



Article

Genetic Dissection of Bentazone Tolerance Loci in Cultivated Soybeans: A Genome-Wide Association Study

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Abstract: Weeds alone cause a 37% loss in attainable soybean production. Bentazone is a postemergence herbicide used to control broadleaf weeds in the cultivation of cereals, legumes, vegetables, and tuber crops. This study aimed to screen the Korean collections of cultivated soybeans in relation to bentazone, identifying the genetic loci controlling bentazone reactions to cultivated soybean collections using a genome-wide association study (GWAS). This study identified bentazone-tolerant and bentazone-sensitive soybean germplasms from 418 cultivated soybean germplasms and found that moderate bentazone tolerance predominated in the Korean collection of cultivated soybeans. The GWAS revealed that 42 SNPs distributed on chromosomes 3, 5, 6, 13, and 20 were strongly associated with the bentazone reaction in 418 cultivated soybean accessions over three years. Of these loci, a genomic region on chromosome 5 contained significant SNPs and was identified as being involved in the bentazone reaction in both 2020 and 2021, based on FarmCPU analysis. By conducting a haplotype analysis, this study identified five putative genes, namely, *Glyma.05g145000* (ATP-binding cassette transporter), *Glyma.05g145100* (unknown), *Glyma.05g145200* (ankyrin repeat family protein), *Glyma.05g145300* (transmembrane amino acid transporter protein), and *Glyma.05g145400* (unknown). Further studies are required to confirm the involvement of the putative genes in the bentazone reaction by comparing their expression levels between bentazone-tolerant and bentazone-sensitive plants. Therefore, the results of this study can be used for marker-assisted selection in programs for the breeding of herbicide-tolerant soybeans.

Keywords: bentazone; NGS-GWAS; herbicide; soybean; resequencing



Citation: Jo, H.; Ali, L.; Song, J.T.; Lee, J.-D. Genetic Dissection of Bentazone Tolerance Loci in Cultivated Soybeans: A Genome-Wide Association Study. *Agronomy* **2023**, *13*, 2345. <https://doi.org/10.3390/agronomy13092345>

Academic Editor: Wensheng Hou

Received: 20 August 2023

Revised: 5 September 2023

Accepted: 7 September 2023

Published: 8 September 2023



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1. Introduction

Soybeans (*Glycine max* L.) are mainly grown for its uses as a protein source in animal feed and as vegetable oil for human consumption. Soybeans are one of the most commercially significant legume crops grown worldwide [1]. The United States, Brazil, Argentina, China, and India are the major producers of soybeans, together accounting for approximately 93% of the world's soybean production [2]. However, soybean yields are threatened by various abiotic and biotic stresses and weeds [3,4]. Weeds alone cause a loss of 37% in attainable soybean production [5]. Effective weed management during soybean cultivation is essential to protect soybean yields from weed competition during the growing seasons [6]. A conventional and modern strategy for cultivating crops is to use inexpensive herbicides that are easy to apply and effective [7]. The global weed control market was worth USD 28.08 billion in 2017 and is predicted to reach USD 44.90 billion by 2026, rising at a compound annual growth rate of 5.35 percent over the forecast period [8]. Herbicides are applied to more than 85 percent of US agricultural crop acres on a regular basis [9]. In

terms of weed control methods, herbicides are dominant in the United States, as shown by the expansion of the Weed Science Society of America (WSSA)'s Herbicide Handbook from 97 herbicides in the first edition of 1967 [10] to 232 in the tenth edition of 2014 [11]. Milne [12] identified 442 herbicides that have been licensed for use in various parts of the world. Herbicides are classified as either preemergence (PRE) or postemergence (POST) herbicides depending on the time of their application [13]. A previous study showed that the application of PRE and POST herbicides together increased soybean yields more than the application of a single herbicide [6]. However, the range of POST herbicides that can be used for soybeans is limited [14], with the currently available POST herbicides being imazethapyr, imazamox, acifluorfen, fomesafen, metolachlor, and bentazone [15,16].

Bentazone [3-Isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide] was first documented in 1975. Bentazone is a POST herbicide used to control broadleaf weeds in the cultivation of cereals, legumes [6], vegetables [17], and tuber crops [18]. Bentazone is a contact herbicide that inhibits the capacity of vulnerable plants to use sunlight in photosynthesis to produce the energy necessary for survival. Depending on the species and dosage, visual injury to the treated surface of the leaves can take anywhere from a few hours to weeks [19,20]. Bentazone's binding to the D1 protein blocks the electron transport from Q_A to Q_B , resulting in the inhibition of CO_2 fixation and the production of ATP and $NADPH_2$ for plant growth [21]. Meanwhile, after tolerant plants including soybeans, corn, and rice (*Oryza sativa*) are treated with bentazone, they metabolize it to glucosyl conjugates of hydroxylated bentazone. However, broadleaf weeds are unable to metabolize it.

