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## Research Paper

# Likelihood assessment for gene flow of transgenes from imported genetically modified soybean (*Glycine max* (L.) Merr.) to wild soybean (*Glycine soja* Seib. et Zucc.) in Japan as a component of environmental risk assessment

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Environmental risk assessment is required for genetically modified (GM) crops before their import into Japan. Annual roadside monitoring along transportation routes from ports to processing facilities for GM soybean (*Glycine max* (L.) Merr.) have been requested as a condition of import only approval because of lack of information on the likelihood of persistence of imported GM soybean for food, feed and processing and the potential for transfer of transgenes into wild soybean (*Glycine soja* Seib. et Zucc.) through gene flow under the Japanese environment. The survey of soybean seeds, plants and wild soybean populations were conducted along transportation routes from unloading ports to processing facilities that provided data to help quantify actual exposure. The survey indicated that the opportunities for co-existence and subsequent crossing between wild soybean populations and imported soybean are highly unlikely. Together the survey results and the comprehensive literature review demonstrated low exposure of imported GM soybean used for food, feed and processing in Japan. This evaluation of exposure level is not specific to particular GM soybean event but can apply to any GM soybean traits used for food, feed and processing if their weediness or invasiveness are the same as those of the conventional soybean.

**Key Words:** *Glycine max*, *Glycine soja*, wild soybean, genetically modified crops, environmental risk assessment, gene flow, exposure assessment.

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## Introduction

Genetically modified (GM) soybean (*Glycine max* (L.) Merr.) was first commercially cultivated in 1996. Over the 18 year period, area cultivated with GM soybean has increased to 90.7 million hectares globally (James 2015). Japan consumes approximately 3 million metric tons of soybean annually, with 90% of these soybeans imported (MAFF 2015, MOF 2015). Japan does not currently cultivate GM soybean, but does import GM soybean. For large-scale cultivation, Environmental risk assessment (ERA) assumes significant exposure, with potential impact scenarios expected to be the most pronounced (Roberts *et al.* 2014, 2015). In practice, however, imported GM crops used for food, feed and processing (FFP) are associated with far

lower exposure compared to large-scale cultivation. It is assumed that spillage of grain during transportation is the only scenario where the receiving environment (*e.g.*, wild native species and endangered species) would be exposed and potentially affected by GM crops in import countries. Given the different exposure levels, ERA for FFP use does not require the assumption of high exposure, but does require a scientific and pragmatic estimation of potential exposure.

Risk assessment is a process that integrates relevant exposure scenarios and potential consequences in terms of harm from those exposures (Wolt *et al.* 2010). Hazard and exposure are characterized separately prior to the formulation of risk estimation (Hill 2005, Nickson 2008). Once both hazard and exposure are identified, the risk is estimated by considering hazard in the context of exposure (Nickson 2008, Wolt *et al.* 2010).

A stepwise approach is proposed for ERA under low exposure scenario including intended use for FFP (Roberts *et al.* 2014). In this approach, it is necessary to understand the characteristics of the GM crop plant, and then to determine

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**Table 1.** Introduction pathway of GM soybeans into Japanese environment and required information for the environmental exposure assessment

Steps of introduction of imported soybean	Information required for exposure assessment
1 Arrival of soybeans at ports	Amount of imported soybean
2 Transportation from unloading ports to food and feed facilities	Amount of imported soybean according to usage (oil, feed, food) Location of food and feed facilities
3 Spillage of soybean seeds during transportation	Transportation routes Frequency/extent of spillage of soybean seeds during transportation
4 Growth of soybean plants from spilled seed	Probability of germination and survival of soybean in the environment near transportation routes Weediness or invasiveness of soybean
5 Growth of soybean adjacent to wild soybean populations	Presence of potential wild soybean growing area near transportation routes
6 Synchrony of flowering period between soybean and wild soybean	Time of flowering period of soybean and wild soybean
7 Outcrossing between soybean and wild soybean	Outcrossing rate between soybean and wild soybean
8 Weediness or invasiveness characteristics of hybrids between soybean and wild soybean	Phenotypic characteristics related to weediness of hybrid between soybean and wild soybean

This table outlines the pathway of potential introduction of imported soybean into the receiving environment. Each step indicates a step for exposure assessment in ERA of GM soybean and information required for the assessment is described in the right column.

the likelihood that the plant will persist or multiply in the environment (Roberts *et al.* 2015). The receiving environment may impact the assessment and should be considered during problem formulation for ERA, particularly in the identification of relevant protection goals (Roberts *et al.* 2014). In Japan, wild soybean (*Glycine soja* Seib. et Zucc.), a cross-compatible relative of soybean, grows naturally. Thus, the likelihood of persistence of a transgene in wild soybean through gene flow from cultivated to wild soybean is another key component that needs to be assessed within the context of an environmental protection goal. For Japan, comprehensive exposure assessments which consider the relevant exposure have not historically been conducted. This is because 16 previously approved GM soybeans conferred only herbicide tolerance (HT) or modified fatty acid components, and thus MAFF and MOE viewed that these traits do not provide selective advantage to wild soybean even if gene flow of transgenes occurred under cultivation use.

