

# Bentazone-Tolerant Soybean

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Bentazone is an herbicide that selectively kills broadleaf weeds by inhibiting photosynthesis. It is widespread in soybean-cultivating areas including genetically modified organism (GMO) and GMO-free regions all around the world. This herbicide carries a double-edged sword since it can also incur damage to crops upon application. The challenge, therefore, lies in the deliberate selection of bentazone-tolerant cultivars.

Keywords: soybean, herbicide tolerance, Bentazone

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## 1. Introduction

Herbicides are selected based on several factors for successful weed control, including weed range, lack of crop injury, effect of agro-ecosystem, economic cost, and policy. Bentazone [3-Isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide] was initially registered in 1975. It has been used for selective POST control of broadleaf weeds and sedges in alfalfa, asparagus, cereals, clover, digitalis, dry peas, flax, garlic, grass, green lime beans, mint, onions, potatoes, snap beans for seeds, sorghum, soybeans, and sugarcane <sup>[1][2][3][4]</sup>. Bentazone is a contact herbicide that interferes with susceptible plants' ability to use sunlight in producing survival energy through photosynthesis. Visible damages on the surface of leaves were usually observed after a few hours to weeks of treatment with bentazone, depending on the species and dosage <sup>[5][6][7]</sup>. Bentazone is absorbed by leaves upon application as a foliar spray, accompanied by rapid break-down or metabolism into natural plant components and metabolites in resistant plants, such as starch, protein, lignin, amino acid, and cellulose <sup>[7]</sup>. When bentazone is absorbed by the roots, it is translocated from the roots to other parts of the plants through xylem, with plant nutrients in the transpiration stream <sup>[8]</sup>. However, the degree of translocation is dependent on plant species after absorption by leaves. In susceptible and resistant soybean genotype, the amount of absorbed bentazone translocated from the treated leaf was 7 to 13 percent of the total absorption <sup>[9]</sup>. In addition, foliar application of bentazone to soybean resulted in slow acropetally translocation of the herbicide <sup>[10]</sup>. In tolerant rice, most of the <sup>14</sup>C-bentazone remained in the treated leaf or translocated to older leaves, whereas most of the <sup>14</sup>C-bentazon was translocated from the treated leaves to younger leaves in susceptible rice <sup>[7]</sup>. Acropetal and basipetal translocation of absorbed <sup>14</sup>C from <sup>14</sup>C-bentazon was equal in corn <sup>[11]</sup>. Most of the absorbed <sup>14</sup>C-bentazone in *capsicum* species remained in the treated leaves, with minimal acropetal and basipetal translocation from the site of application <sup>[12]</sup>. For some susceptible weed species (yellow nutsedge, Canada thistle) the translocation was acropetal transport <sup>[10][13]</sup>.

The adverse effect of bentazone on agro-biodiversity as well as soil fertility is less than that of other selective contact herbicides <sup>[14]</sup>. Bentazone concentration at the field does not considerably affect the microflora even without microbial degradation <sup>[15][16]</sup>. In case of 2,4-D, it adversely affects the activities of *Rhizobium* sp. and glyphosate suppresses the phosphatase activity of purple non-sulfur bacteria in soil, decreasing the growth and activity of azotobacter as well as N-fixation in soybean <sup>[17]</sup>. Compared with other POST broadleaf herbicides, bentazone is quickly degraded in the soil <sup>[18][19]</sup>. For soil fertility, bensulfuron methyl and metsulfuron-methyl reduces the operation of N-mineralization. In addition, prometryn, simazine, and terbutryn inhibit not only the N-fixation, but also reduce the total number of nodules and N in soil. Furthermore, glyphosate, sulfonylurea and atrazine also dramatically alter soil structure with the disturbances in soil earthworm ecology and the inhibition of soil N-cycle under alkaline or low organic matter <sup>[20]</sup>.

