Corporation obtaining approval, the name of its representative, and the address of its main office

Name: Monsanto Japan Limited
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Approved Type 1 Use Regulation

Name of the Type of Living Modified Organism	Drought-tolerant maize (Modified *cspB, Zea mays* subsp. *mays* (L.) Iltis) (MON87460, OECD UI: MON-8746Ø-4)
Content of the Type 1 Use of Living Modified Organism	Provision as food, provision as feed, cultivation, processing, storage, transportation, disposal, and acts incidental to them
Method of the Type 1 Use of Living Modified Organism	—

Outline of the Biological Diversity Risk Assessment Report

I. Information collected prior to assessing Adverse Effect on Biological Diversity

1 Information on preparation, etc. of the genetically modified organisms, etc.

It is generally known that yield of maize is strongly affected by drought stress. Water limitation, particularly during flowering and maturation period is reported to hamper the kernel formation and thus decrease the yield (Boyer and Westgate, 2004; Claassen and Shaw, 1970). In order to control the decrease of maize yield under drought stress, Monsanto Company developed a drought-tolerant maize (Modified *cspB, Zea mays* subsp. *mays* (L.) Iltis) (MON87460, OECD UI: MON-8746Ø-4) (hereinafter referred to as "the transgenic maize").

Under a condition where soil moisture is limited during the period from the later vegetative stage to early reproductive stage, the transgenic maize is proved to control the yield decrease by expressing the target gene, (i.e., the modified low-temperature shock protein *B* gene, or "modified *cspB* gene") (Table 4 on p.16; Table 8 on p.36 of Annex 1). The yield of the transgenic maize under the proper amount of soil moisture content, on the contrary, did not differ from the non-transgenic maize (control) (Table 3 on p.15; Table 6 on p.34 of Annex 1). It is confirmed that the yield of the transgenic maize would be reduced under the condition with limited soil moisture levels compared with the yield under the condition with proper levels of soil moisture (Table 3 on p.15; Table 4 on p.16; Table 6 on p.34 and Table 8 on p.36 of Annex 1).

The drought-tolerant ability of the transgenic maize is conferred by modified cold shock protein B (modified CSPB) coded by modified *cspB* gene from a soil bacterium *Bacillus subtilis*. As shown in the large number of studies on CSPB in bacteria, the CSPB under drought and other kind of stress disassociates the double strand formed on the RNA, and thus stabilizes the RNA and facilitates the translation. Thus, CspB functions as an RNA chaperone that helps cells to maintain normal functions (Graumann *et al.*, 1997; Schindler *et al.*, 1999) (I. 2. (1) (b) ii (i) on p.8–14).

It has been suggested that the CSPB expressed in the transgenic maize also binds with RNA (Fig. 7–10 on p. 38–41 in Annex 2) and helps plants to maintain cellular functions under drought stress. As a result, the modified CSPB in the transgenic maize is suggested to minimize the influence of the drought stress on the physiological abilities (i.e., rate of photosynthesis, stomatal conductance, and quantum efficiency on photosystem II, etc.) and on the efficiency to distribute photosynthetic products to grains (Annex 3; Annex 4) and prevent reduction of the number of grains on the ears, thus controlling the yield reduction (Annex 4).

Limited water availability is the single most important factor that reduces global crop yields. Thus, stabilizing the yields under drought stress is valuable both environmentally and socioeconomically. In North America, it is estimated that 40% of annual crop losses are due to suboptimal water availability (Boyer, 1982).

Maize is an important crop and widely grown in Africa. More than 300 million people depend on maize for their main food resource. In order to seek solution for the influence of the drought stress on maize yields, a public-private partnership project, Water Efficient Maize for Africa (WEMA; http://www.aatf-africa.org), was founded, which has been developing a drought-tolerant maize variety for Africa. Using advanced breeding and genetic recombination technologies, WEMA aims at increase of the maize yields with African

genetic resources. The transgenic maize is one of such drought-tolerant varieties that are planned to be offered as technological transfer.

(1) Information concerning donor nucleic acid

a. Composition and origins of component elements

Composition of the donor nucleic acid that was used for the production of the transgenic maize and the origins of component elements are shown in Figure 1 (p.4) and Table 1 (p.5–12).

The amino acid sequence of the modified CSPB that is expressed in the transgenic maize is identical to that in the wild type CSPB derived from *B. subtilis* (a soil bacterium widely distributed in soil) with the exception of a single amino acid change at the second position from leucine to valine. The change was necessary to add restriction enzyme cutting site for cloning purposes. The estimated amino acid sequence of modified CSPB is shown in Figure 1 of the Annex 5. The *cspB* gene introduced into the transgenic maize is described as "the modified *cspB* gene" and the expressed protein as "the modified CSPB."

b. Functions of component elements

i. Functions of target genes, expression-regulating regions, localization signals, selectable markers, and other component elements of donor nucleic acid

Functions of component elements of donor nucleic acid which were used for the production of the transgenic maize are shown in Table 1 (p.5–12).

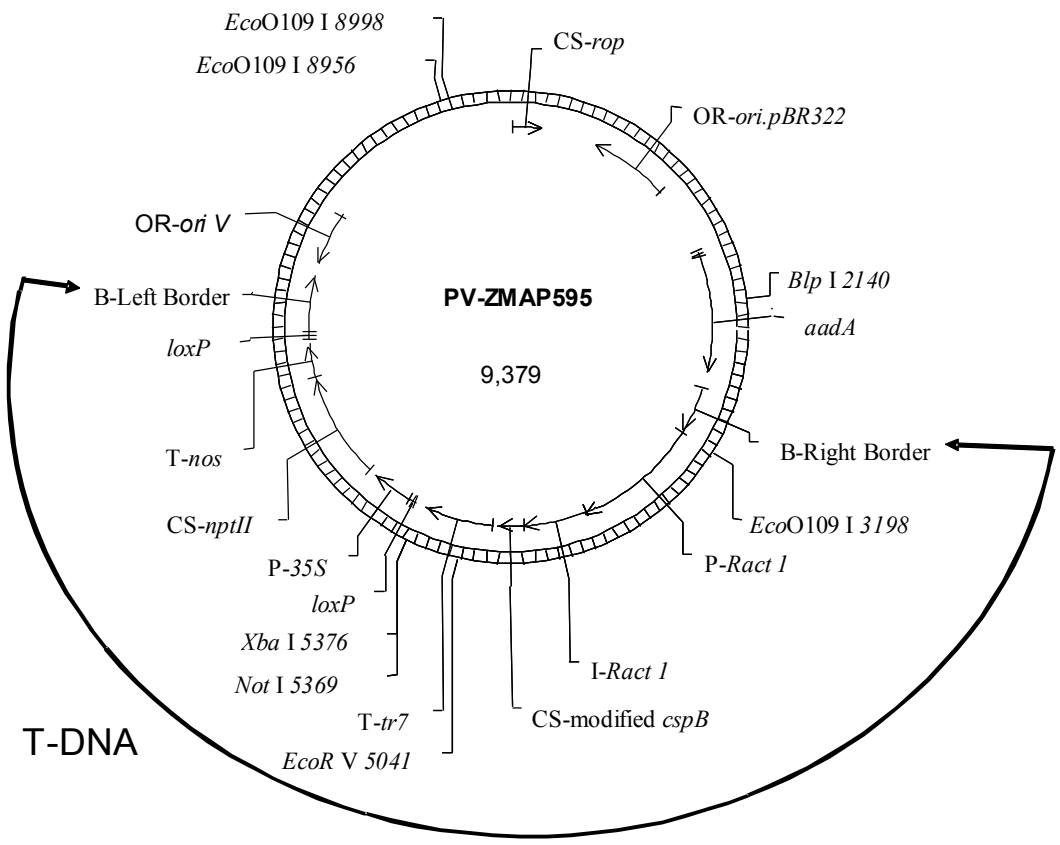


Figure 1. Plasmid Map of PV-ZMAP595 used for the transgenic maize MON87460[1]

[1]Note: All the rights pertinent to the information in the diagram above and the responsibility for the contents rest upon Monsanto Japan Limited.

