acid, \( \beta \)-alanine, arginine, lysine, glutamine, methionine, and a signalling compound methylglyoxal.

**Keywords:** radiation hormesis; GABA; \( \beta \)-alanine; arginine; lysine; glutamine; methionine; methylglyoxal; HPLC; morphological stimulation

1. Introduction

The world will require a dramatic increase in food production in the next 30 years because of the world population growth and the effects of climate change including, but not limited to, increased temperature, changing patterns of rainfall, and elevated levels of CO\(_2\) and ozone [1]. Improving the overall tolerance of plants to environmental stressors is a major focus of agrobiology, while the study of changes in metabolic pathways in response to stressors may be crucial for developing more tolerant plant cultivars [2].

Low-dose \( \gamma \)-irradiation of seeds often leads to a range of positive growth effects, such as increased biomass, accelerated germination and development, and improved immune responses and tolerance to stressors—the phenomenon known as the radiation hormesis [2–5]. A disruption of cellular homeostasis that ultimately leads to the unfolded protein response (UPR) [6] and heat shock response (HSR) [7] is among possible triggers of radiation hormesis. The UPR signalling pathway operates in the endoplasmic reticulum increasing the folding and clearance capacity of a cell by downregulation of the protein synthesis and upregulation of the synthesis of chaperones and components for proteasome degradation [7]. Similar processes in the nucleus and cytoplasm are controlled by the HSR pathway [7]; however, evidence suggests an interconnection between the UPR and HSR pathways in plants [8]. The positive effect on growth and development of low-dose irradiated plants can also be related to the modulation of reactive oxygen species (ROS) levels [5] and phytohormonal balance [9]. ROS-related responses might be connected to changes in phytohormonal signalling pathways since evidence supports ROS as being...
central molecules acting as hubs on different phytohormonal cascades [10]. However, the specific metabolites responsible for radiation hormesis effects still have to be discovered.

The development of “omic” technologies led to a breakthrough in the search for determinants of stress tolerance in plants [11]. Our group studied the hormesis effect after γ-irradiation of barley seeds on biochemical [12], transcriptomic [5], and metabolomic [2] levels. Several metabolites possibly related to the radiation stimulation effect have been established based on the transcriptional activity of genes in embryos of γ-irradiated barley seeds and on metabolic profiles of the roots and shoots of seedlings [2,5]. Low-dose γ-irradiation led to the mobilisation of endosperm resources and reallocation of available nitrogen to facilitate development [2]. To understand if those findings are universal for distinct cultivars, we chose a range of candidate metabolites (a number of proteinogenic amino acids and other stress-responsive metabolites) to study the metabolic responses of different barley cultivars to seed γ-irradiation.

2. Materials and Methods

2.1. Barley Cultivars, γ-Irradiation, and Sampling

Original seeds of seven barley cultivars (Hordeum vulgare L.) were used for assessing concentrations of candidate metabolites in response to γ-irradiation of seeds: Eryoma, Fedos, Grees, Fox 1, Leon, Master, Ratnik. The cultivars were ranked according to their sensitivity to acute irradiation of seeds (Figure S2) [13]. Growth stimulation was observed for the cultivars Fox 1, Ratnik, Eryoma, and Master; the cultivar Leon was inhibited after irradiation; the cultivars Grees and Fedos had no obvious morphological effects after irradiation (Figure S1) [13]. The same batches of seeds for assessing morphological effects were irradiated by 20 Gy for metabolic analysis (dose rate 60 Gy/h). The irradiation of seeds was performed at the γ-facility “GUR-120” (60Co, RIRAE). One thousand seeds of each cultivar in a plastic zip-bag were irradiated and then surface-sterilized using 10% H2O2 for 5 min, then, thoroughly rinsed in distilled water. Non-irradiated seeds were used as control.

Irradiated and control seeds were sown on paper rolls soaked in distilled water immediately after sterilization. Each paper roll contained 100 seeds. For each cultivar, 300 irradiated seeds and 300 control seeds were used. Paper rolls were placed in the growth chamber MIR-254 (Sanyo, Japan) where seedlings grew for seven days at the constant temperature 20 °C, in the dark. Humidity inside the paper rolls reached 100%, inside the growth chamber humidity varied from 75% to 80%. After seven days, tips of shoots (3 cm) and tips roots (4 cm) of 40 random seedlings from a paper roll were sampled for the metabolic analysis. Therefore, one combined root sample and one combined shoot sample (each consisting of 40 plants) were taken from each paper roll and frozen in liquid nitrogen. One g of frozen tissue for each sample was used for the subsequent HPLC analysis. Three biological replicates were used for each experimental condition.