Since bentazone was originally evaluated by the Joint FAO/WHO meeting on Pesticide Residues (JMPR) in 1991, the re-evaluation was periodically conducted until now. The evaluation reported residue of parent bentazone and its metabolites such as 6- and 8-hydroxy-bentazone after its application in seed, food, and feed of plant. In USA, the limit of detection by gas liquid chromatography method for compounds of bentazone and its metabolites in soybean was 0.05 ppm according to a rule by the environmental protection agency in 1919 <sup>[21]</sup>. In Europe, the maximum residue level for soybean was 0.01 ppm based on a report of pesticide residues in food in 2013 <sup>[22]</sup>. In addition, based on carcinogen assessment by environmental protection agency, bentazone was found not to be mutagenic and was classified as a Group E chemical, which means evidence of non-carcinogenicity for humans <sup>[23]</sup>.

## 2. Bentazone

### 2.1. Bentazone Effectivity Modulated by Environmental Conditions

The effective utilization of herbicides is determined by environmental conditions before, during, and after its application [24]. Increasing light intensity at the application of herbicide results in higher photosynthesis and subsequent phloem translocation to increase the movement of foliar-applied herbicides. The stomata remain open at high light intensity, thus increasing tissue penetration of foliar herbicides [24]. Thus, the effectiveness of POST herbicides depends on light intensity during application.

One of the most important environmental factors are air temperature and relative humidity (RH), which alter the performance of herbicides in weed control. Absorption and translocation of bentazone in plant increases with higher temperatures [25]. At high temperatures, herbicide absorption is enhanced through reduced cuticle waxes and increased herbicide diffusion through the cuticle [24]. With high temperatures and high RH levels, the cuticle is highly hydrated, thereby promoting absorption of herbicides [26].

Generally, humidity has a greater effect on herbicide uptake than temperature [27]. Increased humidity increases the uptake of bentazone. For instance, increased uptake of bentazone at 80% RH rather than one at 40% RH was observed in seven plant species [25]. Additionally, high RH levels favor the efficiency of foliar-applied herbicides by affecting herbicide uptake through interactions between the herbicide droplet, leaf cuticle, stomatal opening, and water in or around droplets [28]. The effects of low humidity on bentazone uptake can be overcome by adding adjuvants. For example, enhancement of bentazone uptake by adjuvants was more pronounced at 40% RH than at 80% RH [25]. Bentazone was found to be very mobile in soil at natural RH due to its high-water solubility [28]. Bentazone's herbicidal activity is very low in dry soil; however, its activity increases with increasing soil moisture content and highly active at slightly flooded conditions [28]. Herbicide performance is generally reduced on moisture stressed plants under dry or low soil moisture conditions because plant morphology and physiology are both affected [29]. Understanding of the environmental factors that cause low herbicide efficiency makes it possible to consider the appropriate conditions such as temperature and humidity to spray, thereby maximizing the dose applied and minimizing the processing costs.

### 2.2. Tolerance Mechanism for Bentazone

The WSSA defines herbicide resistance as “the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type, and may be naturally occurring or induced by techniques, such as genetic engineering or selection of variants produced by tissue culture or mutagenesis” [30]. Herbicide resistance mechanisms include structural reforms at the target site of the herbicide, metabolic detoxification, and changes in herbicide uptake, translocation, or compartmentalization [31]. Plant species that are not controlled by a herbicide before any selection pressure or genetic manipulation can be considered naturally tolerant, but not herbicide-resistant [32].