Table 1. Component as well as origin and function of component elements of PV-ZMAP595[2]

Component elements	Origin and function
External backbone region	
Intervening Sequence	Sequence used for DNA cloning.
CS [Note 1]-*rop*	Coding sequence for repressor of primer protein for maintenance of plasmid copy number in *Escherichia coli* (Giza and Huang, 1989).
Intervening Sequence	Sequence used for DNA cloning.
OR [Note 2]-*ori.pBR322*	Origin of replication isolated from pBR322. This confers autonomous replication ability to plasmid in *E. coli*. (Sutcliffe, 1979).
Intervening Sequence	Sequence used for DNA cloning.
aadA	Bacterial promoter, coding sequence, and terminator for the 3′(9)-O-nucleotidyltransferase, the aminoglycoside modified enzyme, derived from transposon Tn7. Confers resistance to spectinomycin or streptomycin. (Fling *et al*., 1985). (GenBank accession X03043)
Intervening Sequence	Sequence used for DNA cloning.
T-DNA region	
B [Note 3]-Right Border	A DNA fragment containing the right border sequence of nopaline type T-DNA region, derived from *Agrobacterium tumefaciens*. The right border sequence is used as the initiation point of T-DNA transfer from *A. tumefaciens* to plant genome (Depicker *et al*., 1982; Zambryski *et al*., 1982).
Intervening Sequence	Sequence used for DNA cloning.
P [Note 4]-*Ract1*	Promoter and leader sequence of actin gene, derived from *Oryza sativa* (rice) (McElroy *et al*., 1990). Constantly induces transcription of the target gene in the entire tissues of plant.
I [Note 5]-*Ract1*	Intron of actin gene, derived from *Oryza sativa* (rice) (McElroy *et al*., 1991). Activates the expression of target gene.
Intervening Sequence	Sequence used for DNA cloning.

[2]Note: All the rights pertinent to the information in the table above and the responsibility for the contents rest upon Monsanto Japan Limited.

Table 1. Component as well as origin and function of component elements of PV-ZMAP595 (continued)

T-DNA region (continued)	
CS-modified *cspB*	Gene coding modified CSPB derived from *B. subtilis* (Willimsky *et al.*, 1992). Detail is shown in I-2-(1)-b-ii.
Intervening Sequence	Sequence used for DNA cloning.
T [Note 6]-*tr7*	3' non-translated transcriptional region of transcription 7 gene derived from *A. tumefaciens*. It induces polyadenylation (Dhaese *et al.*, 1983).
Intervening Sequence	Sequence used for DNA cloning.
loxP [Note 7]	Recombination site of bacteriophage P1. It functions in pairs. When Cre recombinase (DNA recombination enzyme) recognizes the two *lox P* sites, it removes the DNA region existing between them (Russell *et al.*, 1992).
Intervening Sequence	Sequence used for DNA cloning.
P-*35S*	35S promoter region of cauliflower mosaic virus (CaMV) (Odell *et al.*, 1985). Involved in the constant expression of the target gene in the entire tissue of plant.
Intervening Sequence	Sequence used for DNA cloning.
CS-*nptII*	A gene derived from *E. coli* transposon Tn5 (Beck *et al.*, 1982). Encodes neomycin phosphotransferase type II and confers resistance to neomycin and kanamycin on plants. Used as marker to select the transgenic plant during the gene transfer (Fraley *et al.*, 1983).
Intervening Sequence	Sequence used for DNA cloning.
T-*nos*	3' untranscribed region of nopaline synthase (*nos*) derived from *A. tumefaciens* T-DNA. Terminates transcription of mRNA and induces polyadenylation (Bevan *et al.*, 1983).
Intervening Sequence	Sequence used for DNA cloning.
loxP	Recombination site of bacteriophage P1. It functions in pairs. When Cre recombinase (DNA recombination enzyme) recognizes the two *lox P* sites, it removes the DNA region existing between them (Russell *et al.*, 1992).

Table 1. Component as well as origin and function of component elements of PV-ZMAP595 (continued)

T-DNA region (continued)	
Intervening Sequence	Sequence used for DNA cloning.
B-Left Border	A DNA fragment containing the left border sequence (25bp) derived from *A. tumefaciens*. It is the termination point of T-DNA transfer from *A. tumefaciens* to plant genome (Barker *et al.*, 1983).
External backbone region	
Intervening Sequence	Sequence used for DNA cloning.
OR-*ori V*	The replication origin region isolated from the broad-host range plasmid RK2. Permits autonomous replication of vector in *Agrobacterium* (Stalker *et al.*, 1981).
Intervening Sequence	Sequence used for DNA cloning.

Note 1 CS – Coding Sequence

Note 2 OR – Origin of Replication

Note 3 B – Border

Note 4 P – Promoter

Note 5 I – Intron

Note 6 T – 3' non-translated transcriptional termination sequence and polyadenylation signal sequences.

Note 7 *loxP* – The *nptII* gene is used as a marker to select MON87460 strain trait transformant. At the time of the MON87460 strain development, the European Food Safety Authority (EFSA, a safety evaluation authority for genetically modified crops in the EU) and other organizations were promoting development and use of new selection methods to replace antibiotic resistance markers. In this regard, MON87460 was designed to remove *nptII* gene cassette by using *loxP* modified sites that are recognized by Cre recombinase. Later, EFSA announced that the risk of *nptII* gene in genetically modified crops affecting human and livestock health is very low (EFSA, 2004), and therefore the *nptII* gene cassette was not removed from the MON87460 strain.

ii. Functions of proteins produced by the expression of target genes and selective markers, and the fact, if applicable, that the produced protein is homologous with any protein which is known to possess any allergenicity.

[Modified CSPB]

It has been confirmed that the modified CSPB functions as an RNA chaperone and suppresses the decrease of yield by maintaining important physiological functions, including distribution of photosynthetic products into developing ears under the conditions where soil moisture is limited.

Functions of the modified CSPB that are expressed in the transgenic maize are described below.

(i) Functions of cold shock proteins (CSPs) on bacteria and plants

The modified CSPB expressed in the transgenic maize is derived from a soil bacterium *B. subtilis*. Classified as a member of CSP family, the modified CSPB is known to maintain "cold shock domain" (CSD) sequence that binds to RNA.

RNAs expressed in bacteria are generally known to suppress normal cellular functions under various kinds of stress by forming a secondary structure, which in turn decreases protein synthesis (Figure 2-(1) on p.9) (Graumann *et al.*, 1997; Jiang *et al.*, 1997). In contrast, CSPs are considered to work as an RNA chaperone that binds to RNAs (Cristofari and Darlix, 2002) to disassociate the secondary structure of the RNA, and thus stabilizes the translation and improves the cellular functions (Graumann *et al.*, 1997) (Figure 2-(2) on p. 9). Also, there is a report that the RNA chaperone, which is essential for disassociation of the RNA secondary structure, has no role in the maintenance of the first structure of RNA (Cristofari and Darlix, 2002).

It should be noted that CSPs (cold shock proteins) are named so due to the fact that the CSP isolated first was induced a low temperature treatment, while some CSPs in the family for bacteria include those that are expressed also under the optimal temperature condition (Graumann *et al.*, 1997) and those that have roles in maintaining cellular functions by responding to other kinds of environmental stress (Anderson *et al.*, 2006; Etchegaray *et al.*, 1996).

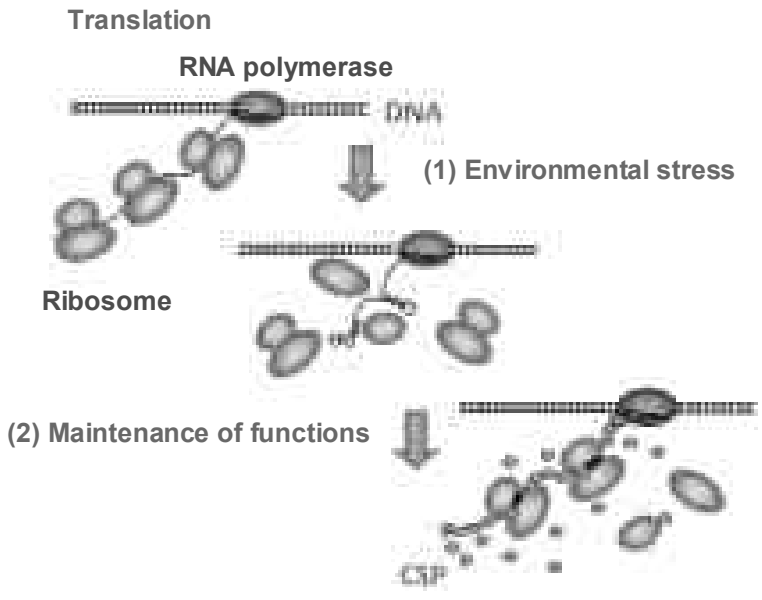


Figure 2. General functions of CSP[3]

Proteins containing CSD are also known to exist in plants as a multidomain protein. These proteins containing CSD closely resemble CSP in bacteria, combining with RNA when environmental stress occurs and helping maintain cellular functions under stress, and thus functioning as an RNA chaperone (Chaikam and Karlson, 2008; Fusaro *et al.*, 2007; Kim *et al.*, 2007; and 2006; Nakaminami *et al.*, 2005).

