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Comparison of Hyperspectral Imagery and Physiological Characteristics of Bentazone-Tolerant and -Susceptible Soybean Cultivars

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Abstract: Bentazone is a broadleaf post-emergence herbicide widely used for crop production that inhibits photosynthetic activity, resulting in phytotoxicity and injury in plants. Evaluating and identifying herbicide-tolerant genotypes is a critical step in plant breeding programs. In this study, we determined the reaction of 138 Korean soybean cultivars to bentazone using visual evaluation, and selected cultivars were further evaluated to determine the effects of bentazone on physiological parameters. For physiological parameters, we measured the normalized difference vegetation index (NDVI) from hyperspectral reflectance images. From 2 to 4 DAT, the NDVI for two sensitive cultivars was between 0.60 and 0.69, while the NDVI for tolerant cultivars was between 0.70 and 0.86. Photosynthesis rate (A), transpiration (E), stomatal conductance (g_{sw}), and total conductance of CO_2 (g_{tc}) were measured using chlorophyll fluorescence. Visual score evaluation showed that moderate bentazone-tolerant cultivars were predominant among the Korean cultivars. For physiological measurements, differences in NDVI were detected between bentazone-tolerant and -sensitive cultivars 2 days after treatment (DAT). However, the A, E, $g_{sw,}$ and g_{tc} levels dramatically decreased 1 DAT in the sensitive cultivars. This study provides insights into the tolerance and sensitivity of soybeans to bentazone.

Keywords: soybean; herbicide; bentazone tolerance; physiological parameters; hyperspectral imagery

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1. Introduction

Soybean (*Glycine max* L.) is an essential commodity worldwide because it is a source of protein and oil for animal feed, aquaculture, industrial uses, and human diet [1–3]. In addition, soybean is a legume grown in 95 countries and is the fourth most widely cultivated crop globally in terms of sown area, after wheat, rice, and maize. Soybean was produced in 120.5 million ha area worldwide in 2019 [4].

Herbicide application is a weed management strategy for increasing crop production [5]. Approximately 37% of achievable soybean production is decreased by weed competitiveness [6]. Pre-emergence (PRE) herbicides are sometimes inadequate for controlling grasses and broadleaf weeds because of heavy rainfall after their application [7]. It is difficult to control weeds using only PRE herbicides. Thus, the application of PRE herbicides and post-emergent (POST) herbicides is a better approach to avoid crop yield losses from weed competition [8,9].

Bentazone (3-isopropyl-1H-2.1.3-benzothiadiazin-4(3H)-one-2,2-dioxide), a benzothiadiazole, is a photosystem (PS) II inhibitor that is widely used for selective POST control of broadleaf weeds in crops such as rice, maize, peanut, beans, and soybean [7,10]. In plants, this substance competes with plastoquinone at its binding site on the D1 protein, blocking electron transport from PS II and resulting in photosynthesis inhibition, oxidative stress, and cell damage [11–13]. The selectivity of an herbicide is based on the ability of the plant to rapidly metabolize it to form nonphytotoxic compounds [10]. However, bentazone shows differential selectivity among plant species and genotypes within plant species [14,15].

In tolerant plants, bentazone is absorbed by the leaves and metabolized into natural components [16]. Herbicide tolerance in germplasm or mutant lines may result from an altered binding site of the target enzyme for bentazone herbicide [17]. Tolerant genotypes detoxify bentazone through hydroxylation and glucose conjugation, whereas sensitive plants are less likely to detoxify bentazone, and certain species cannot detoxify it at all [10]. Different responses to bentazone have been reported for soybeans [7,18,19]. Detoxified bentazone is hydroxylated to 6- or 8-hydroxy-bentazone in tolerant soybeans. This substance is then glycosylated at positions 6 or 8 of its aromatic ring before being oxidized to generate natural plant products, such as starch, protein, lignin, amino acids, and cellulose [7,10].

As part of an advanced weed control scheme, herbicide-tolerant crops are the most productive and sustainable [5]. Genetically modified herbicide-tolerant (GMHT) soybeans are not allowed to grow in non-GMO countries. Atrazine-resistant foxtail millet [20], sulfonylurea-resistant lettuce [21], and triazine-resistant canola [22] are examples of non-GMHT crops in herbicide-based weed management systems. The evaluation and identification of herbicide-tolerant genotypes are important steps in developing non-GMHT soybean cultivars for breeding programs [23]. In many crops, herbicide-tolerant cultivars have been developed by exploiting genetic variability in the germplasm [24]. Evaluation of visible symptoms is not always the best approach for assessing the effects of herbicides on crops [25]. Therefore, the implementation of high-throughput precision technologies is required to generate high-resolution data for understanding plant health under stress conditions [26–28].