There is a recent example in which relative exposure was considered. Lepidopteran insect-protected soybean, MON 87701 (modified *cry1Ac*, *Glycine max* (L.) Merr.) (MON 87701, OECD UI: MON-87701-2), was the first product approved for FFP in Japan after the adoption of the Cartagena Law. MAFF and MOE concluded that it is difficult to assess MON 87701 for cultivation use due to uncertainty of potential gene flow from MON 87701 to wild soybean even though there are no unintended changes related to weediness potential in MON 87701. The potential hazard of the insect-protected soybean against wild soybean was assessed based on two studies. A survey of wild soybean populations in Japan and the experiment using five levels of defoliation (0%, 10%, 25%, 50% and 100%) indicated that seed production of wild soybean is not limited by lepidopteran feeding and has an ability to compensate for defoliation levels observed in nature (Goto *et al.* 2016, MOE 2014). The exposure assessment of imported GM soybean for FFP was

conducted for MON 87701 by following the stepwise approach as shown in **Table 1**. The assessment leads to a conclusion that the exposure level of imported soybean for FFP use to Japan is very low, and thus the potential risk of gene flow from MON 87701 to wild soybean is negligible (MOE 2014). Some values were conservatively estimated as there was some uncertainty in a few steps of the exposure assessment due to lack of available information required for the risk assessment. For example, it was assumed that 100% of spilled imported soybean seeds during transportation were MON 87701 (step 3 in **Table 1**), or that there is 100% probability of soybean growth adjacent to wild soybean populations (step 5 in **Table 1**).

Research presented here was conducted to obtain additional information associated with potential seed spillage during transportation, which helps more accurate evaluation of potential gene flow from imported soybean to wild soybean.

The objective of present research was to conduct the stepwise exposure assessment in import countries like Japan by obtaining information empirically by conducting surveys near port and along roadside and by summarizing published information. This evaluation provided information for likelihood of the persistency of imported GM soybean and a transgene in wild soybean through gene flow under the Japanese environment. This approach would address questions regarding low exposure associated with FFP uses of imported GM soybean and would provide a basis for challenging the necessity of the monitoring imposed to GM soybean for FFP use.

## Materials and Methods

### Roadside survey of routes I, II and III for soybean plants and wild soybean populations

The roadside survey for soybean plants and wild soybean populations was conducted in late August for routes I and II and in early September for route III in 2012, 2013, and

2014. The number of wild soybean plants was, however, unable to be counted as they intertwine. Thus, a group of wild soybean plants grown together was considered as one population and was recorded as such.