In fact, WCSP1, a nucleic acid-binding protein derived from wheat, has a structure resembling CSPA derived from *E. coli* (Karlson *et al.*, 2002), and the volume of expression increases under cold stress as in the case for the expression volume of CSPA derived from *E. coli*, while it does not change under high temperature or salt stress conditions (Karlson *et al.*, 2002). These reports suggest that the two proteins possess similar functions *in vivo* (Karlson *et al.*, 2002). Also notable is that expression of AtGRP2, a CSD-containing protein derived from Arabidopsis, is induced under low-temperature stress and is associated with timing of flowering and seed development (Fusaro *et al.*, 2007). As CSD-containing proteins derived from rice, OsCSP1 and OsCSP2 have been identified. Expression of these proteins is induced under low-temperature stress, and these proteins bind to nucleic acid to be accumulated in reproductive and meristem tissues, thus helping maintain cellular functions (Chaikam and Karlson, 2008).

(ii) Resistance capabilities of *Arabidopsis* and rice induced with the modified *cspB* gene

As described earlier, bacterial CSP or plant proteins containing CSD function as RNA chaperones and confer resistance to environmental stresses. In order to verify the ability of bacterial CSP to confer environmental stress resistance in plants, a modified *cspB* gene derived from *B. subtilis* was introduced into Arabidopsis and rice and the resistance to environmental stress was evaluated.

[3] All the rights pertinent to the information in the diagram above and the responsibility for the contents rest upon Monsanto Japan Limited.

In the study, the Arabidopsis showed resistance to low temperatures, while the rice had resistance to low temperatures, high temperatures, and drought (Castiglioni *et al.*, 2008). However, the resistance of rice to environmental stresses varied among individual plants; while some individuals possessed resistance to all of the three stress treatments, others showed resistance to only one or two treatments (Castiglioni *et al.*, 2008).

Based on the results, it was confirmed that the modified CSPB confers resistance to several kinds of environmental stresses but the conferred resistance varies depending on the events. As the cause of this event-specific environmental stress resistance system, the region of gene attachment was suggested, for it is known that the actions of introduced gene can be influenced by the attachment region (Butaye *et al.*, 2005; De Bolle *et al.*, 2003; Van Leeuwen *et al.*, 2001). These results together suggest that the modified CSPB confers resistance to several kinds of environmental stresses but with variation of the resistance system depending on the gene attachment region.

(iii) Functions of modified CSPB expressed in the transgenic maize

Functions of the modified CSPB in the transgenic maize were tested as below.

First, it was examined whether the modified CSPB expressed in the transgenic maize function as RNA chaperones in the same way as CSPB in *B. subtilis*. The results of *in vitro* experiments indicated that the modified CSPB in the transgenic maize binds to RNA in plants (Figure 7–10 on p.38–41 of Annex 2) and binds with RNA in the same manner as bacterial CSP and plant proteins containing CSD (Figure 9–10 on p.40–41 of Annex 2). It was also confirmed *in vitro* that the modified CSPB disassociates the secondary structure of the nucleic acid as a common function of RNA chaperones (Cristofari and Darlix, 2002). However, the mutant CSPB lacking the binding function (CSPB_F30R and CSPB_F30A) did not bind to nucleic acid and therefore was not able to disassociate the secondary structure (Table 1 on p.31 of the Annex 2). In an immunoprecipitation test with leaves of the maize that expressed a modified CSPB marked with histidine, a complex of the modified CSPB and intrinsic RNA was observed (Figure 11 on p.42 of the Annex 2).

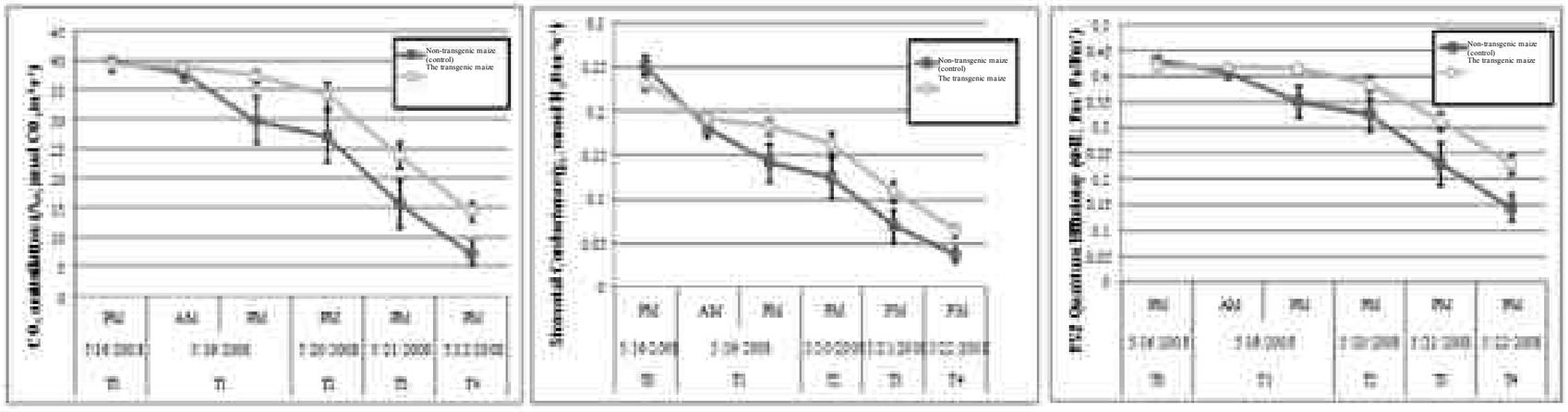
At a cellular level, the modified CSPB is distributed both in the cytoplasm and nucleus of the coleoptiles of the transgenic maize, but not in the vacuole, mitochondria, and chloroplast. These findings indicate the modified CSPB possesses the same kind of localization as seen with bacterial proteins and plant proteins containing CSD (Figure 6 on p.37 of the Annex 2).

Based on the above, it was determined that the modified CSPB has a role of an RNA chaperone in the transgenic maize.

(iv) Physiological characteristics of the transgenic maize in greenhouse

Physiological influence of the modified CSPB on the transgenic maize was studied in a greenhouse in Connecticut, USA.

Using the transgenic maize and non-transgenic maize (control), physiological performance was studied after suspending watering for 6 days starting at the 5-leaf stage (V5). Under the condition with limited soil moisture, the transgenic maize had improved physiological performance, including stomatal conductance, rates of photosynthesis, and quantum efficiency at the photosystem II, compared with the non-transgenic maize (Figure 3 on p.12; Figure 1 on p.6, Figure 2 on p.6, and Figure 4 on p.8 of Annex 3).



A. Rate of photosynthesis B. Stomatal conductance C. Quantum efficiency at the photosystem II

Figure 3. Comparison of physiological performance between the transgenic maize and non-transgenic maize (control) under the condition with limited soil moistures in greenhouse (2008 in the USA)[4]

Watering was suspended for 6 days starting at the 5-leaf stage (V5).

A. Rates of photosynthesis are shown in the putative net assimilation rates of CO_2 /m^2/second (in μmol).

B. Stomatal conductance is shown in the volume of water vapor emission/m^2/second (in mmol).

C. Quantum efficiency at the photosystem II is shown in the flow of electron transport mediated by the photosystem II.

The dates of measurement (shown in the x axis) for A, B, and C are T0 (before the soil watering suspension) and T1–T4 (during the soil watering suspension

[4] All the rights pertinent to the information in the figures above and the responsibility for the contents rest upon Monsanto Japan Limited.

(v) Analysis of factors for the transgenic maize to mediate the yield decline under the limited soil moisture content

In 2006 and 2007, experiments were conducted at the four field locations (Colina: CL, Calera de Tango: CT, Lumbreras: LUM, and Quillota: QUI) in Chile under two soil moisture conditions (well-watered and water-limited) to test seedling vigor, early stand count, days before flowering, stay green, plant height, ear height, dropped ears, stalk lodging, root lodging, final stand count, grain moisture, test weight, and yield. To conduct statistical treatments for the data obtained in all of the fields, only the field plots that met all the following three criteria are used for sampling.