2.2. Candidate Metabolites

In the previous study of γ-irradiated barley cultivar Nur, metabolomic profiles of more than 900 metabolites were obtained [2]. Eleven metabolites were chosen for the validation on a broader range of cultivars, including shikimate, methylglyoxal (MG), pyruvate, lysine, β-alanine, arginine, methionine, glutamine, γ-aminobutyric acid (GABA), serine, asparagine (Table 1). MG was chosen based on the transcriptomic research where an induction of glyoxalase family protein HORVU4Hr1G059270, involved in the MG synthesis, was revealed [5].
Table 1. Fold changes (FC) of candidate metabolites’ concentrations in seedlings of the Nur cultivar after irradiation at stimulating doses (5–20 Gy) (summarized from [5]).

| Metabolite | Organ | Dose, Gy | 5   | 10  | 15  | 20  | Fold Change |
|------------|-------|----------|-----|-----|-----|-----|-------------|
|            |       |          |     |     |     |     |             |
| Asparagine | shoots| 1.70     | 1.56| 1.37| 0.67|     |             |
|            | roots | 0.87     | 0.82| 0.91| 0.43|     |             |
| Arginine   | shoots| 2.36     | 1.42| 1.34| 1.20|     |             |
|            | roots | 0.66     | 0.52| 0.58| 0.47|     |             |
| GABA       | shoots| 2.04     | 1.45| 1.71| 0.88|     |             |
|            | roots | 0.87     | 0.95| 0.90| 0.98|     |             |
| Glutamine  | shoots| 2.14     | 1.70| 1.60| 1.28|     |             |
|            | roots | 0.75     | 0.46| 0.53| 0.34|     |             |
| Lysine     | shoots| 2.05     | 1.80| 1.38| 1.02|     |             |
|            | roots | 1.51     | 1.10| 1.44| 1.08|     |             |
| Pyruvate   | shoots| 1.17     | 1.03| 1.01| 0.89|     |             |
|            | roots | 0.44     | 0.46| 0.39| 0.31|     |             |
| Shikimate  | shoots| 0.57     | 0.83| 0.79| 0.76|     |             |
|            | roots | 0.79     | 0.61| 0.75| 0.64|     |             |
| β-Alanine  | shoots| 1.00     | 0.44| 0.64| 0.24|     |             |
|            | roots | 0.97     | 0.51| 0.60| 0.62|     |             |
| Methionine | shoots| 1.59     | 0.90| 0.94| 0.86|     |             |
|            | roots | 0.70     | 0.49| 0.57| 0.46|     |             |
| Serine     | shoots| 1.45     | 1.77| 1.22| 0.91|     |             |
|            | roots | 0.72     | 0.65| 0.65| 0.50|     |             |

2.3. HPLC Assay

For the qualitative and quantitative analysis, a high-performance liquid chromatograph LC-30 Nexera (Shimadzu, Kyoto, Japan) equipped with a reverse-phase column C18 Nanosphere Eco 80 ODS-1 (Elsico, Moscow, Russia) (5 µm, length 250 mm, diameter 4.6 mm) was used. Standards of metabolites, phenyl isothiocyanate, and triethylamine were purchased from Sigma-Aldrich (Taufkirchen, Germany); hexane was purchased from Honeywell (Seelze, Germany), acetonitrile from Panreac (Darmstadt, Germany). The raw data were analysed using LabSolutions software (Shimadzu, Kyoto, Japan). Standard 0.1 M solutions of metabolites were prepared, and then a series of standard dilutions was created for each metabolite.

From each frozen sample, 0.2 g of tissue was homogenised in liquid nitrogen. After grinding, the sample was placed in a 2 mL tube containing 1.5 mL of ddH₂O. The tubes were placed on a rotator for 15 min, then centrifuged at 14,500 rpm during 10 min. A supernatant was divided into two parts: one was used for amino acid derivatisation, the other for identification of shikimate, MG, and pyruvate.

For amino acid derivatisation, 400 µL of sample extract was placed in a 2 mL tube containing 200 µL of 1M triethylamine and 200 µL of 0.1 M methyl isothiocyanate. After thorough mixing, the tubes were incubated for 1 h at 4 °C. After incubation, 800 µL of hexane were added, the solution was thoroughly mixed. After 10 min incubation, the lower layer was taken and filtered through a 0.45 µm filter [14].