Hyperspectral imagery is an emerging of images and spectroscopy to acquire external and internal characteristics, simultaneously. Recently, hyperspectral imagery is widely used to evaluate the quantitative and qualitative traits of crops [29,30]. Hyperspectral imagery is non-invasive, unbiased, repeatable measurements and measurements of different parameters at once, indicating that this may help minimize time, labor, and expense [31,32]. The common optical spectroscopy in the plant has been used from the visible to the near-infrared spectrum (400–1000 nm) [33]. The raw images with spectroscopy are converted to other indexes at specific wavelengths to measure plant health and growth conditions [34]. Various stresses degrade chlorophyll and pigment content or change cell structures in stressed leaves [35]. Hyperspectral imagery-based vegetative indices are available to detect biotic and abiotic stresses and monitor vegetation due to their good correlation with biomass, photosynthesis, and changes in the chlorophyll-carotenoid ratio [36,37]. For instance, multispectral cameras have been shown to accurately identify viruses on tulip plants [38], and a case of rust was discovered on some Canadian goldenrod [39]. In addition, this technology has been applied for the detection of the water status and chlorophyll content of sunflower [40], powdery mildew on barley [41], and bacterial infection on spinach plants [42]. Hyperspectral imagery has been validated as a reliable method for the identification of plant stresses [43]. One of the key benefits of hyperspectral imagery is that it allows for more precise phenotypic measurement of a large population and for the detection of stresses early on [44].

It is imperative to understand the effects of herbicide stress on morphological, physiological, and biochemical processes in plants [45]. Photosynthesis inhibitor herbicides such as bentazone and atrazine typically alter chloroplast ultrastructure, pigment ratios, and chlorophyll-related protein levels in the photosynthetic apparatus [46]. Photosynthesis, chlorophyll fluorescence, and leaf gas exchange are well-studied physiological parameters for detecting plant responses to herbicide stress [47–50]. Thus, a series of morphological, physiological, biochemical, and metabolic traits has been extensively used for high-throughput phenotype screening to evaluate herbicide tolerance. These traits include plant height [51], biomass production [52], canopy reflectance [53,54], photosynthesis [47,50], stomatal conductance and transpiration [14], and chlorophyll content [50]. All these traits have been demonstrated to be significantly correlated with herbicide tolerance in different plant species [50], and some have been used as selection criteria in breeding programs for abiotic stress-tolerant crops [55,56].

Measuring a combination of parameters, including photosynthesis, gas exchange, pigment content, and agronomic performance, would significantly improve our understanding of the physiological response of soybean to bentazone. Although the differential tolerance response of soybean genotypes to bentazone has been previously reported. However, to date, the assessment of physiological differences through hyperspectral reflectance images and photosynthetic-related parameters for bentazone treatment to soybean remains unexplored. We use two different instruments; a hyperspectral camera and a portable photosynthetic system to measure the differential physiological responses of bentazone-treated tolerant and sensitive soybean cultivars. Therefore, the objectives of this study were: (1) to identify bentazone-tolerant and -sensitive genotypes of 138 Korean soybean cultivars and (2) to evaluate physiological parameters in response to bentazone for selected cultivars using hyperspectral reflectance images and photosynthesis-related measurements.

2. Materials and Methods

2.1. Experiment 1: Evaluation of the Reaction to Bentazone with Visual Scores

2.1.1. Soybean Cultivars and Herbicide Treatments

A total of 138 Korean soybean cultivars were evaluated to determine their response to bentazone at Kyungpook National University (Daegu, 35°52′ N, Republic of Korea). The cultivars were planted in a 50-hole plastic tray (10 holes in length × 5 rows in width) containing horticultural soil (Hanareum, Shinsung Mineral, South Korea). Three seeds from each cultivar were planted in a hole at a depth of 2 cm, with five holes per cultivar. After emergence, one seedling was kept in each hole for growth. Five plants from each accession consisted of one replicate. The tray was watered every alternate day. When soybean plants reached the V2 stage [57], the herbicide Basagran® (40% a.i. bentazone) was applied at the field recommended rate (1200 g a.i. ha^{-1}) on the plant with a handheld manual sprayer (Sumato compression sprayer, MJ Korea). The plot had a completely randomized block design with two replicates. Two assessments as biological replicates were conducted to evaluate the reproducibility of the results.

2.1.2. Visual Determination of Injury

Injury was scored visually 5 days after treatment (DAT) when there were maximum injury symptoms on the leaves of the reference sensitive genotypes (Suwon 98 and PI 97150) [10]. The visual scoring scale is based on the area of leaf burn in our previous study [58]. A phenotypic scoring scale (1–5) was used to evaluate cultivar tolerance. On this scale, the following scores were represented: 1-no evident burn spots; 2-a few burn spots occurred on the plant's leaves; 3-less than half of the plant's leaves had obvious burn spots; 4-more than half of the plant's leaves displayed notable burn spots, and 5-plant leaves of the whole soybean plant exhibited extensive regions of burn and withering. The cultivar with a visual score lower than 2.0 was considered tolerant, from 2.0 to 3.5 was considered moderately tolerant, and more than 3.5 was deemed sensitive.