1. Watering and precipitation data show that water was managed appropriately to impose two differential water treatments.
2. Water monitoring data confirm that the desired well-watered and water-limited treatment levels were established. If direct measurement of soil moisture is not possible, phenotypic responses and growth of commercial variety grown together (commercial reference) indicate the expected influence of the water-limited treatments.
3. A minimum of 15% reduction in yield is exhibited in the water-limited plot compared with well-watered plots in comparison of morphological and developmental characteristics in the commercial reference grown together. Reductions in plant height and ear height, as well as delay of silking, are also assessed and used as supplemental data.

Among the four fields, the field in QUI did not meet the three criteria above (Table 2 on p.14; Table 5 on p.33 in Annex 1), and therefore only fields in CL, CT, and LUM were used for analysis of results.

The results did not show statistically significant difference between the transgenic maize and non-transgenic maize (control) under proper water conditions (Table 3 on p.15; Table 6 on p.34 of Annex 1). When soil moisture was limited in the period from the later vegetative stage to the early reproductive stage, the transgenic maize decreased the final yield (Table 4 on p.16; Table 8 on p.36 of Annex 1). However, statistically significant difference was not confirmed in morphological and developmental characteristics other than the yield decrease between the transgenic maize and the non-transgenic maize used as control under water-limited conditions (Table 4 on p.16; Table 8 on p.36 of Annex 1).

Table 2. Morphological and developmental characteristics of a commercial variety under well-watered and water-limited conditions (2006–2007, Chile)[5]

	CL		CT		LUM		QUI	
Morphological and developmental characteristics	Well-watered	Water-limited	Well -watered	Water-limited	Well -watered	Water-limited	Well-watered	Water-limited
Days to 50% silking	63.1	63.8	66.2	67.3	70.3	73.7*	67.7	67.1
Ear height (inch)	63.4	50.9*	55.0	46.0	50.4	41.8*	63.5	63.4
Plant height (inch)	110.7	79.7*	105.9	92.1	97.9	75.0*	112.0	112.8
Yield (bushel/acre)	185.5	82.3*	236.5	152.3*	213.9	94.4*	203.1	196.3
Loss of yield (%)		56%		36%		56%		3%

* indicates statistically significant differences between properly watered and water-limited conditions in each field ($p \leq 0.05$).

[5] All the rights pertinent to the information in the figures above and the responsibility for the contents rest upon Monsanto Japan Limited.

Table 3. Comparison of morphological and developmental characteristics between the transgenic maize and non-transgenic maize (control) under well-watered conditions (2006–2007, Chile)[6]

Morphological and developmental characteristics	Mean		Range for commercial varieties[1]	
	The transgenic maize	Control	Minimum	Maximum
Seedling vigor[2]	4.9	4.7	4.3	6.0
Early stand count (number/plot)	76.1	73.0	71.0	80.0
Days to 50% pollen shed (day)	66.8	66.7	65.0	74.3
Days to 50% silking (day)	65.2	65.3	62.7	71.0
Stay green[3]	2.4	2.9	1.0	6.7
Ear height (inch)	55.9	52.8	46.1	69.1
Plant height (inch)	101.1	99.0	94.4	116.4
Dropped ears (number/plot)[4]	0.0	0.0	0.0	0.0
Stalk lodged plants (number/plot)[4]	0.0	0.0	0.0	0.0
Root lodged plants (number/plot)[4]	0.0	0.0	0.0	0.0
Final stand count (number/plot)	75.2	74.0	71.3	79.3
Grain moisture (%)	14.8	15.2	10.1	20.2
Grain weight in per bushel (pound/bushel)	56.4	55.8	54.0	61.2
Yield (bushel/acre) [MT/ha][5]	220.7 [13.9]	220.0 [13.8]	166.7 [10.5]	248.4 [15.6]

n = 3

5 Statistically significant difference was not confirmed between the transgenic maize and the non-transgenic maize (control) (variance analysis, $p \leq 0.05$).

[1]The range of the commercial varieties was calculated based on the commercial varieties grown in the fields in CL, CT, and LUM.

[2] The seedling vigor was evaluated with rating scale: 0 = dead and 9 = above average vigor.

[3] The stay green was evaluated with rating scale: 0 = entire plant is dried and 9 = entire plant is green.

10 [4] No statistical comparisons were made for this rating due to lack of variability in the data. The transgenic maize was considered not different from the control because the test and control mean values were identical.

[5] Yields were converted from bushel/acre to MT/ha.

[6]All the rights pertinent to the information in the table above and the responsibility for the contents rest upon Monsanto Japan Limited.

Table 4. Comparison of morphological and developmental characteristics between the transgenic maize and non-transgenic maize (control) under water-limited conditions (2006–2007, Chile)[7]

Morphological and developmental characteristics	Mean		Range for commercial varieties[1]	
	The transgenic maize	Control	Minimum	Maximum
Seedling vigor[2]	5.0	4.8	4.0	6.0
Early stand count (number/plot)	76.8	75.7	67.3	80.7
Days to 50% pollen shed (day)	67.4	68.1	65.7	75.0
Days to 50% silking (day)	67.3	66.8	63.3	74.3
Stay green[3]	4.3	4.7	1.0	7.0
Ear height (inch)	48.0	45.1	40.0	60.5
Plant height (inch)	83.9	78.1	64.9	96.8
Dropped ears (number/plot)[4]	0.0	0.0	0.0	0.0
Stalk lodged plants (number/plot)[4]	0.0	0.0	0.0	0.0
Root lodged plants (number/plot)[4]	0.0	0.0	0.0	0.0
Final stand count (number/plot)	76.7	75.1	71.3	80.7
Grain moisture (%)	19.5	21.3	9.6	25.5
Grain weight in per bushel (pound/bushel)	56.7	56.0	51.3	62.2
Yield (bushel/acre) [MT/ha][5]	114.5* [7.2]	86.7 [5.4]	56.4 [3.5]	167.6 [10.5]

n = 3

The tests were conducted with water limitation in the period between the ca. 10-leaf stage (V10) to Blister stage (R2).

* indicates a statistically significant difference between the transgenic maize and the non-transgenic maize (control) (variance analysis, $p \leq 0.05$).

[1]The range of the commercial varieties was calculated based on the commercial varieties grown in the fields in CL, CT, and LUM.

[2] The seedling vigor was evaluated with rating scale: 0 = dead and 9 = above average vigor.

[3] The stay green was evaluated with rating scale: 0 = entire plant is dried and 9 = entire plant is green.

[4] No statistical comparisons were made for this rating due to lack of variability in the data. The transgenic maize was considered not different from the control because the test and control mean values were identical.

[5] Yields were converted from bushel/acre to MT/ha.

[7]All the rights pertinent to the information in the table above and the responsibility for the contents rest upon Monsanto Japan Limited.

To study the mechanism of the transgenic maize to suppress the yield loss under water-limited conditions, tests with two kinds of irrigation treatments (well-watered and water-limited conditions) were conducted in three fields in Yolo County, California, USA (Harlan South: WHAS, Harlan North: WHAN, Barrios: YBAP). The transgenic maize and a non-transgenic maize (control) were grown under the well-watered and water-limited conditions.

The water-limited treatment was prepared by terminating the irrigation between the late vegetative stage to early maturation stage (ca. 10-leaf stage to milk stage), when yield of maize is reportedly most sensitive to drought stress (Boyer and Westgate, 2004; Claassen and Shaw, 1970). In addition to the morphological and developmental characteristics evaluated in the Chilean study (seedling vigor, early stand count, days to flowering, ear height, plant height, stay green, final stand count, and yield), yield component factors (dry weights of ears, dry weight of foliage, dry weights of aboveground biomass, grain counter per ear, 1000-grain weight, ear count, and harvest index), plant development during the water-limited treatment (dry weights of leaves, stalks, aboveground biomass, and ears, ear diameters, and leaf areas), and physiological characteristics (water potential of leaves and stalks, rate of photosynthesis, stomatal conductance, and quantum efficiency at the photosystem II) were evaluated to analyze the mechanism of yield loss reduction in a more detailed manner.

Since the purpose of the study was to analyze the mechanism of the transgenic maize to reduce yield loss under water-limited conditions, control of soil moisture is needed for all test fields, and yield loss of the transgenic maize must be reduced under water-limited conditions. In the statistical treatments for the data obtained in all of the fields, solely the field plots that met all of the following two criteria are used for sampling.

1. Water treatment efficacy: The yield of the non-transgenic maize (control) under water-limited conditions is reduced at least by 15% compared with the yield of the non-transgenic maize (control) under well-watered conditions.
2. Efficacy of the gene transfer: The yield of the transgenic maize under the water-limited conditions is significantly higher than yield of the non-transgenic maize (control) ($p \leq 0.05$).