Derivatised and non-derivatised extracts were filtered through solid-phase extraction columns ISOLUTE C18 (Biotage, Uppsala, Sweden) using VacMaster-20 (Biotage, Uppsala, Sweden). Ten µL of the resulting extract was used for the HPLC analysis.

The wavelengths for metabolites detection were 210 nm for shikimate, 254 nm for derivatised amino acids, 284 nm for MG, 324 nm for pyruvate. We were unable to split serine and asparagine peaks completely; therefore, the sum of concentrations of these two amino acids is provided. The column temperature was 36 °C; flow rate was 0.7 mL per min. Mobile phase A consisted of 0.1 M CH₃COONa and acetonitrile (14:1).
Mobile phase B consisted of acetonitrile and ddH$_2$O (4:1). Mobile phases were thoroughly mixed and filtered through a 0.45 µm filter. The mode of elution is shown in Table 2.

Table 2. The mode for gradient elution of metabolites.

| Time (min) | Mobile Phase A % | Mobile Phase B % |
|-----------|-----------------|-----------------|
| Amino Acids                        |                |                |
| 0         | 100             | 0               |
| 5         | 91              | 9               |
| 7         | 91              | 9               |
| 12        | 70              | 30              |
| 14        | 70              | 30              |
| 17        | 50              | 50              |
| 25        | 0               | 100             |
| 30        | 100             | 0               |
| Shikimate, MG, Pyruvate           |                |                |
| 0         | 100             | 0               |
| 5         | 91              | 9               |
| 7         | 91              | 9               |
| 9         | 70              | 30              |
| 11        | 70              | 30              |
| 14        | 50              | 50              |
| 16        | 0               | 100             |
| 20        | 100             | 0               |

2.4. Data Analysis

The statistical analysis of the experimental data was based on non-parametric approaches using Microsoft Office Excel 2019 and STATISTICA 8.0 software. Median values of metabolite concentrations are provided in Table S1. The significance of differences was estimated using Mann–Whitney U-test. Spearman’s rank correlation coefficient was assessed using Python 3.8; for the correlation heat map, a library seaborn was used.

3. Results

3.1. The Analysis of Metabolite Contents in Roots and Shoots of Irradiated Plants

Different barley cultivars were characterized by distinct concentrations of target metabolites in response to the radiation exposure. The fold changes of metabolite concentrations in the organs of irradiated plants compared to non-irradiated plants are presented in Table 3.

Table 3. The fold changes of metabolite concentrations in irradiated barley cultivars compared to the control plants.

| Metabolite     | Fox 1 | Ratnik | Eryoma | Master | Fedos | Grees | Leon |
|----------------|-------|--------|--------|--------|-------|-------|------|
| SHOOTS
| Shikimate 0.91 | 0.92 * | 0.90 | 0.74 | 0.91 | 0.80 | 0.96 |
| Methyglyoxal 0.64 | 3.86 | 3.91 * | 1.81 | 0.98 | 1.11 | 4.86 |
| Pyruvate 0.89 | 1.00 | 0.93 | 0.84 * | 0.81 | 0.83 | 1.00 |
| Lysine 1.80 * | 1.23 | 1.01 | 0.93 | 0.85 | 0.93 | 1.29 |
| β-Alanine 102.93 * | 1.12 | 62.36 | 0.76 | 1.91 | 0.83 | 0.72 |
| Arginine 3.45 * | 0.26 | 0.19 * | 0.59 * | 0.84 | 1.29 | 0.40 |
| Methionine 12.22 | 0.37 | 0.55 * | 3.65 | 1.11 | 0.88 | 0.64 |
| Glutamine 0.00 | 0.84 | 0.00 | 0.00 | 1536 ** | 0.94 | 0.98 |
| GABA 1.23 * | 0.59 | 0.45 | 0.79 | 0.85 | 0.92 | 1.36 |
| Serine + Asparagine 0.87 | 1.15 | 1.01 | 0.91 | 0.29 | 0.29 | 1.36 |
Table 3. Cont.