2.2. Experiment 2: Physiological Assessments and Biomass of Bentazone-Treated Soybeans

Soybean Cultivars, Treatment, and Plot Design

Experiment 2 was conducted with two evaluations as biological replicates in a greenhouse at Kyungpook National University (Daegu, 35°52′ N, Korea). The first evaluation was conducted from 26 August to 28 September 2020, and the second evaluation was conducted from 10 November to 16 December 2020. Two tolerant cultivars (Cheongmiin and Cheongja 3) and two sensitive cultivars (Seonam and Hannam) were selected from

Experiment 1 to study their physiological response to bentazone (Table S1; Figure S1). There were 20 pots for each genotype, with half being treated with bentazone and the remaining being untreated and termed control. Ten plants each of treated and untreated cultivars were considered as ten replicates. For both evaluations, the experimental design was 2 × 4 factorial plot design based on completely randomized block with 10 replications. Bentazone and control treatment were applied to 4 soybean cultivars. The experimental unit consisted of 3.7 L plastic pots (10.5 cm upper radius × 6.5 cm lower radius × 16 cm height) with small holes at the bottom for draining excess water. The pots were filled with agronomic soil, and there were no differences in fertility. Four seeds from each cultivar were sown per pot. Soon after emergence, thinning was performed to leave a single plant per pot at the VC stage (unifoliate leaves emergent) [58]. Herbicide treatment was performed as described earlier, and the visual score was evaluated 5 DAT using a 1–5 scale.

2.3. Hyperspectral Imagery Collection

Hyperspectral images were collected from the first day of herbicide application till 4 DAT (from 8:30 a.m. to 12:00 p.m.) using a hyperspectral camera (Specim IQ, Oulu, Finland). Specifications of the hyperspectral camera are described in Table S2. All hyperspectral image was captured with a white panel (90% reflectance) as the reference target. The camera was fixed on a tripod at 2 m above ground, and 10 plants from the treatment and control groups were brought into the focus region beneath the camera. The integration time and recording time were manually set from the touchscreen of the camera to acquire a high-quality image. After recording each image, we saved high quality image based on the color of maximum and minimum intensity histogram. The reflectance transformation was done by changing the intensity threshold value by using the slider on the touchscreen. The intensity slider was adjusted until the reference white panel flashing white and gray. Then the area was selected by pressing the reference white panel on the touchscreen. After confirming that the correct area has been selected, then press set to accept the selection.

2.4. Calculation of Normalized Difference Vegetation Index in Hyperspectral Images

Among vegetative indices, normalized difference vegetation index (NDVI) has broadly used for determination of leaf greenness, so we used NDVI as indicator of leaf damage. To calculate NDVI, hyperspectral imagery was analyzed by ENVI Classic 5.3 (Harris Geospatial Solutions, Broomfield, CO, USA). In the ENVI, only leaf area was manually annotated by a digital pen for hyperspectral data extraction. NDVI was calculated using the following equation [59]:

$$\mathrm{NDVI} = \frac{\mathrm{R}_{800} - \mathrm{R}_{670}}{\mathrm{R}_{800} + \mathrm{R}_{670}} \tag{1}$$

where R_{800} and R_{670} are the reflectances at 800 nm and 670 nm, respectively.

2.5. Determination of Photosynthetic Parameters

In treated and control plants, the gas exchange was estimated using a portable photosynthesis system equipped with a fluorometer, LI-6800 (Li-COR, Lincoln, NE, USA). The overhead chamber featuring with a LED light as the light source to supply uniform light intensity with 90% red and 10% blue light. Of the 5 days of data collection, gas exchange analysis on the 1st day was performed from 8:30 a.m. to 11:30 a.m. before bentazone treatment. The measurements on the succeeding days were conducted at the same time as on the 1st day. The sample CO_2 concentration, relative humidity, and leaf temperature inside the cuvette were set to 400 µmol mol^{-1}, 65%, and 28 °C, respectively, to minimize gradients in the gas exchange system. The photosynthetic photon flux density inside the LI-6800 cuvette was set to 1000 µmol m^{-2} s^{-1} for photosynthesis, transpiration, stomatal conductance of water vapor, and total conductance of CO_2 measurements. A fully expanded leaf of the V2 stage was held in the cuvette until the photosynthesis values were stable, i.e., steady-state, which generally occurred rapidly (from approximately 30 s to 2 min). Before recording the gas exchange data, 20–30 min pre-run was usually required to achieve a stable gas exchange measurement.

2.6. Fresh Weight and Plant Height

Plant height and fresh weight were measured 14 DAT in both the control and treated plants. Plant height was measured from ground level to the leaf base of the highest fully expanded leaf using a ruler. Fresh weight of each plant was measured using an electric balance (Sartorius BS2202S, Germany). Plant height and fresh weight were measured for two tolerant (Cheongmiin and Cheongja 3) and two sensitive (Seonam and Hannam) cultivars.

2.7. Statistical Analysis

Analysis of variance was conducted to determine the difference in visual scores of Korean soybean cultivars and other experiments performed under bentazone treatment using PROC GLM in SAS v9.4 (SAS Institute, Cary, NC, USA, 2013). Least significant difference (LSD) analysis was conducted using the "agricolae" package in R with the "LSD.test" function to identify significant differences in the measured physiological parameters of fresh weight and height. The significance level was set at 5% for comparing means for multiple comparison analyses.