While the non-transgenic maize (control) in all of the three test fields showed at least 15% of yield loss resulted from water limitation (Table 5 on p.20; Table 6 on p.46 of Annex 4), statistically significant differences between the transgenic maize and non-transgenic maize (control) were recognized only in two fields (WHAS and YBAP). Accordingly, only the data from these two fields were included in the analysis (Table 6 on p.20; Table 7 on p.46 of Annex 4).

In the study of the yield component factors, the transgenic maize and non-transgenic maize (control) did not show statistically significant differences in their yields under well-watered conditions (Table 7 on p.21; Table B.1. on p.70, Annex 4). As described before, statistically significant differences were recognized in the yields under water-limited conditions between the transgenic maize and the non-transgenic maize (control) ($p < 0.05$). The yield of the transgenic maize under water-limited conditions (7.6 MT/ha) was significantly higher (11.8%) compared with the yield of the non-transgenic maize (control) (6.8 MT/ha) ($p \leq 0.05$) (Table 8 on p.22; Table 8 on p.47 of Annex 4). The grain count per ear of the transgenic maize (386.8) is approximately 9.1% higher than the grain count per ear of the non-transgenic maize (control) (354.5) ($p \leq 0.05$) (Table 8 on p.22; Table 8 on p.47 of Annex 4). These findings suggested that the difference (approximately 11.8%) in the yields between the transgenic maize and the non-transgenic maize (control) under water-limited conditions is ascribed to the difference in the grain count per ear (approximately 9.1%).

Harvest index was compared between the transgenic maize and the non-transgenic maize (control) to study the mechanisms of the yield loss reduction by the transgenic maize under water-limited conditions. In general, efficiency of a plant to distribute the products

of photosynthesis (photosynthates) to grain can be evaluated by calculating a harvest index, or the ratio of the grain weight to the dry weight of aboveground biomass (Sinclair, 1998). The harvest index of maize grown under proper conditions has been known as approximately 0.50 (Meghji *et al.*, 1984; Russell, 1985; Tollenaar, 1989), which can be lowered under drought stresses (DeLougherty and Crookston, 1979). Reduction of harvest index means reduced distribution of photosynthates to grains, and thus loss of yield (DeLougherty and Crookston, 1979). The harvest index of the transgenic maize under water-limited conditions (0.40) has been confirmed to be significantly higher ($p \leq 0.05$) than that of the non-transgenic maize (control) (0.37) (Table 8 on p.22; Table 8 on p.47 of Annex 4). Based on the higher harvest index (by 8.1%) of the transgenic maize compared with the non-transgenic maize (control), the distribution of photosynthates to grains is considered to be more efficient in the transgenic maize than in the non-transgenic maize (control).

In addition, while both of the dry weight of ears and the dry weight of the aboveground biomass under water-limited conditions are heavier for the transgenic maize than the non-transgenic maize (control) ($p \leq 0.05$), the dry weights of leaves and stalks tend to be lighter for the transgenic maize than the non-transgenic maize (control). This also suggests high efficiency of the transgenic maize to distribute photosynthates to the whole ears compared with the non-transgenic maize (control) (Table 8 on p.22; Table 8 on p.47 of Annex 4). The same can be also said from the fact that the diameter of ears is significantly larger for the transgenic maize than the non-transgenic maize (control) ($p \leq 0.05$) (Figure 5 on p.24; Figure 7 on p.37, Annex 4), while the dry weights of leaves and stalks under water-limited conditions are lower for the transgenic maize than for the non-transgenic maize (control) ($p \leq 0.05$) (Figure 4 on p.23; Figure 5 on p.35, Annex 4).

Based on the findings above, the transgenic maize is considered to reduce yield loss by efficiently distributing photosynthates to grains and ears under water-limited conditions.

It should be noted that the results of the study of physiological characteristics did not show coherent differences in the rate of photosynthesis, stomatal conductance, and quantum efficiency on photosystem II between the transgenic maize and the non-transgenic maize (control) under water-limited conditions (Figure 6 on p.25; Figure 7 on p.26; Figure 8 on p.27; Figure 9–11 on p.39–41 of Annex 4). The reason why the difference found in the greenhouse conditions (described in I-2-(1)-b-ii-(iii)) (Figure 3 on p.12; Figure 1 on p.6, Figure 2 on p.6 of Annex 3, and Figure 4 on p.8 in Annex 3) was not recognized in these tests can be explained as that the physiological test items are more susceptible to change of soil moisture, temperature, transpiration pressure, and other conditions (Beadle *et al.*, 1993), and these conditions are more unstable in the fields than in the greenhouses.

Based on what are described above, the transgenic maize is confirmed to reduce yield loss under water-limited conditions. The significantly higher levels of ear dry weight, ear height, plant height, and harvest index of the transgenic maize demonstrated that the transgenic maize maintains the vegetative development and efficiently distributes the photosynthates to the grains. Thus, the modified CSPB expressed in the transgenic maize is thought to reduce yield loss by efficiently distributing the photosynthates to ears and grains.

Table 5. Criterion 1. Water treatment efficacy: comparison of yield of the non-transgenic maize (control) under water-limited conditions and well-watered conditions (2009, USA)[8]

Test location	Treatment	Yield of control (MT/ha)[1]	Difference in yield (%)	Total irrigation (mm)	Difference in total irrigation (mm)
YBAP	Water-limited	6.0	-52%	339	-156
	Well-watered	12.5		495	
WHAS	Water-limited	8.3	-30%	258	-324
	Well-watered	11.8		582	
WHAN	Water-limited	8.4	-31%	249	-281
	Well-watered	12.1		530	

YBAP: n = 28, WHAS and WHAN: n = 14

Irrigation was limited from ca. 10th leaf stage (V10) to milk stage (R3).

[1] Yields were converted from bushel/acre to MT/ha.

Table 6. Criterion 2. Efficacy of the gene transfer: Comparison of yields between the transgenic maize and the non-transgenic maize (control) under the water-limited conditions (2009, USA)[9]

	Yield (MT/ha)[1]	
Test location	The transgenic maize	Control
YBAP	*6.5	6.0
WHAS	*9.6	8.3
WHAN	8.6	8.4

YBAP: n = 28, WHAS and WHAN: n = 14

Irrigation was limited from ca. 10th leaf stage (V10) to milk stage (R3).

* Statistically significant difference was confirmed between the transgenic maize and the non-transgenic maize (control) (variance analysis, $p \leq 0.05$).

[1] Yields were converted from bushel/acre to MT/ha.

[8] All the rights pertinent to the information in the table above and the responsibility for the contents rest upon Monsanto Japan Limited.

[9] All the rights pertinent to the information in the table above and the responsibility for the contents rest upon Monsanto Japan Limited.

Table 7. Comparison of morphological and developmental characteristics and yield component factors between the transgenic maize and the non-transgenic maize (control) under well-watered conditions (2009, USA)[10]

	Results at YBAP and WHAS	
Morphological and developmental characteristics	The transgenic maize	Control
Seedling vigor[1]	2.82	2.86
Early stand count (number/plot)	85.6	85.2
Days to 50% pollen shed[2]	62.6	62.9
Days to 50% silking (day)[2]	62.7	63.0
Days from silking to flowering[2]	0.1	0.1
Ear height (cm)	*161.1	154.4
Plant height (cm)	*293.8	289.2
Stay green[3]	7.5	7.7
Final stand count (number/plot)	85.7	85.1
Ear dry weight (g/plot)	*16113	15666
Foliage dry weight (g/plot)	9632	9448
Aboveground biomass dry weight (g/plot)	25724	25138
Grain count per ear (number/ear)	535.9	520.0
1000-grain weight (g)	283.0	285.6
Ear count (number/plot)	84.1	83.3
Harvest index[4]	0.50	0.49
Yield (MT/ha) [5]	12.5	12.1

n = 42

* indicates a statistically significant difference between the transgenic maize and the non-transgenic maize (control) (variance analysis, $p \leq 0.05$).

[1] The seedling vigor was evaluated with rating scale: 1 = vigorous 5 = poor.

[2] Days to 50% pollen shed, days to 50% silking, and days from silking to flowering were observed only at YBAP.

[3] The stay green was evaluated with rating scale: 1 = entire plant is dried and 10 = entire plant is green.

[4] Harvest index was calculated as the ratio of grain weight in the plot and the weight of aboveground biomass in the plot.

[5] Yields were converted from bushel/acre to MT/ha.

[10] All the rights pertinent to the information in the table above and the responsibility for the contents rest upon Monsanto Japan Limited.