Bentazone tolerance is mediated by processes including herbicide detoxification, reduced herbicide penetration, and herbicide compartmentalization in plant cells, which reduce the amount of the herbicide and lower the target enzyme to a nonlethal dosage [22]. Nontarget-site herbicide resistance (NTSR) is a complex multigenic trait that triggers various herbicide resistance mechanisms, including decreased absorption, translocation, and detoxification, as well as a less well understood broad-range cytoprotective mechanism [23]. An enhanced herbicide metabolism is considered a critical NTSR mechanism, often involving cytochrome P450 monooxygenase, UDP-glucuronosyltransferases (UGTs), ATP-binding cassette (ABC) transporter, glutathione S-transferase (GST), glycosyltransferase (GT), and peroxidase (POD) [23]. However, NTSR remains poorly understood because of its complexity and diversity, which have led it to be studied less often than target-site resistance, especially in broadleaf weed species [24]. Therefore, genetic dissection for bentazone tolerance or sensitivity loci in soybeans can be used for future breeding programs to develop bentazone-tolerant cultivars.

Genetic studies on bentazone reactions are limited to plants. Zhang et al. [25] showed that sensitivity to bentazone was controlled by a single recessive gene (*bel*). Zhang et al. [26] also identified a cytochrome P450 gene, *CYP81A6*, as *bel* via positional cloning. Moreover, Liu et al. [27] demonstrated that tobacco (*Nicotiana tabacum*) and *Arabidopsis*, both of which are sensitive to bentazone, displayed tolerance to this herbicide after they were transformed with rice *CYP81A6* via agrobacterium-mediated transformation. The sensitivity of rice, corn, and soybeans to bentazone has been demonstrated to be controlled by a single recessive gene [15,28], whereas tolerance in *Capsicum annuum* has been reported to be controlled by a single dominant gene [29]. The genetic control of the bentazone reaction also varies among crop species. Few studies have been conducted with the aim of detecting genes involved in the bentazone response in soybeans using either a biparental mapping population or germplasm collections.

The genetic examination of herbicide tolerance can identify the loci controlling herbicide responses and can be used to improve crop productivity by selecting and pyramiding those favorable loci into elite cultivars [30]. Through genetic studies, several QTLs have been identified as being involved in tolerance or sensitivity to metribuzin herbicides in peas [31], sensitivity to chlorimuron ethyl in soybeans [32], resistance to tembotrione in sorghum [33], and tolerance to metribuzin in wheat [34]. However, there is limited information about bentazone-tolerant QTLs from either biparental mapping or genome-wide

association study (GWAS). Advancements in sequencing technologies and genotyping platforms have facilitated the identification of QTLs and genes controlling many important traits in soybeans [35,36]. An extensive suite of genotyping platforms with various SNPs are now available for most crop species. In addition, the whole-genome resequencing approach is available to detect all genome-wide polymorphisms [37]. The resequencing of the genetic germplasm from the biparental population has led to the identification of genomic regions or putative genes for traits of interest [38]. Therefore, this study aimed to evaluate the Korean collection of cultivated soybeans for bentazone reactions and identify the genetic loci controlling bentazone reactions in these soybeans using GWAS with the available resequencing data.

2. Materials and Methods

2.1. Plant Materials and the Determination of Bentazone Tolerance

The experiments were conducted in the greenhouse of the KNU, Daegu, Republic of Korea (35°53'42" N, 128°36'45" E) from 2019 to 2021. A Korean soybean core collection of cultivated soybeans consisting of 430 accessions was made publicly available in the Republic of Korea by Jeong et al. [39]. From the Korean core collection of cultivated soybeans, 418 accessions were used in this study. In three consecutive tests, seeds were sown in a 50-hole plastic tray containing horticulture soil (Hanareum; Shinsung Mineral, Goesan, South Korea). Three seeds of each soybean accession were placed in the soil at a depth of 2 cm; finally, one seedling was kept for the evaluation of the bentazone reaction. These five plants of each accession in a row represent one replication. The experimental design was a complete block design with two replications. The foliar application of a bentazone, Basagran® (a.i. bentazone 40%), was examined at a field-recommended rate (1200 g a.i. per ha) when the plants reached the V2 growth stage [40], approximately 20 days after planting. The herbicide was sprayed on the top of the plant with a handheld sprayer. For each replication, to assess tolerance and sensitivity, control plants were included to serve as a basis for comparison [41,42]. The response of each individual plant was recorded 5 days after the application of the herbicide. The phenotypic evaluation was based on a visual scoring scale of 1–5 [41]. On this scale, the following scores were represented: 1—no evident burn spots; 2—a few burn spots on the plant's leaves; 3—less than half of the plant's leaves have obvious burn spots; 4—more than half of the plant's leaves display notable burn spots; and 5—the leaves of the whole soybean plant exhibit extensive regions of burn and withering. Cultivars with a visual score lower than 2.0 were considered tolerant, those scoring from 2.0 to 3.5 were considered moderately tolerant, and those scoring more than 3.5 were deemed sensitive.