| Metabolite            | Fox 1 | Ratnik | Eryoma | Master | Fedos | Grees | Leon  |
|-----------------------|-------|--------|--------|--------|-------|-------|-------|
| **ROOTS**             |       |        |        |        |       |       |       |
| Shikimate             | 0.83  | 0.91   | 0.80   | 0.87   | 1.34  | 1.19  | 0.81  |
| Methylglyoxal         | 1.28  | 1.53   | 1.79   | 0.65 * | 1.29  | 0.75  | 0.94  |
| Pyruvate              | 1.23  | 0.91   | 1.30   | 1.15   | 1.07  | 1.48  | 0.97  |
| Lysine                | 0.82  | 1.43   | 1.53   | 1.04   | 0.95  | 1.04  | 2.93  |
| β-Alanine             | 1.63  | 0.29   | 1.89   | 2.35   | 0.00  | 0.42  | 1.86 *|
| Arginine              | 1.00  | 1.11   | 1.07   | 1.01   | 0.98  | 1.04  | 3.62 *|
| Methionine            | 0.16  | 0.99   | 1.12   | 0.14   | 2.35 *| 1.79  | 1.45  |
| Glutamine             | 0.52  | 2.71 * | 1.69   | 1403 **| 3.74  | 1.15  | 1.23  |
| GABA                  | 0.77  | 1.67 * | 2.07   | 0.75   | 3.94  | 1.30  | 1.27  |
| Serine + Asparagine   | 1.54 *| 1.85 * | 1.23   | 0.71   | 0.56  | 1.55  | 1.46 *|

Note: *—the statistically significant ($p < 0.05$, Mann–Whitney U-test) changes in the concentrations of metabolites in organs of irradiated plants compared to the non-irradiated. **—glutamine was identified in one control sample and in all irradiated samples.

3.2. Methylglyoxal, Shikimate, Pyruvate, γ-Aminobutyric Acid, and β-Alanine

A significant decrease in concentrations of shikimic acid in shoots of the Ratnik cultivar was found (Table 3, Figure 1). A significant three-fold increase in methylglyoxal concentrations was found in shoots of the Eryoma cultivar, while in roots of the Master cultivar a decrease in the MG root concentration was observed (Table 3, Figure 1). A significant decrease in the concentrations of pyruvic acid in shoots of the Master cultivar was noted (Table 3, Figure 1). A significant increase in the γ-aminobutyric acid (GABA) content was found in the roots of the Ratnik cultivar and shoots of the Fox 1 cultivar (Table 3, Figure 1). A drastic increase in the concentration of non-proteinogenic amino acid β-alanine in shoots of barley cultivars Fox 1 and Eryoma, and in roots of the Leon cultivar was found (Table 3, Figure 2).

3.3. Free Proteinogenic Amino Acids

A significant two-fold increase in the concentration of lysine was found in shoots of the Fox 1 cultivar (Table 3, Figure 2). We found a significant decrease in arginine concentrations in shoots of barley cultivars Eryoma and Master, and three-fold increases in shoots of the Fox 1 cultivar and in the roots of the Leon cultivar (Table 3, Figure 2). The concentrations of methionine was significantly decreased in shoots of the Eryoma cultivar, and increased in the roots of the Fedos cultivar (Table 3, Figure 2). We found a significant increase in the glutamine content in roots of the Ratnik cultivar (Table 3, Figure 2).

We were unable to completely separate the peaks of polar uncharged molecules of serine and asparagine; therefore, the data are presented as the sum of two peaks. A significant increase in the content of these amino acids was found in roots of cultivars Fox 1, Leon, and Ratnik (Table 3, Figure 2).

3.4. Relationships between Changes in Metabolites Concentrations and Changes in Morphological Traits of Irradiated Plants

Data on the morphological traits of the studied cultivars in response to irradiation are presented in Figure S1 [13]. The following significant correlations were revealed: fold change (FC) of lysine in shoots and FC of shoot biomass ($r = 0.61$), FC of β-alanine in shoots and FC of root biomass ($r = 0.71$), FC of glutamine in shoots and FC of shoot length ($r = -0.91$), FC of glutamine in shoots and FC of shoot biomass ($r = -0.63$) (Figure 3); FC of shikimate in roots and FC of shoot biomass ($r = -0.64$), FC of methylglyoxal in roots and FC of root biomass ($r = 0.82$), FC of β-alanine in roots and FC of shoot biomass ($r = 0.79$), FC of methionine in roots and FC of shoot biomass ($r = -0.86$), FC of GABA in roots and FC of shoot biomass ($r = -0.64$), FC of serine+asparagine in roots and FC of root length ($r = 0.68$) (Figure 4).
Figure 1. The fold changes of shikimic acid, methylglyoxal, pyruvic acid, and GABA concentrations in roots and shoots of barley after γ-irradiation of seeds compared to the control. *—the statistically significant ($p < 0.05$, Mann–Whitney U-test) changes in the concentrations of metabolites in organs of irradiated plants compared to the non-irradiated. Note: The fold change is assessed as a median of molar concentrations in roots (shoots) of irradiated plants with respect to a median of molar concentrations in roots (shoots) of non-irradiated plants. Cultivars Fox 1, Ratnik, Eryoma, and Master are γ-stimulated; the cultivar Leon is γ-inhibited; the cultivars Grees and Fedos have no obvious morphological changes after irradiation.
Figure 2. The fold changes of proteinogenic amino acids and β-alanine concentrations in roots and shoots of barley after γ-irradiation of seeds compared to the control. *—the statistically significant ($p < 0.05$, Mann–Whitney U-test) changes in the concentrations of metabolites in organs of irradiated plants compared to the non-irradiated. Note: The fold change is assessed as a median of molar concentrations in roots (shoots) of irradiated plants with respect to a median of molar concentrations in roots (shoots) of non-irradiated plants. Cultivars Fox 1, Ratnik, Eryoma, and Master are γ-stimulated; the cultivar Leon is γ-inhibited; the cultivars Grees and Fedos have no obvious morphological changes after irradiation.