3. Results

3.1. Phenotypic Distribution of Bentazone Tolerance in Korean Soybean Cultivars

Analysis of variance was conducted to identify significance for test, cultivar, and test by cultivar interaction. Bentazone response for the visual measurement of 138 soybean cultivars with two biological replicates (test) differed significantly among cultivars, tests, and cultivar by test interactions (Table 1). The mean values of the bentazone response score of both tests were 2.2 and 2.6, with a range of 1.0–4.9. On an average, 20% (*n* = 28), 67% (*n* = 92), 8% (*n* = 11), and 5% (*n* = 7) of the total soybean cultivars exhibited visual ratings of 1.0–1.9, 2.0–2.9, 3.0–3.9, and 4.0–4.9, respectively (Figure 1). The results indicate that moderate bentazone tolerance is predominant in Korean soybean cultivars. Soybean cultivars such as Cheongmiin, Cheongja 3, Sinpaldal, Jungmo 3009, and Daechan showed a high tolerance to bentazone, whereas Hannam, Seonam, Beakchun, and Pokwang had a high sensitivity to bentazone in the two tests (Figure 1, Table S1). Among these cultivars, Cheongmiin, Cheongja 3, Hannam, and Seonam were subjected to further physiological evaluation (Experiment 2) based on their visual scores.

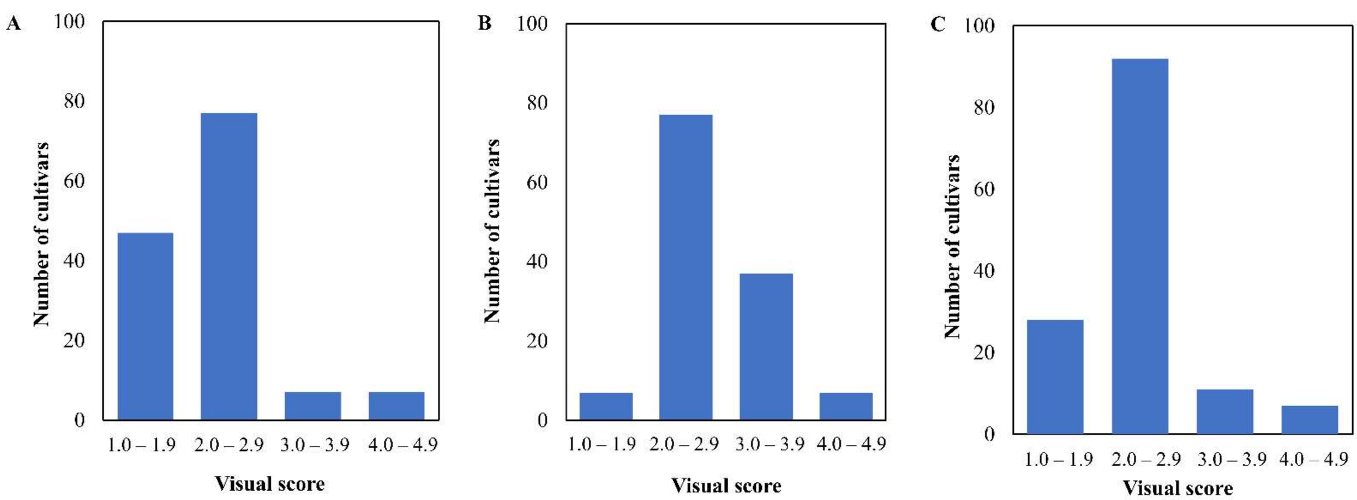

Figure 1. Frequency distribution of the response to herbicide bentazone with 138 Korean soybean cultivars. (**A**) Distribution of visual score in test 1. (**B**) Distribution of visual score in test 2. (**C**) Distribution of averaged visual score of the two tests. Visual score < 2.0, tolerance; 2.0–3.5, moderate tolerance; and >3.5, sensitive.

Table 1. Analysis of variance for reaction to bentazone on Korean soybean cultivars.

Source of Variation	Degree of Freedom	Sum Square	Mean Square	F-Value	*p*-Value
Test	1	17.8	17.8	87.2	<0.0001
Replication in test	2	3.9	1.9	9.5	0.0001
Cultivar	137	138.7	1.0	5.0	<0.0001
Test × Cultivar	127	118.7	0.9	4.6	<0.0001
Error	264	53.8	0.2		

3.2. Determination of Leaf Greenness Using Hyperspectral Images of NDVI

NDVI was used to measure leaf greenness. There were no statistical differences between the control and treatment in the two tolerant cultivars (Cheongmiin and Cheongja 3) on each day during the 5 days (Figure 2A, Table S3). Reduction rates in NDVI values at 4 DAT were −2.7% and 1.3% for Cheongmiin and Cheongja 3, respectively (Table S3). In contrast, two bentazone-sensitive cultivars, Seonam and Hannam, showed a trend of gradual decrease in NDVI values during treatment. For the two sensitive cultivars, NDVI values were significantly decreased 2 DAT compared with the control (Figure 2A; Table S3). NDVI values decreased continually from 1 to 3 DAT for the two sensitive cultivars. However, the NDVI value of bentazone-treated Hannam at 4 DAT was slightly increased. In particular, the NDVI value was significantly lower for the Seonam (18.9% reduction rate) and Hannam (10.5% reduction rate) cultivars than for the control (Table S3). The range of NDVI observed in two sensitive cultivars from 2 to 4 DAT, 0.60 to 0.69, were used as the critical point for the determination of sensitive soybean cultivars after bentazone treatment.