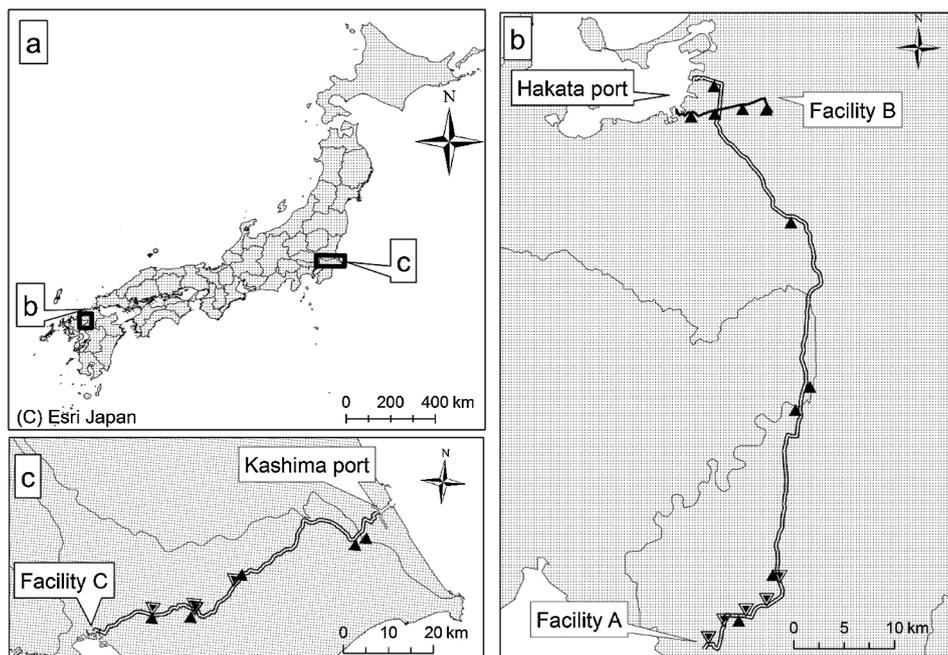
**Observation routes**

A total of 25 inland roads from 11 Japanese ports were identified as transportation routes used to move large quantities of imported soybean grain to feed facilities. Of those, Hakata and Kashima ports were selected in this research because MAFF observed a higher number of soybean plants within the approximately 5 km radius of the unloading site in their roadside surveys (MAFF 2011a, 2011b, 2012, 2013, 2014). There are two inland roads from the Hakata port, and both were included in this survey (Fig. 1). Route I is

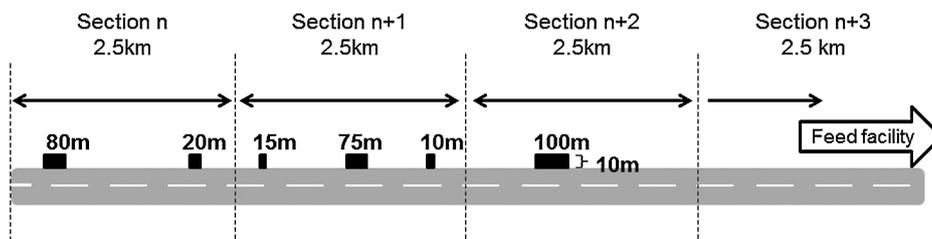
73.4 km starting at the Hakata port in the north and ends at feed facility A in the south. Route II is 11.7 km starting at the Hakata port in the west and ends at feed facility B in the east. There are seven inland roads identified that originate from the Kashima port. Among them, the longest route which is 87.2 km was chosen as observation route III (Fig. 1).

**Observation sites**

Each 2.5 km segment of the observation routes was defined as a section, and each section was numbered according to the distance from the ports. Route I, II and III were divided into 30, 5 and 35 sections respectively. In each section, 10 m width from the left edge of the road when facing to the feed facility was surveyed since soybean seeds are unlikely to spread more than 10 m (Fig. 2). Land use of roadside



**Fig. 1.** Monitoring routes surveyed in this study. Fig. 1a shows two areas in Japan that were surveyed in this study. Fig. 1b is enlarged view of area b in Fig. 1a and shows routes I and II. Route I (white line) starts at the Hakata port and ends at feed facility A. Route II (black line) starts at the Hakata port and ends at feed facility B. Fig. 1c is enlarged view of area c in Fig. 1a and shows route III (white line) that starts at the Kashima port and ends at feed facility C. The white arrows (∇) on the routes in Fig. 1b and 1c denote places where wild soybean populations were observed in this study. The black arrow (▲) on the routes in Fig. 1b and 1c denote places where each route crosses rivers or runs along rivers.