To follow metabolic reallocation after irradiation, root/shoot (R/S) ratios of metabolite concentrations were assessed for irradiated and control plants (Table 4). Generally, the R/S ratio of MG diminished after irradiation, suggesting increased accumulation of this compound in shoots. β-Alanine’s R/S ratio drastically decreased in the γ-stimulated cultivars after irradiation and increased in the γ-inhibited cultivar Leon (Table 4). Arginine and glutamine reallocations to roots were often observed after irradiation.
3.4. Relationships between Changes in Metabolites Concentrations and Changes in Morphological Traits of Irradiated Plants

Data on the morphological traits of the studied cultivars in response to irradiation are presented in Figure S1 [13]. The following significant correlations were revealed: fold change (FC) of lysine in shoots and FC of shoot biomass ($r_s = 0.61$), FC of $\beta$-alanine in shoots and FC of root biomass ($r_s = 0.71$), FC of glutamine in shoots and FC of shoot length ($r_s = -0.91$), FC of glutamine in shoots and FC of shoot biomass ($r_s = -0.63$) (Figure 3); FC of shikimate in roots and FC of shoot biomass ($r_s = -0.64$), FC of methylglyoxal in roots and FC of root biomass ($r_s = 0.82$), FC of $\beta$-alanine in roots and FC of shoot biomass ($r_s = 0.79$), FC of methionine in roots and FC of shoot biomass ($r_s = -0.86$), FC of GABA in roots and FC of shoot biomass ($r_s = -0.64$), FC of serine+asparagine in roots and FC of root length ($r_s = 0.68$) (Figure 4).

Figure 3. Correlations of changes in morphological traits of irradiated plants and changes in metabolic concentrations in shoots of irradiated plants. Note: SL—fold change of the shoot length of irradiated plants; RL—fold change of the root length of irradiated plants; SB—fold change of the shoot biomass of irradiated plants; RB—fold change of the root biomass of irradiated plants.

Figure 4. Correlations of changes in morphological traits of irradiated plants and changes in metabolic concentrations in roots of irradiated plants. Note: SL—fold change of the shoot length of irradiated plants; RL—fold change of the root length of irradiated plants; SB—fold change of the shoot biomass of irradiated plants; RB—fold change of the root biomass of irradiated plants.
Table 4. The ratio of the metabolite concentrations in roots to the concentration in shoots (R/S ratio) for irradiated and non-irradiated barley cultivars.