2.2. Publicly Available Resequencing Data of 418 Cultivated Soybean Accessions

We obtained the SNP data for 418 accessions of the Korean cultivated soybean collection from Kim et al. [43] (https://figshare.com/projects/Soybean_haplotype_map_project/76110 (accessed on 01 November 2022)). The physical positions of the SNPs were based on the reference genome Williams 82 (Wm82.a2.v1) [44]. The unusual SNPs, including those with >20% missing calls, >10% heterozygosity, and monomorphic SNPs, and >1% a minor allele frequency were filtered out. Finally, we retained 10,597,683 high-quality SNPs to perform further analysis [43].

2.3. GWAS

GWAS was conducted to identify the loci controlling the bentazone reaction in soybeans, followed by candidate gene identification. A mixed linear model (MLM) was used to perform the association analysis. The MLM was used to evaluate the dataset using the Genome Association and Prediction Integrated tool-R (GAPIT) [45]. Both PCA and the kinship coefficient matrix from GAPIT were used. Based on the MLM results, FarmCPU separately analyzed chromosomes 3, 5, 6, 13, and 20 using GAPIT [46]. A threshold value of $-\log_{10}(p) \geq 7.01$ for chromosome 5 was adopted to indicate a significant association of

SNPs with the bentazone reaction, based on the Bonferroni correction method. To analyze the linkage disequilibrium (LD) on a genomic region of chromosome 5, which contained the significant SNPs, Haploview tools were used with the default Haploview parameters [47].

2.4. Statistical Analyses

Analysis of variance (ANOVA) was conducted using PROC GLM in SAS v9.4 (SAS Institute, Cary, NC, USA), and the descriptive statistics, mean, standard deviation, coefficient of variation (CV%), kurtosis, skewness, and phenotypic frequency distribution for the bentazone reaction were analyzed using SPSS 20.0 (SPSS for Windows, standard version 20.0; SPSS Inc., Chicago, IL, USA) based on Pearson's correlation analysis.

3. Results

3.1. ANOVA for the Bentazone Reaction with Cultivated Soybean Accessions

The bentazone response of the cultivated collection of soybeans was evaluated for three consecutive years (2019, 2020, and 2021), which were considered to represent three environments. The results of the ANOVA and descriptive statistics of the bentazone reaction of 418 soybean accessions in the three environments are presented in Tables 1 and 2, respectively. The ANOVA revealed that the environments, soybean accessions, and their interaction had significant effects on the bentazone reaction to soybean accessions (Table 1). The means of the bentazone reaction of the cultivated soybeans were 2.54, 2.54, and 2.71 in 2019, 2020, and 2021, respectively (Table 2).

Table 1. Analysis of variance for the bentazone reaction of 418 cultivated soybean collections.

Source of Variation	Degree of Freedom	Sum of Square	Mean of Square	F-Value	p-Value
Year (Y)	2	10.982	5.491	87.2	<0.0001
Replication in Y	3	0.001	0.003	9.5	0.0001
Genotype (G)	417	776.784	1.862	5.0	<0.0001
G × Y	696	667.568	0.959	4.6	<0.0001
Error	2216	1517.100	0.680		

Table 2. Descriptive statistics of the bentazone reaction in the three different environments tested.

Year	Mean	SD	CV	SK	KUR	Kolmogorov-Sminov
2019	2.54	0.87	34%	−0.187	−0.638	0.23 **
2020	2.54	0.79	31%	0.276	−0.368	0.18 **
2021	2.71	0.67	25%	−0.136	−0.094	0.30 **

SD, standard deviation; CV, coefficient of variation; SK, skewness; KUR, kurtosis. ** Significant at the 0.01 probability level.

3.2. Bentazone Reactions to 418 Cultivated Soybean Accessions

The phenotypic distributions of the bentazone reaction of the cultivated soybeans in the three environments are shown in Figure 1. In all of the years included in this study, most accessions displayed visual scoring at a level of 2–3 (Figure 1). Overall, 13.2%, 31.2%, 43.4%, and 12.2% of the evaluated accessions showed visual scores of 1–2, 2–3, 3–4, and 4–5, respectively, in the year 2019. The corresponding values in 2020 were 8.6%, 48.3%, 32.8%, and 10.3%, respectively, while those in 2021 were 2.8%, 32.9%, 54.8%, and 9.4%, respectively. These results indicated that most cultivated soybean accessions in this study showed a moderate bentazone response, with a visual score of 2–3. Based on the visual scores over the three years, 11 accessions, namely, Daepung, Boseok, KAS531-5, KAS625-19, KLS087160, IT178037, IT104887A, Clark, KAS_102-2, KAS651-37, and Cheongja3, showed strong tolerance to the bentazone treatment (Table S1). Meanwhile, Hannam, Haman, Hadaedu, Dowling, Bancheongdu, KLS419, and IT104887A were extremely sensitive to bentazone (Table S1).