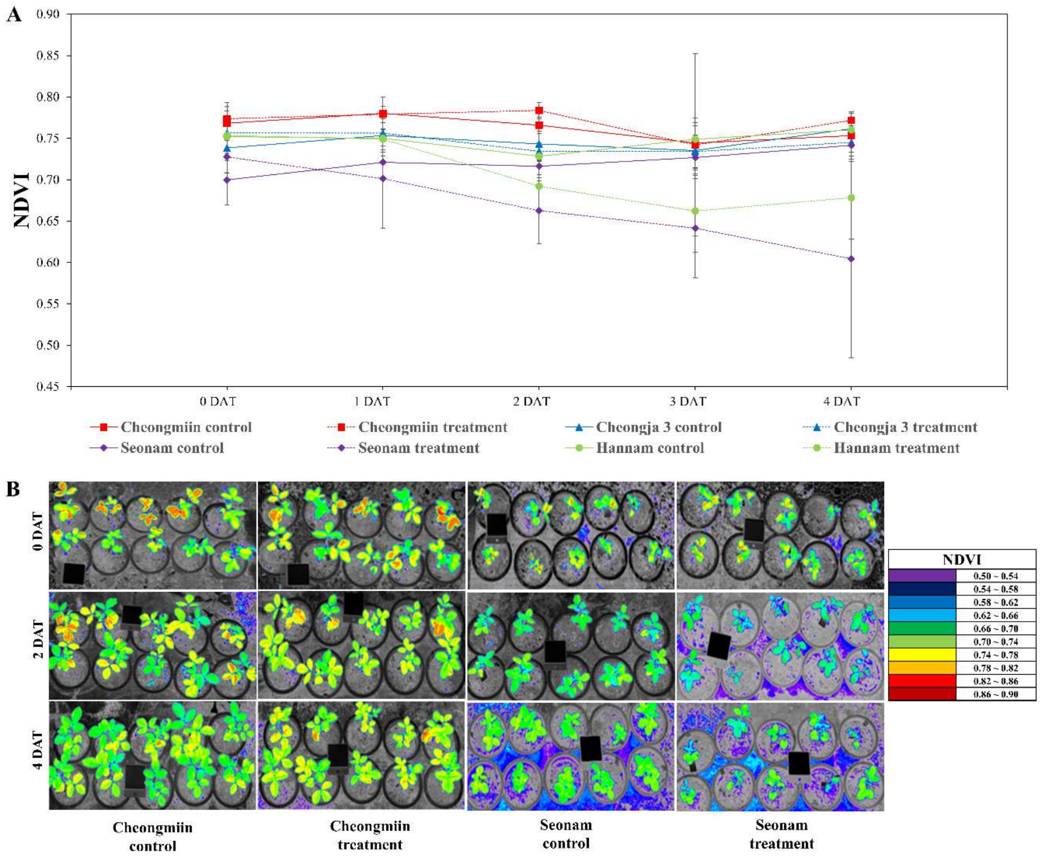

Figure 2. Vegetative index and hyperspectral images of bentazone-treated and control soybean plants. (**A**) Normalized difference vegetative index (NDVI). The 0 days after treatment (DAT) corresponds to the date before treatment. The bars represent standard deviations. Different colors represent the

different soybean cultivars. Solid lines are the soybean cultivars with control treatment and dotted lines are soybean genotypes with bentazone treatment (**B**) Differences in hyperspectral images between control and treated tolerant cultivar, Cheongmiin, and sensitive cultivar, Seonam, as shown by computed spectral rations for NDVI.

We visualized soybean plants using the NDVI to determine the stress response. Hyperspectral images of NDVI are shown for bentazone-tolerant Cheongmiin and sensitive Seonam under control and treatment conditions (Figure 2B). The 10-color band represents the typical range for the NDVI values of the leaves. The 10-color band of NDVI values ranged from 0.50 to 0.90 (Figure 2B). Colors ranging from 0.70 to 0.86 were shown in bentazone-tolerant cultivar, whereas the hyperspectral images of Seonam at 2 and 4 DAT had blue, dark green, and light green bands in the treatments.

3.3. Investigation of Photosynthesis-Related Parameters

The net photosynthesis rate (A), transpiration rate (E), stomatal conductance of water vapor (g_{sw}), and total conductance of CO_2 (g_{tc}) were used as the photosynthesis-related parameters for the four cultivars selected in this study (Figure 3). The mean net photosynthetic rate was lower under the treatment than under the control conditions (Figure 3A, Table S4). For the bentazone-tolerant and -sensitive cultivars, the lowest A values were observed 1 DAT and the A values increased thereafter. The reduction rates were different between bentazone-tolerant cultivars and bentazone-sensitive cultivars. Compared with the control, the reduction in A values 4 DAT in treated cultivars was 22.6% and 8.7% for Cheongmiin and Cheongja 3, respectively (Table S4). However, the two bentazone-sensitive cultivars showed dramatically decreased A values 1 DAT. Moreover, higher reduction rates during treatment at 4 DAT were observed in Seonam (84.6%) and Hannam (62.2%) than those in the control.