| Metabolite      | Fox 1 (R/S) | Ratnik (R/S) | Eryoma (R/S) | Master (R/S) | Fedos (R/S) | Grees (R/S) | Leon (R/S) |
|-----------------|-------------|--------------|--------------|--------------|-------------|-------------|------------|
|                 | 0 Gy 20 Gy  | 0 Gy 20 Gy  | 0 Gy 20 Gy  | 0 Gy 20 Gy  | 0 Gy 20 Gy  | 0 Gy 20 Gy  | 0 Gy 20 Gy  |
| Shikimate       | 0.1 0.1     | 0.1 0.1      | 0.1 0.1      | 0.0 0.0      | 0.1 0.1      | 0.0 0.0      | 0.1 0.1     |
| Methylglyoxal   | 0.1 0.4     | 0.2 0.2      | 0.2 0.2      | 0.2 0.2      | 0.3 0.7      | 0.3 0.3      | 0.3 0.3     |
| Pyruvate        | 0.4 0.7     | 0.2 0.7      | 0.1 0.1      | 0.1 0.1      | 0.1 0.3      | 0.2 0.2      | 0.2 0.2     |
| Lysine          | 0.6 0.6     | 0.2 0.2      | 0.5 0.5      | 0.7 0.6      | 0.3 0.3      | 0.3 0.3      | 0.3 0.3     |
| β-Alanine       | 60.0 1.0    | 0.2 0.2      | 1330 1.0     | 0.5 1.0      | 2.0 2.0      | 2.4 2.4      | 0.2 0.2     |
| Arginine        | 4.8 4.8     | 1.2 2.4      | 5.2 1.2      | 7.8 1.2      | 2.7 2.7      | 2.7 2.7      | 2.9 2.9     |
| Methionine      | 6.3 0.1     | 0.6 0.6      | 1.7 1.7      | 1.0 2.1      | 8.9 0.3      | 0.3 0.7      | 0.1 0.3     |
| Glutamine       | 1.0 1.0     | 0.8 0.8      | 2.7 2.7      | 2.1 1.0      | 1403 100     | 485 1815     | 1623 1.2    |
| GABA            | 3.4 2.4     | 0.5 0.5      | 1.3 1.3      | 0.2 0.2      | 0.9 0.9      | 1.8 1.8      | 0.7 1.7     |
| Ser + Asp       | 0.3 0.3     | 0.6 0.6      | 0.5 0.5      | 0.8 0.8      | 0.9 0.9      | 1.0 1.0      | 0.3 0.3     |

4. Discussion

Several earlier findings for the barley cultivar Nur [2] were confirmed on a broader range of barley cultivars. These include the possible involvement of GABA, methylglyoxal, β-alanine, and free proteinogenic amino acids, such as arginine, lysine, glutamine, and methionine in the establishment of morphological stimulation after the application of low-dose γ-irradiation to crop seeds.

The results obtained for shikimate, pyruvate, serine, and asparagine metabolites did not support the significant involvement of these metabolites in growth responses to low-dose ionizing radiation. Shikimic acid metabolism in plants provides a carbon skeleton for the synthesis of the aromatic amino acids L-tryptophan, L-phenylalanine, and L-tyrosine [15]. There was revealed a weak correlation of root concentrations of shikimate and root length (Figure 4); however, similar patterns of shikimate response in γ-stimulated and γ-inhibited cultivars (Figure 2) do not allow choosing this metabolite as a target. No apparent regularities were found for either concentrations of pyruvic acid, which participates in photosynthesis and the Krebs cycle [16], or for the amino acids serine and asparagine.

4.1. Stress-Responsive Metabolites

γ-Aminobutyric acid. GABA is a non-proteinogenic amino acid, a key metabolite of primary and secondary metabolic pathways that rapidly accumulates in plant tissues in response to biotic and abiotic stresses [17]. We observed an increase in GABA concentrations in roots of the γ-stimulated cultivar Ratnik and in shoots of the γ-stimulated cultivar Fox 1. γ-Aminobutyric acid is involved in the general nitrogen metabolism and in the storage and transportation of nitrogen [18]. During its catabolism, GABA is converted to succinic semialdehyde (SSA), of which concentrations were decreased in the metabolic profile of the Nur cultivar roots after irradiation at stimulating doses of 10–15 Gy and increased in all other conditions, demonstrating the opposite pattern with shoot concentrations and having a negative correlation with root length [2]. It was hypothesized that the degradation of GABA could limit the accumulation of ROS under oxidative stress conditions [18]. Catabolism of GABA could be used for the rapid generation of succinate and energy (as NADPH) via the Krebs cycle after removal of the stress factor [19]. This suggests the role of GABA catabolism under radiation exposure as a possible source of energy and/or ROS production limiter. The protective effect of the application of GABA or its isomer, β-aminobutyric acid, enhances the abiotic stress tolerance in plants [20]. The increase in GABA levels in organs of γ-stimulated cultivars suggests a possible role of this non-proteinogenic amino acid in the stimulating effect occurrence (Table 3).

Methylglyoxal. Methylglyoxal is a dicarbonyl compound, which accumulates in cells as a by product of various metabolic pathways, including glycolysis, and plays signalling roles via Ca²⁺, ROS, and ABA signalling [21]. MG concentrations were generally increased in roots of γ-stimulated cultivars (except Master, Figure 1), and the positive correlation was
found between an increase in MG root concentrations and the biomass of roots ($r_s = 0.82$, Figure 4). MG was chosen as a candidate metabolite based on the whole-transcriptome analysis of irradiated barley embryos of the Nur cultivar [5] where the induction of glyoxyrase, the enzyme of MG metabolism, was shown in barley embryos 48 h after the irradiation of seeds. Recently, global gene expression profiling has shown that MG could induce signalling cascades, and an overlap between MG-responsive and stress-responsive signalling events might exist in plants [22].