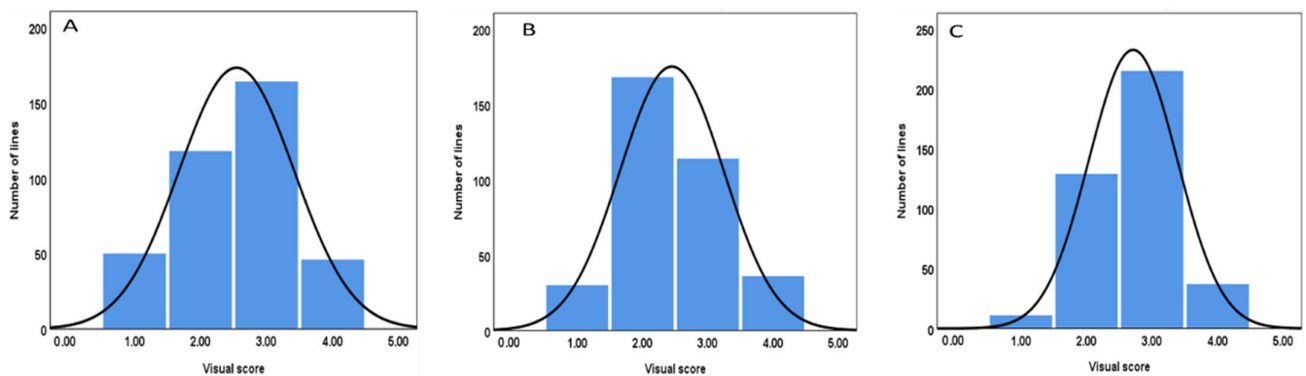


Figure 1. Phenotypic distribution of bentazone reactions in 418 cultivated soybean collections in 2019 (A), 2020 (B), and 2021 (C).

3.3. GWAS for Bentazone Reactions in Cultivated Soybeans

With a total of 10,597,683 SNPs, a GWAS was performed with the MLM (Figure 2). The summarized results of the MLM analyses and SNPs with the $-\log_{10}(p) \geq 5.0$ for the bentazone reaction across the three years are presented in Table 3. In 2019, an SNP on chromosome 20 was associated with the bentazone reaction of cultivated soybean accessions at a $-\log_{10}(p)$ value of 5.0 based on the MLM association analysis (Figure 2A). Meanwhile, in 2020, four SNPs were detected on chromosomes 5 and 6 (Figure 2B). A total of 37 SNPs distributed on chromosomes 3, 5, and 13 were associated with the bentazone reaction in 418 cultivated soybean accessions (Figure 2C). Overlapping QTL regions on chromosome 5 across two years were associated with the bentazone reaction in cultivated accessions.