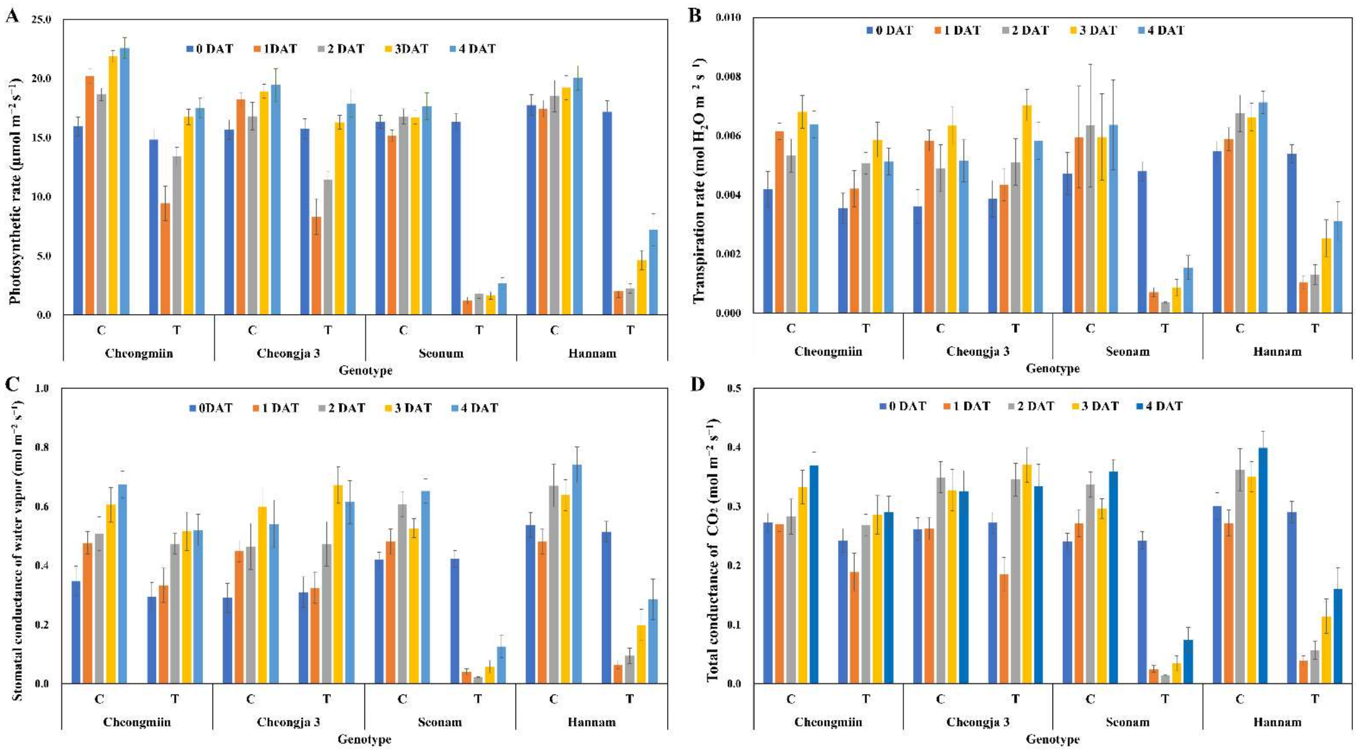

Figure 3. Leaf gas exchange parameters of bentazone-treated and control soybean plants. (**A**) Net photosynthetic rate. (**B**) Transpiration rate. (**C**) Stomatal conductance of water vapor. (**D**) Total conductance of CO_2. "C" and "T" represent control and treatment, respectively. The 0 days after treatment (DAT) corresponds to date before treatment. Error bars indicate the standard error.

For the E values of the two bentazone-tolerant cultivars, there was no significant difference between the control and treatment each DAT except Cheongmiin 1 DAT (Figure 3B, Table S4). In contrast, the E values differed significantly from 1 to 4 DAT for the two bentazone-sensitive cultivars. Higher reduction rates of E values in treatments at 4 DAT were observed for Seonam (76.2%) and Hannam (56.3%) than bentazone-tolerant cultivars. There was no significant difference in the g_{sw} and g_{tc} values of the two bentazone-tolerant cultivars under control and treatment conditions (Figure 3C,D, Table S4). The g_{sw} and g_{tc} values of the two bentazone-sensitive cultivars were significantly lower from 1 to 4 DAT than those of the control. Simultaneously, the two tolerant cultivars did not have significant differences on each DAT, except for Cheongmiin at 1 DAT for g_{tc} value. Reduction rates in g_{sw} values at 4 DAT were observed in Cheongmiin (21.6%), Seonam (79.4%), and Hannam (64.1%).