This compound has a dual role: an overaccumulation provokes ROS generation, while lower concentrations provide signalling functions [23]. MG is connected with glutathione-related redox regulation, which is crucial for plant defence and adaptive responses under changing environmental conditions [22]. Cadmium (Cd) stress decreased wheat morphological traits in a concentration-dependent manner, while toxic effects of Cd were alleviated by exogenously applied MG [23], suggesting that MG could mitigate Cd toxicity in wheat. Similarly, spraying with 10 mM methylglyoxal did not decrease the fresh weight of wheat plants, while photosynthetic parameters even improved and chlorophyll content increased in some cases [24]. Increased transcript levels of glutathione-S-transferases and omega-3 fatty acid desaturases were detected after the MG spraying [24]. Considering the increase in MG concentrations in organs of stimulated cultivars, it is plausible to consider the role of this molecule as a player in the network for hormetic effect establishment.

$\beta$-Alanine. Another non-proteinogenic amino acid, $\beta$-alanine, is accumulated in plants as a general stress-responsive molecule involved in plant protection from extreme temperature, hypoxia, drought, heavy metal shock, and some biotic stresses [25]. Strong induction of $\beta$-alanine was observed in shoots of $\gamma$-stimulated cultivars Fox 1 and Eryoma (Figure 2). Changes in $\beta$-alanine concentrations in shoots correlated with changes in root biomass after irradiation ($r_s = 0.71$, Figure 3), and the same pattern was observed for FC of $\beta$-alanine in roots and FC of shoot biomass ($r_s = 0.79$, Figure 4). The exact role of $\beta$-alanine in stress tolerance remains unknown, though the tolerance mechanisms may involve the increase in coenzyme A synthesis and the role of $\beta$-alanine in secondary metabolism, including lignin biosynthesis [25]. The role of cell wall components in the radiation hormesis effect was demonstrated in transcriptomic studies of irradiated barley embryos [5].

4.2. Free Proteinogenic Amino Acids

Lysine, alanine, arginine, methionine, and glutamine belong to proteinogenic amino acids. Generally, high abundant amino acids such as proline, arginine, asparagine, glutamine, and GABA are synthesized during abiotic stress, while most of the low abundant amino acids are accumulated due to increased protein turnover rather than synthesis induction [26].

Arginine. In plants, arginine is involved in the storage and transport of nitrogen and is a precursor of polyamines and nitric oxide [27]. A disturbance in nitrogen metabolism under stress is a well-known consequence of stress exposure [28]. Under stress conditions, the number of specific nitrogen-containing compounds with diverse functions can accumulate in cells, including free amino acids, amides, diamines, and polyamines [29]. Among the 21 proteinogenic amino acids, arginine has the highest nitrogen to carbon ratio, which makes it especially suitable as a storage form of organic nitrogen [27]. Arginine concentrations were drastically increased in shoots of the most stimulated and in roots of the most inhibited cultivar (Table 3). The metabolomic analysis of the Nur cultivar also revealed the sufficiently increased concentrations of arginine in shoots, especially after irradiation at stimulating doses of 15 and 20 Gy [2]. Those data also suggested the radiation-induced redistribution of available nitrogen from roots to shoots [2]. However, the redistribution of arginine to shoots was found only for the most stimulated cultivar Fox 1, while other cultivars had a rather opposite pattern, accumulating arginine in roots after irradiation (Table 4). It is probable that the $\gamma$-stimulated cultivars Fox 1 and Nur have an increased synthesis of arginine in leaves, since the synthesis in chloroplasts via ornithine
is apparently the only operational pathway to provide arginine in plants, and the rate of arginine synthesis is tightly regulated by various feedback mechanisms in accordance with the overall nutritional status [27].