Bentazone tolerance requires mechanisms that limit the amount of active herbicide entering the target site, mechanisms that decrease herbicide uptake, and mechanisms that improve herbicide metabolism. Differential absorption and metabolism in treated bentazone-susceptible line leaflets resulted in a higher bentazone concentration than that of tolerant species, revealing that differential metabolism is the key cause of different responses to bentazone [11][33]. Tolerant plants can easily detoxify bentazone through rapid aryl hydroxylation followed by glucose conjugation, whereas susceptible plants metabolize bentazone to a lesser extent, and several species are unable to metabolize it at all. The absorbed bentazone was hydroxylated to either 6-hydroxy-bentazone or 8-hydroxy-bentazone and then glycosylated at positions 6 or 8 of its aromatic ring, which was eventually oxidized to produce natural plant products, such as starch, protein, lignin, amino acid, and cellulose [34]. Based on the species, the positions of its aromatic ring of hydroxylation and glycosylation are different. Bentazone is readily metabolized in rice and other gramineous plants by detoxifying this herbicide primarily by 6-hydroxylation or 6- and 8-hydroxylation and glucose conjugation of its hydroxylated positions [35]. Sorghum and Johnsongrass [36] rapidly metabolizes bentazone by producing 6-hydroxylbentazone, whereas wheat [35], corn [37], and soybean [9] metabolizes it by producing 6- and 8-hydroxyl-bentazone after hydroxylation followed by glycosylation at the 6 and 8 positions of its aromatic ring.

Metabolic resistance of bentazone are generated by endogenous cytochrome P450 mono-oxygenases, glucosyl transferases (GTs), glutathione S-transferases (GSTs), and/or other enzyme systems, such as aryl acylamide [38]. Hydroxylation of aromatic rings or alkyl groups by a family of enzymes known as cytochrome P450 mono-oxygenases and then, glucose conjugation at positions of its aromatic ring by GSTs or GTs is the most common way to detoxify herbicides in plants [39][40].

### 2.3. Inheritance and Genes Controlling Bentazone Tolerance in Crops

Several studies have reported different responses to bentazone in crops, such as rice [41], maize [42], potato [43], sweet potato and pumpkin [44], cowpea [45], red bean [46], lentil and pepper [47], peanut [48], clover [49], and blueberry [50].

In previous studies, the cytochrome P450 family has been identified to detoxify bentazone in various plants [4][37][51]. In rice, a *Bel*-encoded cytochrome P450 mono-oxygenase, *CYP81A6*, mediated resistance to bentazone. The loss of bentazone resistance in rice resulted from a single base-pair deletion of *CYP81A6* [4][52]. Additionally, Fang et al. [53] reported that a 519-kD transcription factor-like protein (B9FDA1) containing two histidine kinase-like ATPase domains and a zinc-finger type C3HC4 RING domain can serve as a transcription factor for the expression of bentazone tolerance genes in rice.

In soybean, the gene expression pattern of certain cytochrome P450 homologs, such as *CYP94A1*, *CYP82A2*, *CYP71D8*, and *CYP81E8*, occurred when plants were treated with bentazone [9]. Bernard and Wax [54] reported that the inheritance of bentazone sensitivity in soybean was controlled by a single recessive gene. A single recessive gene (*bnz-1*) was mapped to chromosome 16 for bentazone sensitivity of soybean, where a single base-pair deletion in the coding region of *GYP81E22* (*Glyma.16G149300*) at +1465 bp downstream from the translation start codon led to a premature stop codon and loss of function of cytochrome P450 hydroxylase [55]. Several compounds inhibit both soybean aryl hydroxylation and cytochrome P450 reactions, provided by in vivo evidence of bentazone metabolism facilitated by cytochrome P450 [51]. For GTs, Leah et al. [51] isolated and purified two enzymes from bentazone-tolerant soybean to produce the glycosylated 6- or 8-hydroxybentazone.

POST application of bentazone reported initially the susceptibility of various in-breeds in the study of inheritance of single crosses of corn, which may be attributed to a single recessive gene, named *ben* [42]. Several studies supported that the *ben* locus may be responsible for bentazone sensitivity by cytochrome P450 monooxygenase in white-kernel inbred [56] and sweet corn [57]. In addition, another study of an in vitro assay with corn reported that bentazone aryl hydroxylation of maize microsomes is catalyzed by cytochrome P450 mono-oxygenase [37].