3.4. Effects on Plant Growth

Bentazone treatment resulted in growth retardation and reduced biomass production. Therefore, the fresh weight and plant height of the bentazone-tolerant (Cheongmiin and Cheongja 3) and bentazone-sensitive (Seonam and Hannam) cultivars were compared between the control and treatment groups 14 DAT (Figure 4). There was no significant difference between the fresh weight of the two bentazone-tolerant cultivars Cheongmiin and Cheongja 3 in the treatment and control groups. However, statistically significant differences were observed between the Seonam and Hannam cultivars (Figure 4A). The decrease in fresh weight was the lowest for the tolerant cultivars (11–12%), whereas the decrease for the sensitive cultivars ranged between 57–71%. There was no significant difference in plant height between the control and treated Cheongmiin and Cheongja 3 (Figure 4B). Significant differences in plant height were observed between the control and treated Seonam and Hannam cultivars (Figure 4B). The lowest reduction rates in plant height were for the two bentazone-tolerant cultivars, with differences of 0.5% and 2.0% for Cheongmiin and Cheongja 3, respectively. Regarding the sensitive cultivars, the observed decreases in plant height ranged between 15.0–29.0%.

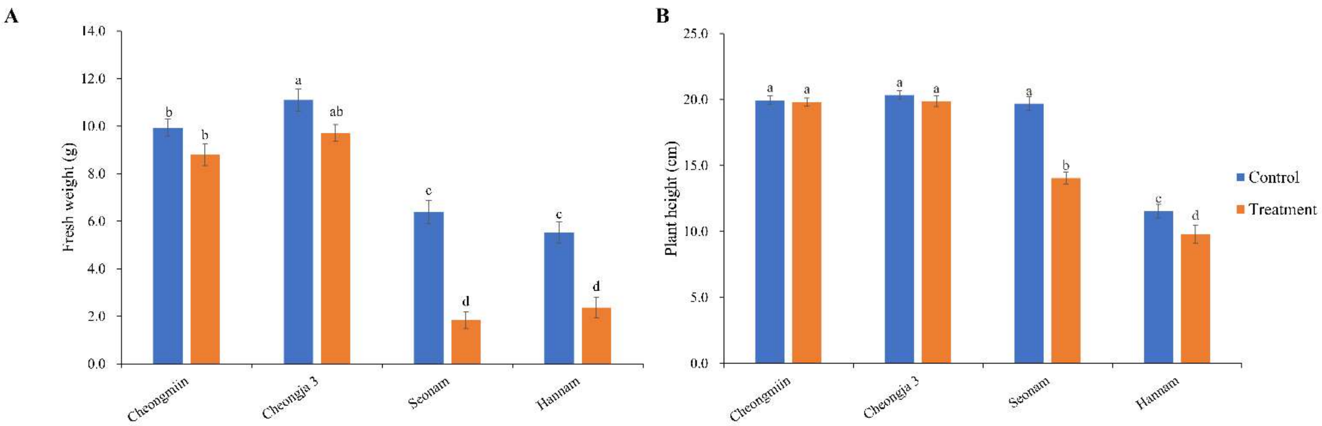

Figure 4. Growth parameters of (**A**) fresh weight and (**B**) plant height of bentazone-treated and control plants measured 14 days after treatment. Error bars indicate standard error. Different letters denote statistically significant differences at $p < 0.05$.

4. Discussion

As weeds cause a 37% decrease in soybean yield, weed control is critical for achieving expected soybean production [60]. Bentazone has been used as a broadleaf herbicide in soybean fields in France, Germany, Greece, Italy, Spain, and the USA. Ali et al. [10] suggested that the application of graminicides and bentazone could simultaneously control both grass and broadleaf weeds in bentazone-tolerant soybean fields. This strategy results in a cost-effective and less labor-intensive alternative to glyphosate application in GMHT soybean fields. Therefore, it would be prudent to develop bentazone-tolerant soybeans.

Searching for variations in genetic resources is crucial for identifying valuable genetic materials and developing new bentazone-tolerant cultivars. Wax et al. [61] evaluated the responses of 338 U.S. and Canadian soybean cultivars to bentazone in a field, greenhouse, and growth chamber for three years. They demonstrated that soybean cultivar, Hurrelbrink, is bentazone-sensitive. Kato et al. [7] found that 155 out of 164 soybean cultivars were tolerant to bentazone. In the present study, we classified three types of responses to bentazone: tolerant, moderately tolerant, and sensitive. Studies reported that bentazone symptoms of Williams 82 were recovered after bentazone treatment [7,16]. Ali et al. [58] mentioned that Williams 82 is a moderate-bentazone cultivar. In this study, moderate bentazone-tolerant cultivars were predominant in Korean soybeans, with a visual score ranging from 2.0–2.9 (Table S1). A total of 28 soybean cultivars (approximately 20% of tested cultivars) were bentazone-tolerant, including Cheongmiin, Cheongja 3, Sinpaldal, Jungmo 3009, and Daechan. However, eight soybean cultivars were highly sensitive to bentazone (Table S1). These cultivars could be used as genetic resources to identify genes controlling bentazone tolerance or sensitivity, and in soybean breeding programs to develop bentazone-tolerant soybean cultivars.