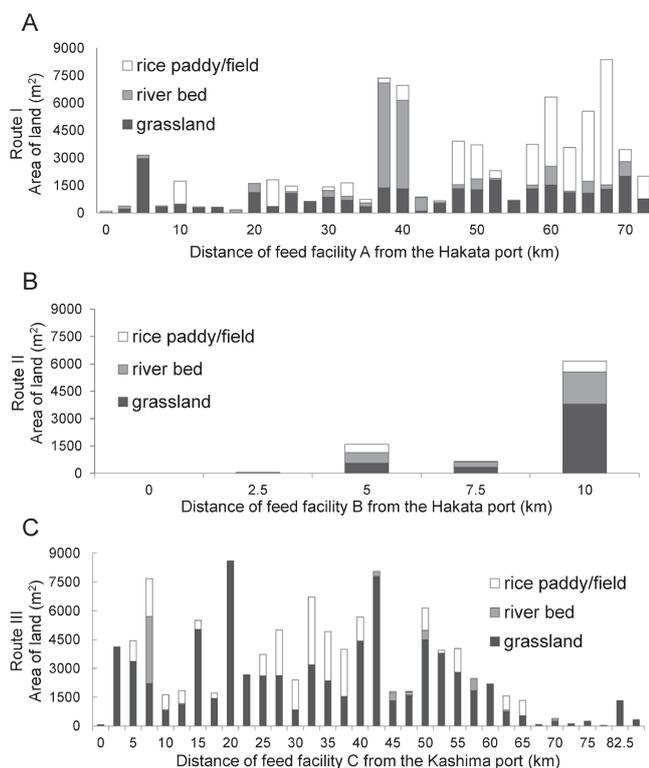


**Fig. 2.** Schematic diagram of sections and observation sites of roadside area along the transportation routes. Transportation routes shown as gray line were divided 2.5 km sections. The section number is assigned according to the distance from port. Observation sites were shown as black square above the transportation route. In each section, an area of approximately 100 m in length was selected as observation sites. Observation sites were set as 10 m width from the left edge of the transportation routes. Observation sites were selected as 100 m in continuous length or in total. For example, total length of observation sites were 100 m = 15 m + 75 m + 10 m in the section n + 1.

areas along the surveyed routes was assessed from air photos using ArcGIS® for Desktop version 10.0 (Esri Japan Corporation, Japan). Eight land use categories were defined according to the Geospatial Information Authority of Japan as: 1. rice paddy/field, 2. dry river bed, 3. grassland, 4. park/public green space, 5. residential zone (garden/planting), 6. bare ground, 7. brush, and 8. land covered by cement or blacktop (Geospatial Information Authority of Japan 2015). Probability of wild soybean growth is assumed to be high for categories 1–3 and low for categories 4–8. In 2012, areas with higher potential for growth of wild soybean (land categories 1–3), were identified as observation sites up to approximately 100 m in length per section along the observed routes. If the selected areas did not reach a total of 100 m in length, areas in other categories were selected for the remainder of the length (Fig. 2).

### Survey near Kashima and Hakata ports and routes I, II and III for soybean seed

The survey for soybean seed was conducted at 11 sites (6 sites at Hakata port and 5 sites at Kashima port) within a 5 km radius near Hakata ports on September 2–4 and Kashima on August 26–28, 2015 (Fig. 4a, 4b). The observation sites were selected based on the monitoring conducted by MAFF (MAFF 2011a, 2011b, 2012, 2013, 2014).



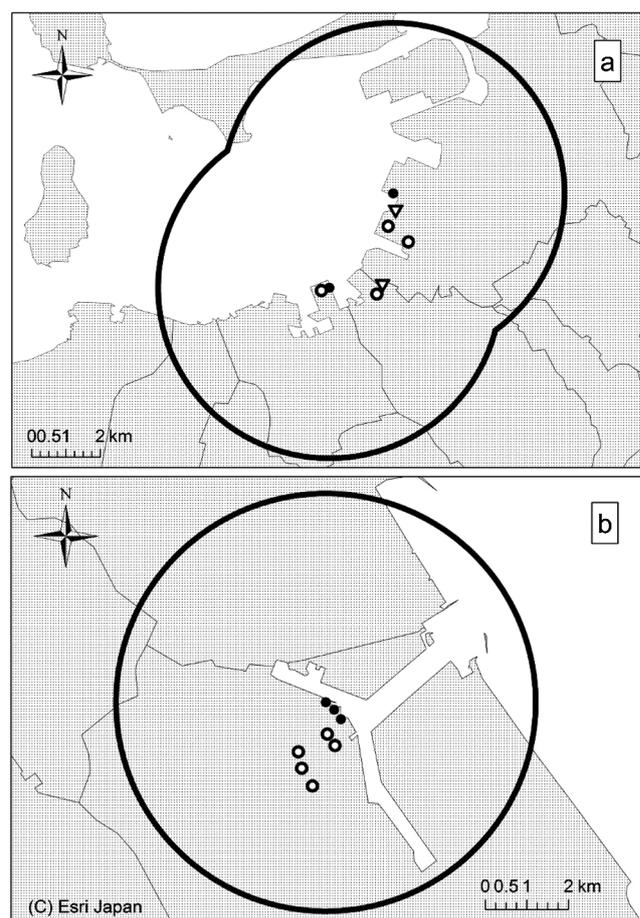
**Fig. 3.** Potential growing area for wild soybean ( $m^2$ ) along transportation routes I (A), II (B) and III (C). Categories 1. rice paddy/field, 2. dry river bed and 3. grassland were considered as high potential wild soybean growing area. The potential wild soybean growing area is sum up for each 2.5 km section.