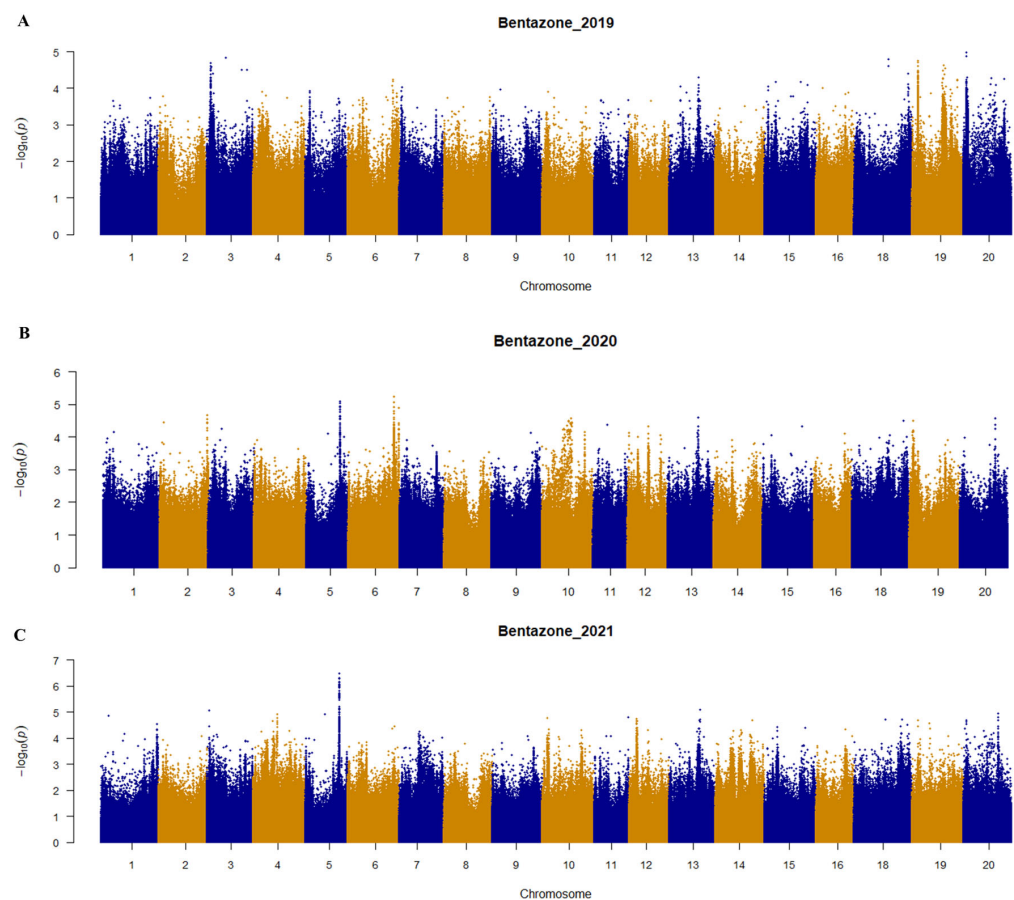


Figure 2. Manhattan plots of the bentazone reaction of 418 cultivated soybean collections over three years: (A) 2019, (B) 2020, and (C) 2021.

Table 3. SNP loci associated with bentazone reactions over three years.

Year	Chromosome	Position (Wm82.a2.v1)	$-\log_{10}(p)$	Minor Allele Frequency	R^2 of Model without SNP	R^2 of Model with SNP	Allelic Effect
2019	20	3,180,608	5.0	0.26	0.007	0.072	−1.073
2020	5	33,890,084	5.0	0.31	0.023	0.094	−0.626
	5	33,890,132	5.1	0.36	0.023	0.095	1.187
	6	45,537,285	5.2	0.32	0.023	0.097	−1.191
	6	45,542,880	5.1	0.31	0.023	0.095	−1.165
2021	3	2,396,680	5.1	0.26	0.011	0.071	1.077
	5	33,888,469	6.1	0.33	0.011	0.085	1.149
	5	33,889,712	6.5	0.33	0.011	0.091	1.196
	5	33,889,777	6.1	0.37	0.011	0.086	−0.611
	5	33,890,196	5.7	0.33	0.011	0.080	1.122
	5	33,893,210	5.6	0.34	0.011	0.079	0.575
	5	33,897,411	6.0	0.37	0.011	0.083	−0.599
	5	33,898,865	6.3	0.36	0.011	0.088	−0.639
	5	33,906,810	6.1	0.37	0.011	0.085	0.604
	5	33,909,318	5.6	0.32	0.011	0.078	1.109
	5	33,910,238	5.7	0.34	0.011	0.079	−0.598
	5	33,910,587	5.6	0.33	0.011	0.079	−0.585
	5	33,911,240	5.7	0.37	0.011	0.080	−0.587
	5	33,911,700	6.1	0.37	0.011	0.085	−0.612
	5	33,911,822	5.9	0.33	0.011	0.083	0.597
	5	33,912,867	5.8	0.35	0.011	0.081	1.134
	5	33,913,504	5.7	0.18	0.011	0.080	−1.082
	5	33,919,165	6.0	0.32	0.011	0.084	1.145
	5	33,919,559	6.0	0.32	0.011	0.083	1.147
	5	33,919,921	5.6	0.32	0.011	0.078	−0.104
	5	33,920,300	6.3	0.32	0.011	0.088	1.198
	5	33,920,327	6.0	0.38	0.011	0.084	−0.607
	5	33,920,665	5.5	0.32	0.011	0.077	−0.568
	5	33,922,937	6.1	0.38	0.011	0.085	−0.612
	5	33,923,052	5.6	0.32	0.011	0.078	0.579
	5	33,923,330	6.2	0.38	0.011	0.086	−0.620
	5	33,923,680	5.2	0.32	0.011	0.072	1.061
	5	33,924,925	5.7	0.38	0.011	0.080	−0.586
	5	33,925,495	5.1	0.35	0.011	0.072	1.051
	5	33,925,711	5.6	0.33	0.011	0.078	0.574
	5	33,927,103	5.7	0.32	0.011	0.080	0.796
	5	33,929,254	5.1	0.32	0.011	0.072	−0.555
	5	33,931,369	5.1	0.35	0.011	0.072	1.065
	5	33,931,839	5.5	0.39	0.011	0.077	0.600
	5	33,932,264	5.2	0.32	0.011	0.073	0.558
	5	33,934,052	5.1	0.33	0.011	0.071	0.546
	13	30,701,036	5.1	0.21	0.011	0.072	−0.678