The vegetation indices of hyperspectral reflectance images are accurate, time-saving, and nondestructive measures. Because of these features, they have been used for high-throughput phenotyping measurements and acquisition of phenotypic data from large populations instead of visual scores [62]. Light absorption and reflectance are essential features of the physiological processes in plants. The NDVI is highly correlated with plant health and vitality. There has been considerable interest in studying the effects of canopy vitality on plant reflectance [63]. Several studies have indicated that NDVI is decreased by herbicides such as glyphosate and paraquat [64–66]. Hinojosa et al. [67] reported that abiotic stress-tolerant genotypes of quinoa (*Chenopodium quinoa* willd.) have higher NDVI values than sensitive genotypes. In this study, we observed that the herbicidal effects on NDVI 2 DAT may determine bentazone-tolerant and -sensitive characteristic of soybeans (Figure 2). There was a similar trend between the control and treated bentazone-tolerant soybean from 2 to 4 DAT, whereas significant differences between the control and treatment were observed from 2 to 4 DAT for the sensitive cultivars (Figure 2). Thus, NDVI values from 2 to 4 DAT were prone to determine the response of soybean to bentazone treatment.

PS I, PS II, CO_2 reduction pathways, photosynthetic pigments, and electron transport systems are essential components of the photosynthetic machinery, and any impairment in these pathways results in the inhibition of overall photosynthesis [68]. The effects of herbicides on the photosynthetic performance of different crops have been reported [46,47,69]. Photosynthesis and stomatal conductance of bentazone-treated resistant potato cultivars are higher than those of the sensitive genotype [70]. Vivancos et al. [71] observed that photosynthesis is unaffected by glyphosate in tolerant soybeans, whereas it is rapidly inhibited in sensitive genotypes subjected to glyphosate treatment. The photosynthetic parameters of the herbicide-tolerant genotype are superior to those of the sensitive genotype of hairy fleabane [72]. The bentazone-tolerant cultivar Williams 82 exhibits symptoms 1 DAT but rapidly recovered 2 DAT [7,16]. However, the expression levels of bentazone-related genes in Williams 82 decreased from 4 to 8 h after treatment. In this study, photosynthesis, transpiration, stomatal conductance, and total CO_2 conductance were dramatically decreased 1 DAT in sensitive cultivars (Figure 3), although phenotypic differences were not observed (Figure 2). Thus, 1 DAT may be the starting point for identifying soybean responses to bentazone treatment, based on photosynthesis-related measurements of photosynthesis, transpiration, stomatal conductance, and total conductance of CO_2.

Herbicides can interrupt plant growth, causing growth reduction. The negative impact of bentazone on photosynthesis results in growth retardation and reduced biomass production, as the total biomass of plants depends on the energy supplied by photosynthesis. Similar inhibition of growth after herbicide application of metribuzin, isoproturon, and glyphosate has been previously reported in soybeans [48,52,73,74]. In this study, although the fresh weight decreased following bentazone treatment compared with that in the control, there were statistically significant differences between the control and treatment in the fresh weight in cultivars with sensitivity (Figure 4). Although there were statistically significant differences between the control and treatment in the plant height in sensitive cultivars, Seonam under control treatment showed similar plant height to bentazone-tolerant cultivars. Interestingly, fresh weight of two bentazone-sensitive cultivars in the control showed smaller than ones in bentazone-tolerant cultivars.

In conclusion, this study was primarily designed to determine bentazone-tolerant and bentazone-sensitive cultivars and their differential responses to bentazone. Tolerant and sensitive cultivars responded differently to bentazone treatment. Tolerant cultivars showed better performance in terms of biomass, photosynthesis, and other physiological parameters than sensitive cultivars. Based on NDVI from hyperspectral images, we inferred that NDVI values from 2 to 4 DAT were prone to determine the response of soybean to bentazone treatment. Moreover, 1 DAT may be the starting point to identify soybean responses to bentazone treatment based on the photosynthetic-related traits, such as photosynthesis rate, transpiration, stomatal conductance, and total conductance of CO_2. Furthermore, there were statistically significant differences between the control and treatment in the fresh weight and plant height in cultivars with sensitivity (Figure 4). Bentazone has been extensively used to control broadleaf weeds in farmlands of different crops. A limited number of scientific reports have been published on the differential physiological response of the tolerant and sensitive genotypes of different crops. But to date, the assessment of physiological differences through hyperspectral images and photosynthetic-related parameters for bentazone treatment in plants including soybean remains unexplored. The findings of our research provide basic knowledge of the responses of bentazone to soybean. It also provides insights into the methodological merits of measuring bentazone reaction, which will aid in the comprehension of the mechanism involved and the identification of the genes responsible for bentazone reaction. Moreover, this study provided that hyperspectral imagery could be a possible alternative for phenotyping a large population as it requires less time for data generation. In the future, research studies should be conducted under field conditions to confirm the validity of our results and their wide-scale applicability. Furthermore, studies are required to identify the xenobiotic detoxification pathways in both sensitive and tolerant cultivars and the genetic mechanisms underlying bentazone tolerance in soybean.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12102241/s1, Figure S1: Bentazone reaction of bentazone-tolerant cultivar and -sensitive cultivar. A. Bentazone treated and untreated soybeans after 5 days. B. Bentazone treated and untreated soybeans after 14 days; Table S1: List of Korean soybean cultivars and their response to bentazone treatment in two different evaluations; Table S2: Technical specifications of hyperspectral camera; Table S3: Descriptive statistical analyses for normalized difference vegetation index of bentazone treated and control plants; Table S4: Descriptive statistic of photosynthesis-related traits of bentazone treated and control plants.

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