Bentazone tolerance of *Capsicum annuum* was also controlled by a single dominant gene [58]. A dominant *Bzt* gene responsible for bentazone tolerance in pepper cultivar “Santaka” may be the same gene or may be closely linked to the *Bzt* locus that confirms bentazone tolerance in “Bohemian Chili” [47].

New technologies involved in genetically modifying herbicide-resistant cultivars help in managing alternatives to glyphosate. Several of them that are still being tested may be available for release on short notice [59]. Bentazone-resistant species of cotton [60][61], tobacco, and Arabidopsis [62] were developed through genetic transformation of the bentazon-resistant gene (*Cyp81A6*) from rice. Additionally, rat *CYP1A1* was transformed for potato bentazone-resistant cultivars [63].

## 2.5. Utilization of Bentazone in Soybean Production

Bentazone is a POST broadleaf herbicide that is widely used in soybeans. Annual broadleaf weed species can reduce soybean yield by more than 50%. *Ambrosia* spp. and *Amaranthus* spp. are frequently reported in literature and regarded as the most noxious broadleaf weeds in soybean. Bentazone prevents weed growth in grain-type soybean as well as vegetable soybean fields [64]. Soybean yield and harvestable soybean losses were prevented by pre-harvest application of a mixture of bentazone and acifluorfen, as well as bentazone and imazamox. The total weed control output of bentazone in conjunction with imazamox and typhenesulfuron was 95.04% [65]. Application of glyphosate tank mixtures with bentazone was more selective to Roundup Ready soybean when applied at younger stages [66].

Bentazone responses in soybean plants were classified into three types, namely tolerant, moderately sensitive, and highly sensitive [67]. While bentazone application causes minor injuries in tolerant soybean cultivars, plants gradually recover with growth, resulting in a marginal reduction in yield [68]. Compared to other broadleaf herbicides, application of bentazone produces less leaf injury and yield reduction in soybean [6][69][70][71][72][73][74][75][76][77].

## 3. Future Perspective and Conclusion

Since genetically modified (GM) soybean was developed, growers have switched to adopt the new GM cultivars because of its simplicity and flexibility of weed control that depends on only one herbicide to control a broad spectrum of weeds without crop injury. Globally, GM soybeans accounted for about 82% of the total soybean production area [78]. However, many countries are not deliberately growing genetically modified soybean, because of concern over its adverse impact on human health and environmental problems. The demand for non-GM soybean is relatively high in the European Union (EU), compared with other parts of the world. Of the number of soybean and soybean-derived products consumed by the EU, 95% are imported. The main use of soybean products in the EU is for animal feed [79]. Base on the Food and

Agriculture Organization (FAO), bentazone was registered to use as herbicide for soybean seed and forage in France, Germany, Greece, Italy, Spain, the Netherlands, and the USA. In non-GMO (genetically modified organism) areas in EU and Asian countries, application of graminicides and bentazone can control simultaneously both grass and broadleaf weed in bentazone-resistant soybean field, which save time, labor, and application cost. Thus, it is important to develop bentazone-resistant soybeans for non-GM soybean production area.

Soybean resistance or tolerance to POST herbicide is essential. Soybean is a broadleaf plant, and weed control was mainly carried out by the PRE + POST herbicide application system before herbicide-resistant soybeans were produced mainly through genetic modification. However, there is a difference in the responses to bentazone among soybean genotypes; hence, it is necessary to select tolerant soybeans for breeding and for genetic studies. It is also necessary to understand inheritance of resistance to bentazone through population genetic studies such as a genome-wide association study. Several studies have reported on the genes that control bentazone tolerance in several crops; however, few genetic studies have been conducted in the past to clarify the molecular mechanisms of bentazone tolerance in soybean. This is most likely due to the advent of genetically modified herbicide tolerance as a significant selectivity method, as well as the production of strong target-site-specific mutant crops for specific herbicides.

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