3.4. Putative Genes Predicted to Be Involved in the Bentazone Reaction

The most significant SNPs in 2020 and 2021, based on the FarmCPU analysis, were located on chromosome 5 (Figure 3). Most of the SNPs on chromosome 5 in 2019 and 2020 were within a haplotype block, which was located between 33,888,343 bp and 33,930,815 bp (Figure 3A–C). For the bentazone-treated cultivated soybean accessions, there were five putative genes in the LD block on chromosome 5, where the most significant SNPs were located (Figure 3D). Among them, the functions of two putative genes (*Glyma.05g145100* and *Glyma.05g145400*) were unknown (Table 4). The other three putative genes were *Glyma.05g145000* (ABC transporter), *Glyma.05g145200* (ankyrin repeat family protein), and *Glyma.05g145300* (transmembrane amino acid transporter protein).

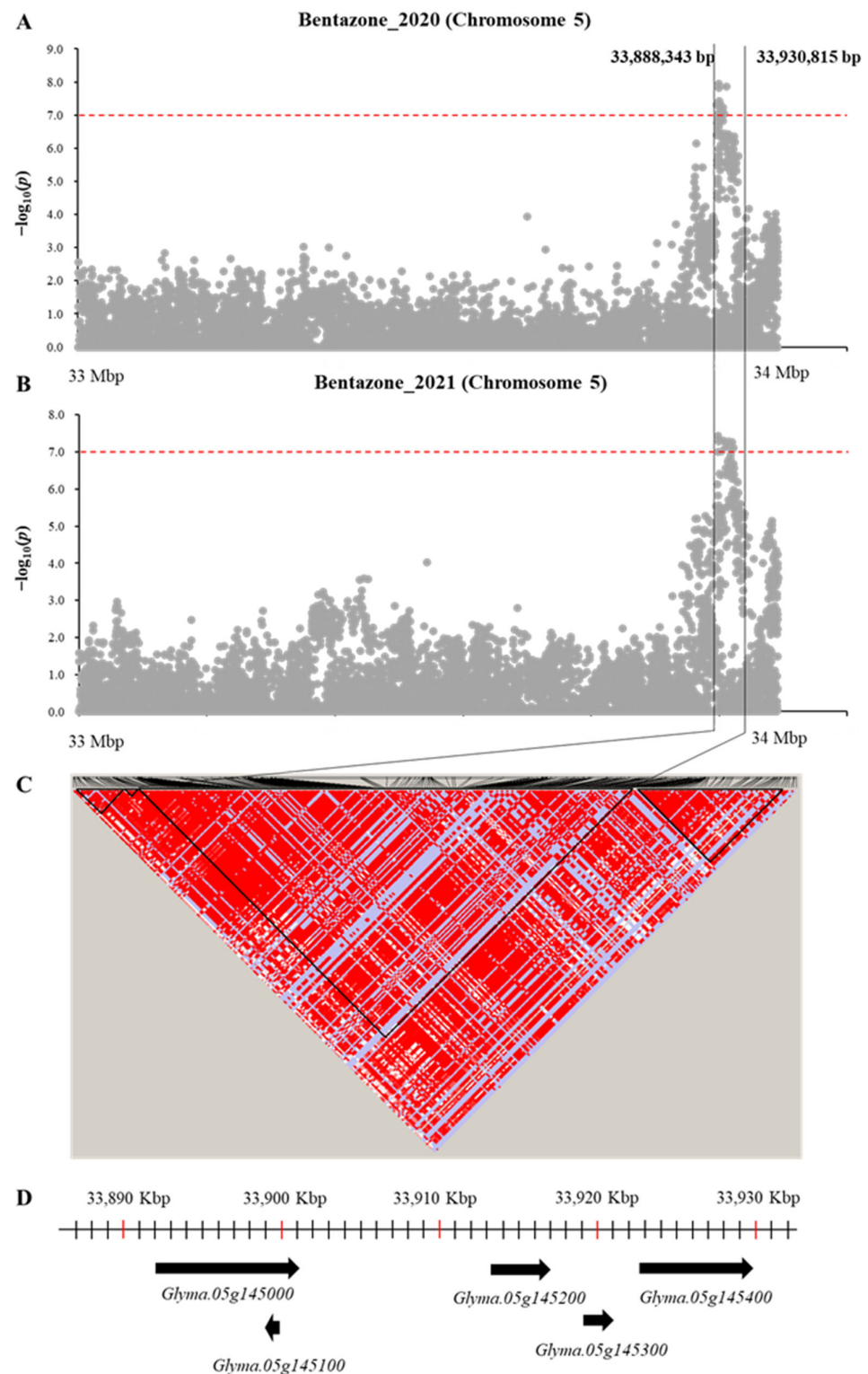


Figure 3. Linkage disequilibrium block of the most significant SNPs on chromosome 5 in cultivated soybean collections based on FarmCPU analysis. (A) Manhattan plot for 2020. The locations in the linkage disequilibrium (LD) block with the most significant SNPs on chromosome 5 are indicated by solid lines. (B) Manhattan plot for 2021. The location in the LD block with the most significant SNP on chromosome 5 is shown by black solid lines. (C) Haplotype blocks on chromosome 5. (D) Putative genes in the LD block. A total of five putative genes are in the LD block. The encoded genes are shown as arrows in the LD block between 33,888,343 bp and 33,930,815 bp (Wm82.a2.v1).

Table 4. Predicted genes present in the linkage disequilibrium block on chromosome 5.

Gene	Chromosome	Start Position (Wm82.a2.v1)	End Position (Wm82.a2.v1)	Predicted Protein (Pfam)
<i>Glyma.05g145000</i>	5	33,892,148	33,900,808	ATP-binding cassette (ABC) transporter
<i>Glyma.05g145100</i>	5	33,899,542	33,899,844	Unknown
<i>Glyma.05g145200</i>	5	33,913,231	33,916,617	Ankyrin repeat family protein
<i>Glyma.05g145300</i>	5	33,919,262	33,920,958	Transmembrane amino acid transporter protein
<i>Glyma.05g145400</i>	5	33,922,696	33,929,901	Unknown

4. Discussion

The application of herbicides is essential for weed control in most agricultural production systems today. Broadleaf weed management is essential for large-scale soybean production systems. However, phytotoxicity is a major concern in the application of POST herbicides, which might disrupt soybean vegetative growth and yield [48]. Bentazone is a selective-contact herbicide that is commonly employed in the production of soybeans, wheat, and rice. It is one of the few available selective-contact herbicides for the POST treatment of soybeans that shows no symptoms in bentazone treatment. This study confirmed that herbicide-tolerant soybean cultivars are indeed necessary for the extensive application of bentazone. We evaluated the reaction of 418 cultivated soybean accessions to bentazone in three independent environments. ANOVA