Also, soybean seed was surveyed against 162 observation sites in route I–III selected in previous section in 2015.

## Results

### Land usage along observation routes

More than 80% of the roadside area was land category 8 (other) for all three observation routes, which included area covered by cement or blacktop (Table 2). The area of potential to grow for wild soybean (categories 1–3) (rice paddy/field, dry river bed, and grassland) was relatively small, comprising only 12.0%, 8.4% and 12.9% for route I, II and III, respectively (Table 2). Route I runs urban area in section 1–6 and section 18–19, and crosses rivers at section 1, 17, 23, 24 and 27. Also, route I runs along river in section 8 and section 15–16. Route II runs urban area in section 1–3, and crosses a river at section 2, 3, 4, and runs along a pond



**Fig. 4.** Observation sites for soybean seed and location where soybean seeds were observed in Hakata port area and Kashima port area. The map shows an area of Hakata port (a) and Kashima port (b) where imported soybean is unloaded. The circle (○) denote observation sites but soybean seed was not found. The arrows (▽) denote observation sites where soybean seeds were found in Hakata or Kashima port area at the survey in 2015. The circle denotes 5 km radius from soybean unloading areas at Hakata and Kashima port.

**Table 2.** Area (m<sup>2</sup>) associated with each category along the transportation routes

Land category*	Route I		Route II		Route III		Routes I–III	
	Area (m <sup>2</sup> )	%						
1. Rice paddy/field	40,059	5.2	1,145	0.9	27,078	3.0	68,282	3.8
2. Dry river bed	18,608	2.4	3,556	2.8	9,161	1.0	31,325	1.7
3. Grassland	33,836	4.4	6,044	4.8	81,664	9.0	121,544	6.7
<b>Total (Categories 1–3)</b>	<b>92,503</b>	<b>12.0</b>	<b>10,744</b>	<b>8.4</b>	<b>117,903</b>	<b>12.9</b>	<b>221,150</b>	<b>12.2</b>
4. Park/public green space	4,360	0.6	1,758	1.4	3,207	0.4	9,325	0.5
5. Residential zone	12,037	1.6	6,156	4.8	22,981	2.5	41,174	2.3
6. Bare ground	1,596	0.2	–	–	1,604	0.2	3,200	0.2
7. Brush	1,742	0.2	828	0.7	1,268	0.1	3,838	0.2
8. Others**	655,591	85.4	107,746	84.7	764,499	83.9	1,527,836	84.6
<b>Total (Categories 4–8)</b>	<b>675,326</b>	<b>88.0</b>	<b>116,488</b>	<b>91.6</b>	<b>793,559</b>	<b>87.1</b>	<b>1,585,373</b>	<b>87.8</b>
<b>Total (Categories 1–8)</b>	<b>767,829</b>	<b>100.0</b>	<b>127,232</b>	<b>100.0</b>	<b>911,462</b>	<b>100.0</b>	<b>1,806,523</b>	<b>100.0</b>

\* Each land use category was defined according to Geospatial Information Authority of Japan (Geospatial Information Authority of Japan 2015).

\*\* Others is the land area covered by cement or blacktop.

Percentage of each category is calculated as: (area of each category/Total (Categories 1–8)) × 100.

**Table 3.** Number of observation sites, soybean seed and wild soybean populations found in surveys along the three transportation routes (I, II and III)

	Category of Land Use								Total
	rice paddy/ field	dry river bed	grassland	park/public green space	residential zone (garden/planting)	bare ground	brush		
<b>Observation site (2015)*</b>	<b>21</b>	<b>9</b>	<b>38</b>	<b>5</b>	<b>7</b>	<b>73</b>	<b>9</b>	<b>162</b>	
Soybean seed	0	0	0	0	0	0	0	0	
<b>Observation site (2012–2014)*</b>	<b>59</b>	<b>63</b>	<b>165</b>	<b>22</b>	<b>13</b>	<b>154</b>	<b>17</b>	<b>493</b>	
Wild soybean populations									
2012									
Hakata—Feed facility A (route I)	3	0	0	0	0	0	0	3	
Hakata—Feed facility B (route II)	0	0	0	0	0	0	0	0	
Kashima—Feed facility C (route III)	0	1	1	0	0	0	0	2	
2013									
Hakata—Feed facility A (route I)	1	0	3	0	0	1	0	5	
Hakata—Feed facility B (route II)	0	0	0	0	0	0	0	0	
Kashima—Feed facility C (route III)	0	0	1	0	1	0	0	2	
2014									
Hakata—Feed facility A (route I)	2	1	2	0	0	2	0	7	
Hakata—Feed facility B (route II)	0	0	0	0	0	0	0	0	
Kashima—Feed facility C (route III)	0	1	4	0	0	2	0	7	
<b>Total</b>	<b>6</b>	<b>3</b>	<b>11</b>	<b>0</b>	<b>1</b>	<b>5</b>	<b>0</b>		

\* Number of sites for 2012–2014 and 2015 is shown as sum of observation sites for three years.