Table 8. Comparison of morphological and developmental characteristics and yield component factors between the transgenic maize and the non-transgenic maize (control) under water-limited conditions (2009, USA)[11]

	Results at YBAP and WHAS	
Morphological and developmental characteristics	The transgenic maize	Control
Seedling vigor[1]	2.48	2.62
Early stand count (number/plot)	85.5	85.5
Days to 50% pollen shed[2]	63.3	63.2
Days to 50% silking (day)[2]	64.8	65.0
Days from silking to flowering[2]	1.5	1.8
Ear height (cm)	*157.5	151.8
Plant height (cm)	*262.2	259.2
Stay green[3]	6.6	6.6
Final stand count (number/plot)	84.5	85.0
Ear dry weight (g/plot)	*10104	9260
Foliage dry weight (g/plot)	8950	9132
Aboveground biomass dry weight (g/plot)	*19053	18392
Grain count per ear (number/ear)	*386.8	354.5
1000-grain weight (g)	260.4	257.4
Ear count (number/plot)	76.1	75.6
Harvest index[4]	*0.40	0.37
Yield (MT/ha) [5]	*7.6	6.8

n = 42

Irrigation was limited from ca. 10th leaf stage (V10) to milk stage (R3).

* İndicates a statistically significant difference between the transgenic maize and the non-transgenic maize (control) (variance analysis, $p \leq 0.05$).

[1] The seedling vigor was evaluated with rating scale: 1 = vigorous 5 = poor.

[2] Days to 50% pollen shed, days to 50% silking, and days from silking to flowering were observed only at YBAP.

[3] The stay green was evaluated with rating scale: 1 = entire plant is dried and 10 = entire plant is green.

[4] Harvest index was calculated as the ratio of grain weight in the plot and the weight of aboveground biomass in the plot.

[5] Yields were converted from bushel/acre to MT/ha.

[11] All the rights pertinent to the information in the table above and the responsibility for the contents rest upon Monsanto Japan Limited.

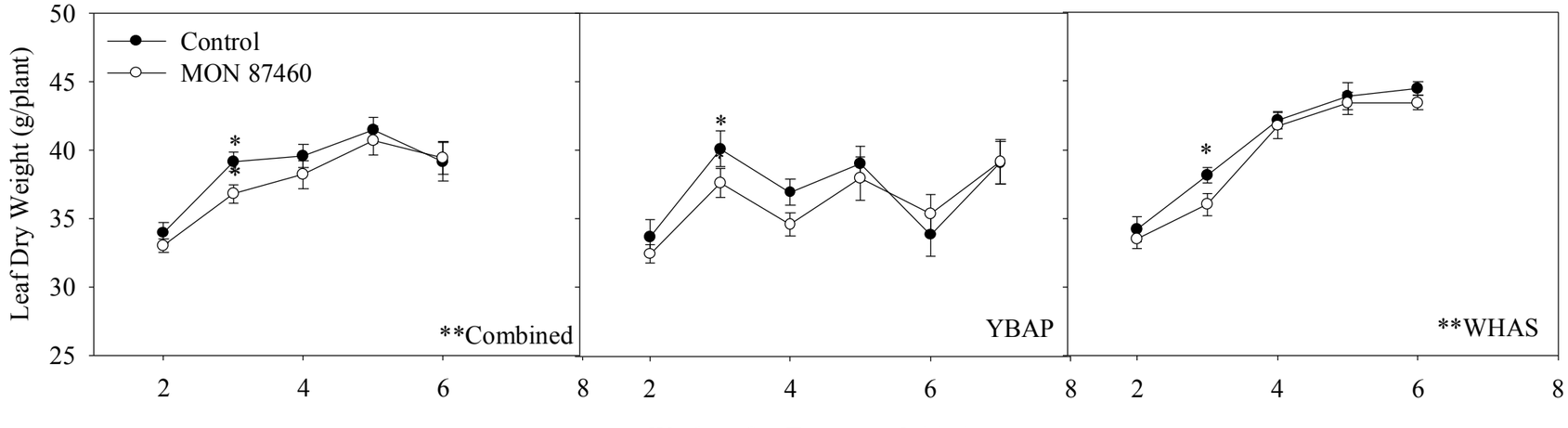


Figure 4. Change of leaf dry weight under water-limited conditions (2009, USA)[12]

Change of leaf dry weight with time for the transgenic maize (○) and the non-transgenic maize (control) (●) under water-limited conditions at all fields (Combined), Barrios (YBAP), and Harlan South (WHAS). Leaf dry weights were measured from 2 to 4 samples taken from each plot every week between the 2nd and 7th week during the water-limited period. Each value shows the mean ± 1 × standard error.

* indicates a statistically significant difference between the transgenic maize and the non-transgenic maize (control) was recognized on the measuring day ($p \leq 0.05$).

** indicates a statistically significant difference between the transgenic maize and the non-transgenic maize (control) was recognized on all measuring days at the field ($p \leq 0.05$). Mean leaf dry weight for the transgenic maize and the non-transgenic maize (control) was 37.9 g and 38.8 g, respectively, for all the fields, and 39.6 g and 40.6 g, respectively, for WHAS. The water limitation treatment was started at the 8th leaf stage (V8). Flowering occurred 3 to 4 weeks after the beginning of water limitation treatment.

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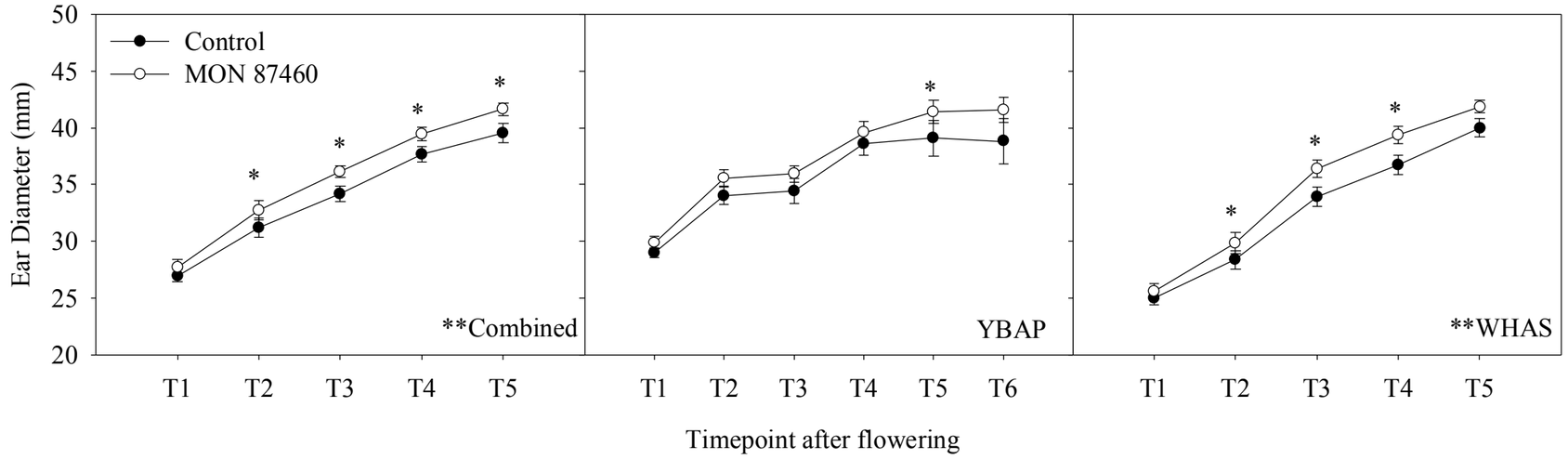

Figure 5. Change of ear diameter under water-limited conditions (2009, USA)[13]

Change of ear diameter with time for the transgenic maize (○) and the non-transgenic maize (control) (●) under water-limited conditions at all fields (Combined), Barrios (YBAP), and Harlan South (WHAS). Each value shows the mean ± 1 × standard error.

* indicates a statistically significant difference between the transgenic maize and the non-transgenic maize (control) was recognized on the measuring day ($p \leq 0.05$).

** indicates a statistically significant difference between the transgenic maize and the non-transgenic maize (control) was recognized on all measuring days at the field ($p \leq 0.05$). Mean ear diameter for the transgenic maize and the non-transgenic maize (control) was 36.0 mm and 34.1 mm, respectively, for all the fields, and 34.6 mm and 32.8 mm, respectively, for WHAS.