indicated that genotype, year, and genotype-by-year interactions had a significant impact on the bentazone reaction ($p = 0.001$) (Table 1). In addition, we identified bentazone-tolerant and bentazone-sensitive soybean germplasms from 418 cultivated soybean germplasms and found that moderate bentazone tolerance is predominant in the Korean collection of cultivated soybeans (Figure 1). Indeed, our previous study reported a similar result, finding that most of the released Korean cultivars showed moderate bentazone tolerance [42]. Based on the visual score assigned over the three years considered in this study, 11 accessions, namely, Daepung, Boseok, KAS531-5, KAS625-19, KLS087160, IT178037, IT104887A, Clark, KAS_102-2, KAS651-37, and Cheongja 3, showed strong tolerance to bentazone treatment. Meanwhile, Hannam, Haman, Hadaedu, Dowling, Bancheongdu, KLS419, and IT104887A were extremely sensitive to it. These bentazone-tolerant accessions may be used directly or indirectly in soybean breeding efforts to minimize bentazone toxicity at the field level.

Generally, relatively little genetic research has been conducted on soybean herbicide tolerance. This is probably due to the development of genetically modified herbicide-resistant crops and the production of robust target-site-specific mutant crops for certain herbicides. Since the development of genetically modified (GM) soybeans, producers have shifted to new GM cultivars due to the ease and versatility of weed control, which requires just one chemical to cover a wide range of weeds without causing crop damage. Overall, GM soybeans contribute around 82 percent of total soybean output. However, GM organisms have numerous drawbacks, including herbicide resistance genes moving from resistant cultivars to weedy relatives and existing mutants approaching their fitness boundaries. In addition, concerns about the impact of the intensive use of herbicides on human health and the environment due to the cultivation of GM crops have led to a global ban on the planting of GM soybeans. Weed management in soybean fields is mainly accomplished via the use of PRE and POST herbicides. Therefore, soybean resistance or tolerance to POST herbicides is essential in soybean production.

Since the reactions of soybean genotypes to bentazone differ, it is necessary to choose bentazone-tolerant and bentazone-sensitive soybeans for breeding and genetic research. The development of a cultivar with a faster ability to detoxify herbicides as the basis for herbicide tolerance will be beneficial to soybeans. Secondly, GWAS and QTL mapping

are important to understanding the genetic loci on the soybean genome for bentazone reactions. Several investigations have been conducted to identify the genomic loci or genes responsible for bentazone reactions in many crops. Few genetic studies have sought to explain the molecular mechanisms underpinning bentazone tolerance in soybeans.