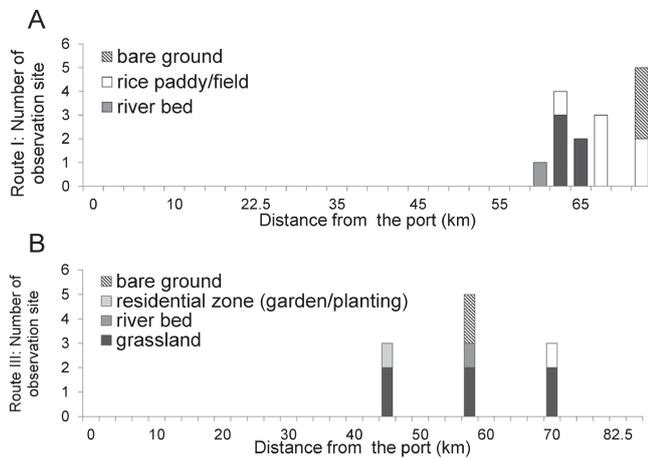
in section 5. Route III crosses rivers at section 4, 18, 24 and 29, and runs urban area in section 31–35.

### Survey near Kashima and Hakata ports and routes I, II and III for soybean seeds

Soybean seeds were found at two out of six observation sites within 5 km from Hakata port, whereas no soybean seeds were found in five observation sites near Kashima port (Fig. 4a, 4b). Not a single soybean seed was found in 162 observation sites along the three transportation routes in 2015 (Table 3).

### Roadside survey of routes I, II and III for soybean plants and wild soybean populations

Not a single soybean plant was found in 493 observation sites of the three transportation routes in any of the survey years (2012, 2013 and 2014; Table 3). Across 493 observations, wild soybean populations were found on routes I and III in all the three years (2012, 2013 and 2014), but no wild soybean population was found on route II in any of the observation years (Table 3). There were 15 wild soybean populations observed on route I and 11 populations observed on route III. Of the total of 26 wild soybean populations, 20 of them were found in the areas with higher potential for growth of wild soybean (land categories 1–3). More specifically, six were found in observation sites categorized as rice



**Fig. 5.** The land use categories where wild soybean populations were observed along transportation routes I (A) and III (B) over a three year (2012–2014) survey. Number of observation site where wild soybean populations is sum up for each 2.5 km section. The number of wild soybean population is shown as total of three years' survey (2012–2014).

paddy/field, three were found in observation sites categorized as dry river bed, and 11 were found in observation sites categorized as grassland (Table 3). A few wild soybean populations were found in areas with lower potential for growth of wild soybean. One wild soybean population was found in the observation site categorized as 5. residential zone (garden/planting), and five populations were found in the observation sites categorized as 6. bare ground (Table 3). Along route I, all wild soybean populations were found at 60 km or greater distance from the Hakata port. Along route III, all wild soybean populations were found at 45 km or greater distance from the Kashima port (Fig. 5).

## Discussion

In this paper, the likelihood of gene flow of transgenes from GM soybean used as FFP to wild soybean in Japan has been evaluated considering eight distinct steps (Table 1). The potential exposure level for steps 1, 2, 6, 7 and 8 were determined based on comprehensive review of published information and steps 3, 4 and 5 were addressed based on the surveys of soybean seeds, plants and wild soybean populations near ports and along transportation routes conducted herein.

### Exposure assessment

#### Step 1. Arrival of soybeans at ports

In Japan, approximately 3 million tons of soybeans are consumed annually, over 90% of which is imported (MAFF 2015, MOF 2015). Soybean import fluctuates from 4.2 million tons in 2005 to 2.8 million tons in 2014. High percentage of soybean grain imported from the U.S.A. and Brazil is GM. Top 10 ports importing soybean account for more than 95% of total soybean imported into Japan (MOF 2015), and

MAFF has conducted roadside survey for soybean plants considering these 10 ports.