Lysine. In higher plants, lysine is synthesized by a metabolic pathway starting with aspartate, during which threonine, methionine, and isoleucine are also formed [30]. FC of concentrations of free lysine gradually decreased from shoots of γ-stimulated cultivars to shoots of “no effect” cultivars (Figure 2), and drastically increased in roots of the γ-inhibited cultivar Leon (Figure 2). Changes in lysine concentrations in shoots correlated with changes in shoot biomass ($r_s = 0.61$, Figure 3). For Arabidopsis thaliana stress-response studies, several amino acids including lysine showed particularly high fold increases under different stress conditions, and, based on bioinformatic studies, a strong induction of their synthesis has been postulated [26]. Accumulation of the normally low abundant amino acids is a consequence of increased protein turnover during abiotic stress [26], while increased protein turnover is a part of the unfolded protein response (UPR) and can be one of the reasons of hormesis effects [6]. In high-lysine rice it was shown that lysine accumulation was also associated with UPR [31]. Several findings suggest that an excessive increase in lysine content also affects other metabolic pathways, thereby affecting the growth and development of the target plant [32]. Meanwhile, the results of metabolomic and transcriptomic analyses suggested significant increases in TCA cycle metabolites in germinated seeds with a high free lysine level, potentially affecting energy metabolism in the germinated seeds and subsequent seedling establishment [33].

Due to its complex structure, the oxidation of lysine produces high amounts of ATP, which might be essential for survival during stress conditions provoking carbohydrate starvation [34]. In addition, lysine is the precursor for N-hydroxy-pipeolic acid, a transmitter during the establishment of systemic acquired resistance [35]. Pipeolic acid, a product of lysine catabolism, showed an increase in roots and shoots of barley under various stress treatments [36]. However, in our previous work, where the analysis of barley metabolome after irradiation was performed, the concentrations of pipeolate were increased only after irradiation at a very high dose, 100 Gy [2]. Therefore, the increase in free lysine after increased protein turnover may indeed be related to the enhanced production of ATP.

Glutamine. The amino acid glutamine is the central molecule of nitrogen metabolism, which accepts reduced nitrogen in the nitrogen assimilation pathways and acts as an amino group donor in central and secondary metabolism. In addition, glutamine can act as a signalling molecule in plants [37,38]. The glutamine concentrations in shoots negatively correlated with shoot length ($r_s = −0.91$, Figure 3) and shoot biomass ($r_s = −0.63$, Figure 3), and the R/S ratio of glutamine concentrations was increased in almost all γ-stimulated cultivars (Table 4). Glutamine is a major amino donor for the synthesis of amino acids, nucleotides, and other nitrogen-containing compounds in all organisms. Application of glutamine to the roots of rice plants demonstrated that glutamine may also function as a signalling molecule to regulate gene expression in plants [37]. Levels of glutamine in rice rapidly decreased within 15 min of nitrogen starvation treatment, indicating that part of the N-deficient signals could be mediated by glutamine [39]. Low concentrations of free glutamine in γ-stimulated cultivars (Table 3) suggest efficient usage of this amino acid during germination after seed irradiation.

Methionine. Methionine plays an important regulatory role at several levels of cellular metabolism, participating in the initiation of mRNA translation and having a regulatory role in the form of S-adenosylmethionine (SAM) [40]. Methionine concentrations were drastically increased in the shoots of γ-stimulated Fox 1 and Master cultivars (Figure 2). Changes in methionine concentrations in roots negatively correlated with changes in shoot biomass ($r_s = −0.86$, Figure 4). As a donor for methyl groups, methionine through SAM regulates essential cellular processes such as cell division, cell wall synthesis, chlorophyll synthesis, and membrane synthesis [41]. Moreover, SAM is the source of the propylamine group in the synthesis of the polyamines spermidine and spermine, which play crucial roles
in many aspects of plant growth, including cell proliferation and differentiation, apoptosis, homeostasis, and gene expression [41].

5. Conclusions

Seven metabolites that may be involved in the growth stimulation after γ-irradiation of barley seeds were revealed based on metabolomic screening and subsequent validation of the results on several barley cultivars. Most of these belong to free amino acids: γ-aminobutyric acid, β-alanine, arginine, lysine, glutamine, methionine, and also includes a signalling compound methyleglyoxal. Concentrations of these metabolites (except glutamine) were increased in the cultivars that showed growth stimulation after γ-irradiation of seeds. The increased concentrations of free amino acids may be a consequence of the UPR response after radiation stress. The role of GABA, MG, β-alanine, lysine, and methionine metabolism under radiation exposure probably includes ATP production, antioxidant defence, and cell wall structural changes. Arginine and glutamine most likely participate in nitrogen redistribution and reallocation, creating conditions for faster growth after moderate stress exposure.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agriculture11100918/s1, Figure S1: Morphological traits of barley cultivars, Figure S2: Ranking results reflecting radiosensitivity of the H. vulgare cultivars after γ-irradiation of seeds, Table S1: Median values of metabolite concentrations (µg/mL).

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