T1 occurred 3 to 4 days after flowering. The ear measurements were conducted twice a week between the start of flowering and about 18 to 19 days after flowering. The study was carried out between

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4th and 7th week after the start of the water limitation treatment. Since the measurement of ear diameter can be repeatedly conducted on the same ear, more accurate data can be obtained by using ear diameter measurement than the measurement of ear dry weight. Also, the measurement can be done with a larger number of samples because the ears do not have to be cut off for measurement.

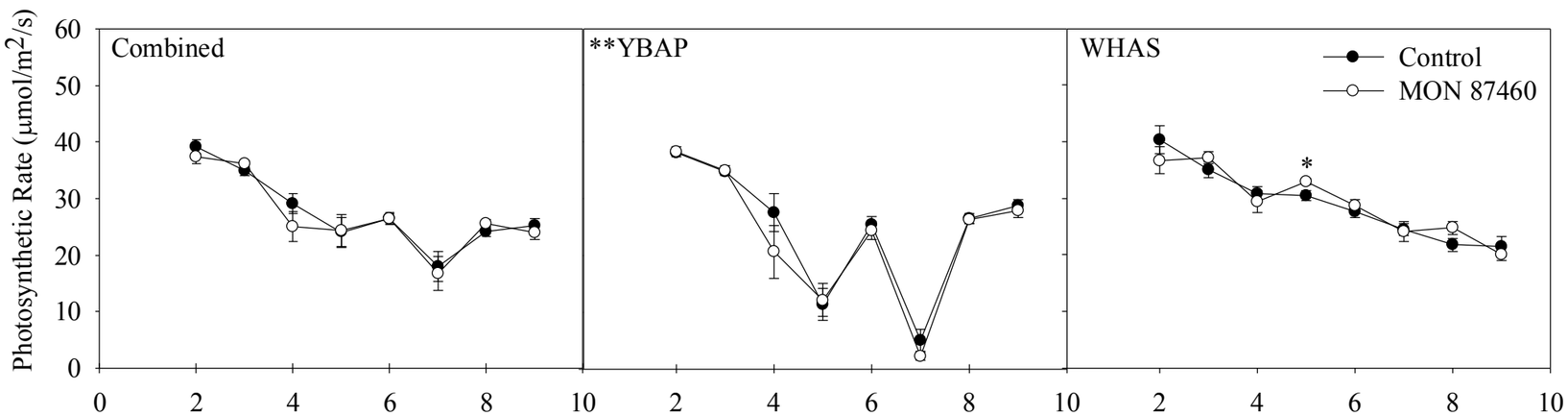

Figure 6. Change of photosynthesis rate under water-limited conditions (2009, USA)[14]

Change of photosynthesis rate with time for the transgenic maize (○) and the non-transgenic maize (control) (●) under water-limited conditions at all fields (Combined), Barrios (YBAP), and Harlan South (WHAS). Each value shows the mean ± 1 × standard error.

5 * indicates a statistically significant difference between the transgenic maize and the non-transgenic maize (control) was recognized on the measuring day ($p \leq 0.05$).

** indicates a statistically significant difference between the transgenic maize and the non-transgenic maize (control) was recognized on all measuring days at the field ($p \leq 0.05$). Mean photosynthesis rate for the transgenic maize and the non-transgenic maize (control) was 25.4 μmol/m^2/s and 27.1 μmol/m^2/s, respectively, for YBAP.

Water limitation was started at ca. 8th leaf stage (V8). The flowering started about 3 to 4 weeks after the beginning of the water limitation period. In the measuring period, the 2nd to 7th weeks were in the water-limited period. The 8th and 9th weeks were designed to achieve transpiration rate of 100%.

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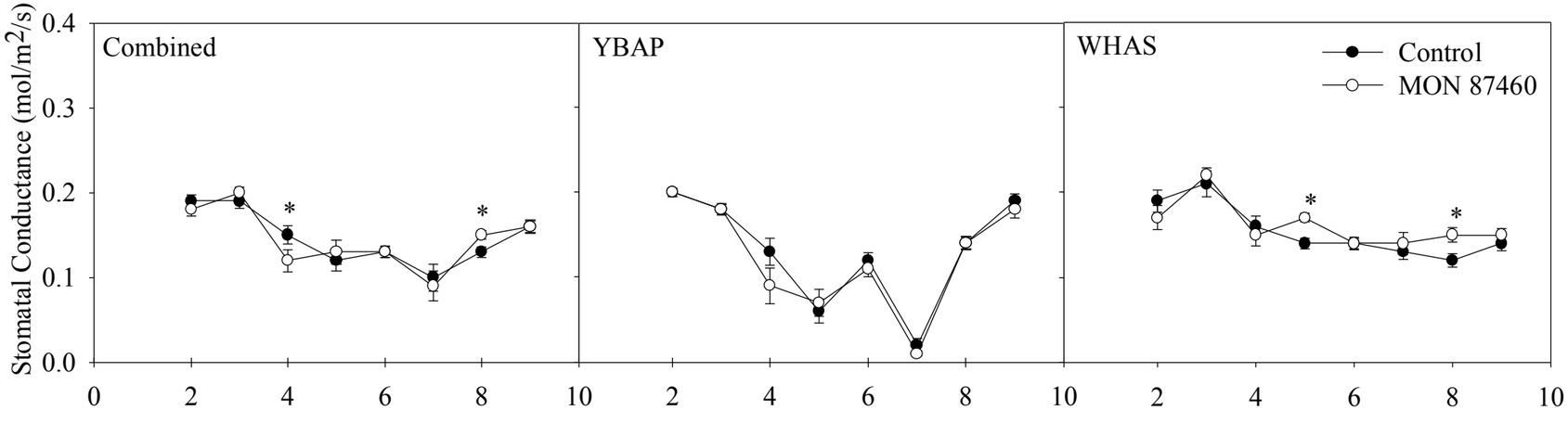

Figure 7. Change of stomatal conductance under water-limited conditions (2009, USA)[15]

Change of stomatal conductance with time for the transgenic maize (○) and the non-transgenic maize (control) (●) under water-limited conditions at all fields (Combined), Barrios (YBAP), and Harlan South (WHAS). Each value shows the mean ± 1 × standard error.

* indicates a statistically significant difference between the transgenic maize and the non-transgenic maize (control) was recognized on the measuring day ($p \leq 0.05$).

Water limitation was started at ca. 8th leaf stage (V8). The flowering started about 3 to 4 weeks after the beginning of the water limitation period. In the measuring period, the 2nd to 7th weeks were in the water-limited period. The 8th and 9th weeks were designed to achieve transpiration rate of 100%.

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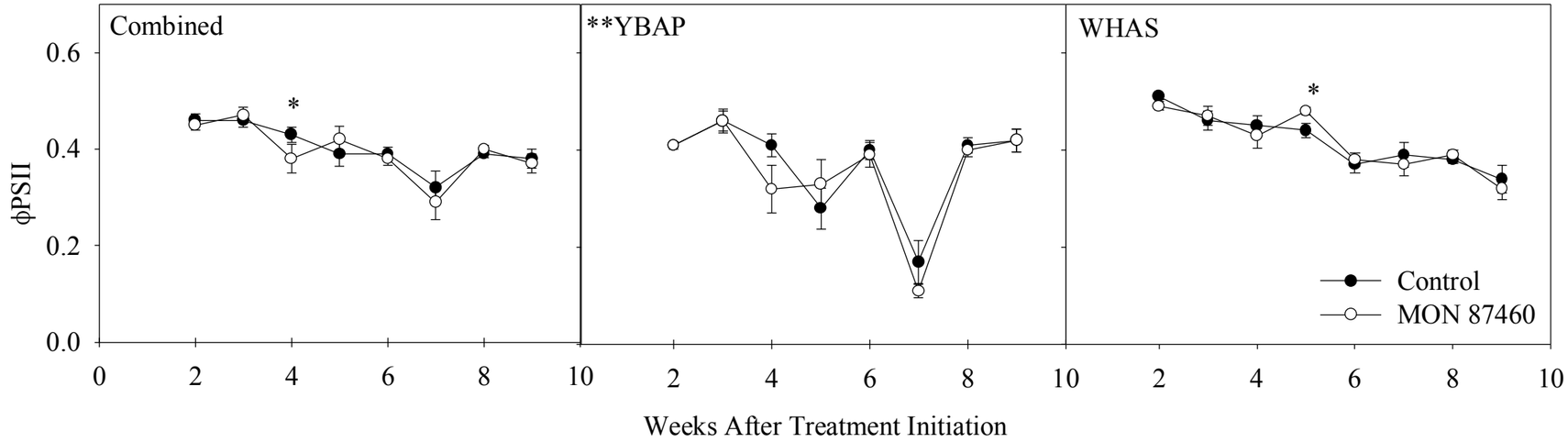

Figure 8. Change of quantum efficiency at the photosystem II under water-limited conditions (2009, USA)[16]

Change of quantum efficiency at photosystem II with time for the transgenic maize (○) and the non-transgenic maize (control) (●) under water-limited conditions at all fields (Combined), Barrios (YBAP), and Harlan South (WHAS). Each value shows the mean ± 1 × standard error.