#### Step 2. Transportation from unloading ports to food and feed facilities

The intended use of soybean falls roughly into three categories: 1) oil, 2) feed, and 3) food (excluding oil). Imported GM soybean is used for oil and feed, whereas non-GM soybean is separately imported and handled as identity-preserved (IP) soybean, and used for food (MAFF 2015). About 1.9 million tons of soybeans, which accounts for 69.2% of the total import amount, is used for oil. Processing facilities for soybean grain are located near the unloading ports in order to increase efficiency of vegetable oil production (MAFF 2016); therefore no environmental exposure inland. Approximately 0.1 million tons of soybean, which accounts for only 3.8% of the total import amount, is used for feed (MAFF 2015) and potentially transported inland. The soybean for food purposes, including tofu and natto, comprises approximately 0.9 million tons, which accounts for 33.9% of total imported soybean, and they consist almost entirely of non-GM soybean (Yamaura 2011).

#### Step 3. Spillage of soybean seeds during transportation

Generally, transportation of grain by vehicles is considered the main contributor to spillage for many plant species (Nishizawa *et al.* 2009, von der Lippe and Kowarik 2007a, 2007b, Yoshimura *et al.* 2006a). It was reported that the number of oilseed rape seeds spilled from seed trailers decreased with distance from a field in France (Bailleul *et al.* 2012).

In the present study, soybean seed was surveyed both in the area near ports and along the transportation routes from ports to feed facilities. Soybean seeds were found at two out of six observation sites near Hakata port, but no soybean seeds were found at five sites near the Kashima port. Furthermore, no soybean seeds, or soybean plants were found along the transportation routes from the ports to feed facilities. Only 8–16 soybean plants were found by the roadsides within 5 km radius from imported soybean unloading sites at 10 major soybean import ports during monitoring conducted by MAFF (MAFF 2011a, 2011b, 2012, 2013, 2014). Based on the study discussed in this paper, it is concluded that the spillage of imported soybean along the roadsides occurs only near a starting point of transportation.

#### Step 4. Growth of soybean plants from spilled seed

Soybean is a highly domesticated crop and has lost some characteristics typical of weedy species (OECD 2000). It is showed that soybean seeds imbibe water and rot quickly before becoming volunteer plants in a cultivation field (Owen 2005). Furthermore, the storage temperature and humidity from harvest to arrival at a port would likely negatively impact the germination and vigor of soybean seeds (de Alencar *et al.* 2006, Mbofung *et al.* 2013). In addition, physical damage to soybean seeds like splits and cracks, increase during transportation (Lusas 2004).

In this study, imbibed and germinated soybean seeds were observed only near Hakata port, but not along the

transportation routes. MAFF has reported the sites where soybean plants were identified were not the same as those observed in previous years' suggesting that observed soybean plants were due to spillage from a recent grain handling and they have not self-sustained in nature (MAFF 2011a, 2011b, 2012, 2013, 2014).

**Step 5. Growth of soybean plants adjacent to wild soybean populations**

It was reported that outcrossing occurs in the condition of overlapped flowering and proximal growth (Mizuguti *et al.* 2009, 2010, Nakayama and Yamaguchi 2002). Thus, a critical step in evaluating the potential exposure of imported soybeans to wild soybean is to consider the potential overlap (coexistence) of cultivated soybean and wild soybean. In this study, 20 of 26 wild soybean populations were found in rice paddy/field, dry river bed, and grassland along three transportation routes for imported soybean (Table 3). This is consistent with the environment that was previously reported as wild soybean growing areas (Asano 1995, Kikuchi *et al.* 2005, Numata and Yoshizawa 1975, Ohashi 1999, Saruta *et al.* 2007, Takahashi *et al.* 1996). Six wild soybean populations were observed in residential zone (garden/ planting) and on the bare ground. These wild soybean populations were observed in areas after construction or land development near the observation areas. Our observation coincident with the previous reports that areas disturbed by floods or human activities such as agriculture and construction are considered as a suitable growing area of wild soybean populations (Hajika *et al.* 2003, Yamada *et al.* 2012).

Wild soybean populations on routes I and III were located 60 km from Hakata port and over 45 km from Kashima port, respectively (Fig. 5). No wild soybean populations were found along the transportation routes close to the ports. Because the transportation routes close to ports run business and residential areas which provide limited opportunities for wild soybean growth. Our survey showed that potential growing area of wild soybean (*i.e.*, rice paddy/ field, dry river bed, and grassland) was 0.94, 0.01 and 8.4% around 5 km from the ports for the route I, II and III, respectively (Fig. 3). Soybean seeds were observed germinating and growing only within 5 km from the ports, and not along the transportation routes. Based on the fact, it is unlikely, the growing area of soybean and wild soybeans did overlap along the transportation routes and soybean plant grows adjacently to wild soybean.