The GWAS approach has been extremely useful in the genetic dissection of complex traits in plants. It has been successfully used in various plant species, including rice [49], wheat [50], maize [51], tomatoes [52], and soybeans [53–55]. However, little information has been reported on the detection of the genomic regions of soybeans associated with sensitivity or resistance to herbicides through GWAS. Moreover, few genetic studies on the bentazone treatment of soybeans have been reported.

To date, the cytochrome P450 hydroxylase (*CYP81E22*) in soybeans, which confers high sensitivity to bentazone, has been identified on chromosome 16 [28]. In the present study, GWAS identified 42 SNPs from different years, which were present on five chromosomes. Among these identified SNPs, a genomic region on chromosome 5 overlapped between the findings from 2020 and 2021 (Table 3). A haplotype analysis revealed that there were five putative genes in the LD block on chromosome 5. Of these putative genes, three genes—*Glyma.05g145000* (ABC transporter), *Glyma.05g145000* (ankyrin repeat family protein), and *Glyma.05g145000* (transmembrane amino acid transporter protein)—were reported the gene functions.

Generally, there are four phases of plant detoxification against toxic chemicals for NTSR [23]. Phase I is the detoxification to help activated herbicide molecules be exposed to phase II enzymes. The typical detoxification phase, phase I, comprises oxidation that is involved in P450 monooxygenases. Phase II detoxification involves the conjugation of a hydrophilic molecule to the activated herbicides. Conjugations of glutathione, homol glutathione, or sugar molecules to the herbicides by enzymes (GST and GT) are typical examples of phase II detoxification. In contrast to phase I and phase II detoxification, phase III confers herbicide resistance through compartmenting conjugated molecules or herbicides into the vacuole or extracellular space. ABC transporter genes are the most common transporters in phase III detoxification. Phase IV detoxification involves the degradation of herbicides or their metabolites in the vacuole or extracellular spaces. These detoxification processes have been reported to involve cytochrome P450 monooxygenase, UGTs, the ABC transporter, GST, GT, and POD [23]. The present study identified a putative gene, *Glyma.05g145000*, on chromosome 5, annotated as an ABC transporter (Table 4). Previous studies reported that the ABC transporter gene was involved in compartmentalizing herbicides and their metabolites in higher plants [55,56]. Moreover, knockout of the ABC transporter (*EcABCC8*) from a glyphosate-resistant population of *Echinochloa colona* (jungle rice) enhanced sensitivity to glyphosate in rice [57]. In addition, the overexpression of *EcABCC8* orthologous genes was reported to confer glyphosate resistance in rice, maize, and soybeans. Furthermore, based on an RNA-sequencing analysis, Qiao et al. [58] recently reported that the expression levels of ABC transporter genes in rice were induced by bentazone treatment. Thus, *Glyma.05g145000* (ABC transporter) may confer bentazone tolerance in the cultivated soybean collection examined in this study.

Further studies are required to confirm the involvement of putative genes in the bentazone reaction through biparental mapping analysis and a comparison of the expression levels of putative genes between bentazone-treated and control soybean plants. The herbicide-tolerant, herbicide-sensitive accessions and herbicide tolerance loci discovered in this study might speed up gene mapping, and assist in the map-based cloning of a herbicide tolerance gene, as well as shedding light on how herbicide responses are regulated in soybeans.

In conclusion, this study revealed the phenotypic distribution of cultivated soybean collections and possible putative genes for bentazone reactions in soybeans. The results determined bentazone-tolerant and bentazone-sensitive soybean germplasms from 418 cultivated soybean germplasms and found that moderate bentazone tolerance predominated in the Korean collection of cultivated soybeans. By conducting a GWAS with publicly avail-

able resequencing data, we identified five putative genes, namely, *Glyma.05g145000* (ABC transporter), *Glyma.05g145100* (unknown), *Glyma.05g145200* (ankyrin repeat family protein), *Glyma.05g145300* (transmembrane amino acid transporter protein), and *Glyma.05g145100* (unknown). The results of this study can be used in breeding programs for herbicide-resistant soybeans.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy13092345/s1>, Table S1: The information of soybean accessions and betanzone reaction over three years.

Author Contributions: Conceptualization, J.-D.L.; formal analysis, H.J.; investigation and methodology, L.A.; writing—original draft preparation, H.J. and L.A.; writing—review and editing, H.J., J.T.S., and J.-D.L. supervision and project administration, J.-D.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by Kyungpook National University Development Project Research Fund, 2020.

Data Availability Statement: The datasets generated in this study are available from the corresponding author upon reasonable request.

Acknowledgments: The authors acknowledge the personnel from the Plant Genetics and Breeding Laboratory at Kyungpook National University.

Conflicts of Interest: The authors declare no conflict of interest.

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