**Step 6. Synchrony of flowering period between soybean and wild soybean**

It has been reported that synchrony of flowering period has a strong correlation with the number of hybrids produced between cultivated and wild soybeans (Ohigashi *et al.* 2014). Therefore, in addition to geographic overlap of soybean and wild soybean along transportation routes, temporal overlap (*i.e.*, synchrony of flowering period between soybean and wild soybean) is another critical factor to assess a likelihood of hybridization. The flowering time of wild soybean varies depending on the genotype (Nakayama

**Table 4.** Summary of published literature on observed hybridization frequency between cultivated soybean (*Glycine max*) and wild soybean (*Glycine soja*) in Asia

Country	Distance between cultivated and wild soybean plants (m)	Observed hybridization frequency (%)	Reference
China	5	0.03	(Liu <i>et al.</i> 2012)
	29	0.001	
Japan	0.05	0–0.097	(Mizuguti <i>et al.</i> 2010)
	2	0–0.013	
	4	0–0.013	
	6	0–0.013	
	8	0.00	
	10	0.00	
Japan	0.05	<0.01	(Mizuguti <i>et al.</i> 2009)
Japan	Close proximity	0.00*	(Kuroda <i>et al.</i> 2008)
Russia		0.00*	(Dorokhov <i>et al.</i> 2004)
Korea		0.00*	(Kim <i>et al.</i> 2003)
Japan	0.5	0.73	(Nakayama and Yamaguchi 2002)

\* No hybridization observed from GM soybeans to wild soybean.

and Yamaguchi 2002), but the peak flowering time has been observed from August to September (Kaga *et al.* 2006, Nakayama and Yamaguchi 2002, Suda and Shirasawa 1995). Considering the duration of soybean growth from germination to flowering, it follows that only soybean grain transported from spring to early summer could potentially bloom during the flowering period of wild soybean.

**Step 7. Outcrossing between soybean and wild soybean**

Many studies in the published literatures covering various locations, years and soybean varieties demonstrate that naturally occurring pollen-mediated gene flow from cultivated to wild soybean is 0–0.73% even when soybean and wild soybean grew adjacent to each other and showed flowering synchrony (Table 4). Also, the outcrossing rate is decreased when cultivated soybean and wild soybean are further apart from each other and no hybrid was produced in the distance more than 8 m (Table 4).

**Step 8. Weediness or invasiveness characteristics of hybrids between soybean and wild soybean**

Soybean domestication characteristics are not conducive to species survival in nature (OECD 2000). It has been shown that the number of hybrid plants seedlings established in semi-natural condition was 0.98/m<sup>2</sup>, whereas wild soybean was 2.87/m<sup>2</sup> in a three-year monitoring study (Oka 1983). Furthermore, it has been also observed that wild soybean has a higher rate of seed dormancy due to impermeability of the hard seed coat based on the fact that seed germination rate was 88.7% in hybrid seeds and 35.5% in wild soybean seeds and more pod shattering than their hybrids with cultivated soybean (hybrid: 4–5.3% vs. wild soybean: 83–94%) (Chen and Nelson 2004, Oka 1983). Kuroda *et al.* (2010) reported that hybrids between cultivated soybean and wild soybean disappeared from wild soybean growing area within one to three years. This was likely due to a number of inferior fitness-related characteristics of the hybrid plants compared to wild soybean, such as seed coat

characteristics, seed survival in winter, and competitiveness with other plants (Kuroda *et al.* 2010). It has been also reported that the presence of soybean genes related to inferior winter survival reduces the probability of introgression of a neutral transgene (Kitamoto *et al.* 2012). Furthermore, it has been reported that the fertility of pollen and ovules produced by hybrid plants is to 5.5–48.5% and reduced compared to that of wild soybean (Kozak 2009) suggesting level of genetic incompatibility between the two species.

It had been qualitatively discussed that the potential exposure of GM soybean resulting from its intended use as FFP was low based on the understanding that exposure level of import soybean is relatively lower than cultivation. In this paper, we could show the low exposure of the imported soybean quantitatively based on stepwise approach by the literatures and our surveys. This evaluation of exposure level is not specific to particular GM soybean event but can apply to any GM soybean traits used for food, feed and processing if their weediness or invasiveness are the same as those of the conventional soybean.

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