* indicates a statistically significant difference between the transgenic maize and the non-transgenic maize (control) was recognized on the measuring day ($p \leq 0.05$).

** indicates a statistically significant difference between the transgenic maize and the non-transgenic maize (control) was recognized on all measuring days at the field ($p \leq 0.05$). Mean quantum efficiency at photosystem II for the transgenic maize and the non-transgenic maize (control) was 0.37 and 0.39, respectively, for YBAP.

Water limitation was started at ca. 8th leaf stage (V8). The flowering started about 3 to 4 weeks after the beginning of the water limitation period. In the measuring period, the 2nd to 7th weeks were in the water-limited period. The 8th and 9th weeks were designed to achieve transpiration rate of 100%.

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(vi) Conclusion on the functions of the modified CSPB

Studies have shown that the modified CSPB binds to RNA and disrupts secondary structure of RNA. It is also known that the modified CSPB is localized in the nucleus and cytoplasm and is especially abundant in the meristem tissues (Annex 2). These findings are also recognized in proteins containing bacterial CSP and plant CSD (Chaikam and Karlson, 2008; Fusaro *et al.*, 2007; Sasaki *et al.*, 2007), suggesting that the modified CSPB acts on response pathways to confer drought tolerance on the transgenic maize. Photosynthesis rate, stomatal conductance, and quantum efficiency at photosystem II of the transgenic maize were improved compared with the non-transgenic maize (control) under water-limited conditions in environment-controlled greenhouse (Annex 3). The results of the field tests conducted in California in 2009 indicated that the transgenic maize efficiently distributes photosynthates under water-limited conditions, which results in higher ear dry weight, grain count per ear, yield, and harvest index than the non-transgenic maize (control) (Annex 4). These findings together suggest that the modified CSPB functions as an RNA chaperone under water-limited conditions and, hence, maintains the growth and development of the transgenic maize, resulting in a reduction of yield loss (Figure 9 on p.29).

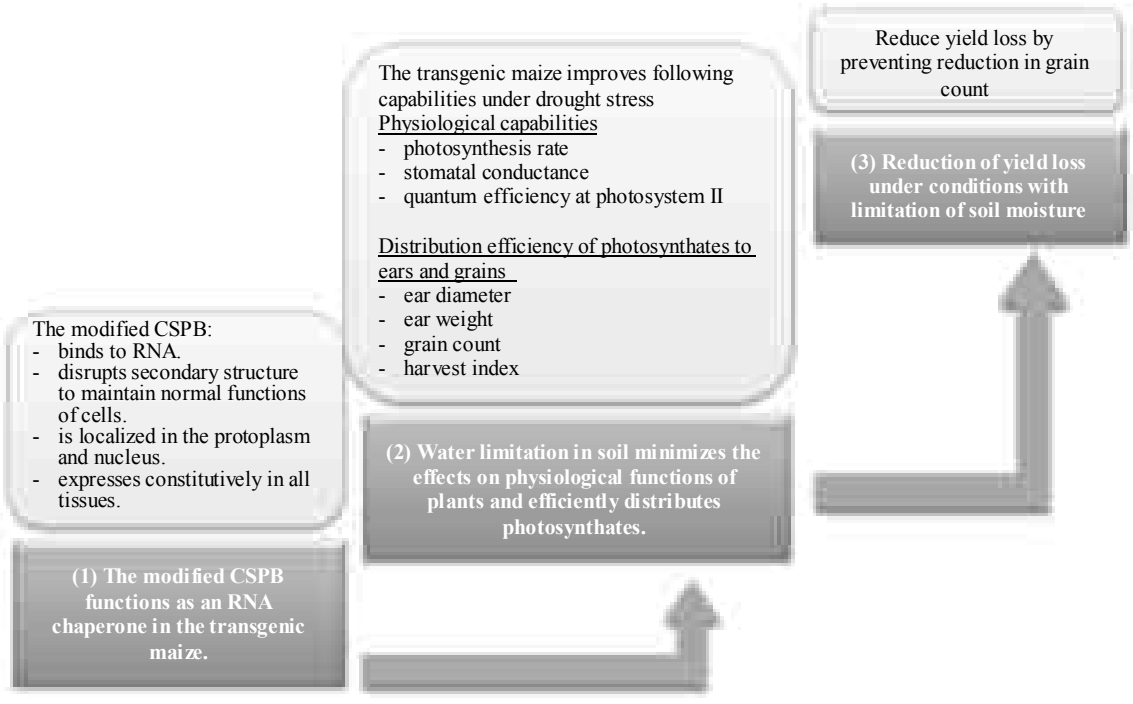

Figure 9. Function of the modified CSPB expressed in the transgenic maize[17]

No similarities were found in the modified CSPB and known allergens in comparison to determine whether the modified CSPB shares functionally important amino acid sequences with known allergens using the allergen database (AD8) with the FASTA type algorism.

[NPTII protein]

nptII gene, an antibiotic tolerant marker gene introduced to select transformants, is derived from an *E. coli* transposon Tn5. The encoded NPTII protein inactivates aminoglycoside antibiotics (e.g., kanamycin) through phosphorylation, conferring tolerance to these antibiotics on the transformant. As a result, application of kanamycin to media will enable selection of transformed cells (Beck *et al.*, 1982; Nap *et al.*, 1992; Shaw *et al.*, 1993).

Recent studies have concluded that NPTTII protein is not homologous with known allergens, toxins, or other proteins that induce side effects in animals or humans (Hileman and Astwood, 2000; McCoy and Silvanovich, 2005). No similarities were found in NPTII protein and known allergens in comparison to determine whether NPTII protein shares functionally important amino acid sequences with known allergens using the allergen database (AD 2009) with the FASTA type algorism.

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iii. Specific modifications of host metabolism, if any

[Modified CSPB]

Bacteria-derived CSPs including CSPB nonspecifically bind to RNA and function as an RNA chaperone (Herschlag, 1995; Jiang *et al.*, 1997). As a result, CSP plays a role in maintaining translation under conditions where translation is inhibited otherwise (Graumann *et al.*, 1997). CSPB does not have functions to directly induce transcription (Schindler *et al.*, 1999; Weber *et al.*, 2001), and there is no report showing that CSPB has enzymatic activity.

Thus, it is unlikely that expression of the modified CSPB in the transgenic maize results in production of new metabolites by enzyme activities of the modified CSPB.

[NPTII protein]

NPTII protein is an enzyme that catalyzes phosphorylation reaction of hydroxyl group in aminoglycosides contained in aminoglycoside antibiotics (Shaw *et al.*, 1993). NPTII protein is reported to have association only in phosphorylation reactions in limited aminoglycoside antibiotics, including neomycin, kanamycin, promomycin, ribostamycin, and butirosin (Davies, 1986; Davies and Smith, 1978; Price *et al.*, 1974). A study of structural activities of NPTII protein demonstrated that the protein cannot use aminoglycoside antibiotics as a substrate due to minute changes (e.g., removal of the hydroxyl group or modification of amino group) of aminoglycoside structure in aminoglycoside antibiotics (Price *et al.*, 1974).

Thus, it is unlikely that expression of NTPII protein in the transgenic maize results in production of new metabolites.

[Modified CSPB + NPTII protein]

As described above, modes of action differ between the modified CSPB and NPTII protein. Also, NPTII protein has high substance specificity, and the modified CSPB does not possess an aminoglycoside structure that allows it to act as a substrate to NPTII protein. Thus, these proteins are considered to act independently and are unlikely to interact in plants. This suggests that the two kinds of proteins expressed in the transgenic maize do not produce new metabolites in maize.

Whether expression of the modified CSPB and NPTII protein in the transgenic maize results in production of new metabolites (Annex 6) was experimentally determined. The experiment was conducted in 2006 and 2007 using the aboveground biomass and grain samples taken from the transgenic maize and non-transgenic maize (control) that were grown under two soil moisture conditions (well-watered conditions and water-limited conditions) in three field locations (Calera de Tango: CT, Colina: CL, Lumbreras: LUM). The test plots designated for the well-watered conditions received proper levels of irrigation, while irrigation in the test plots designated for water-limited conditions was limited in the period from the later vegetative stage to the early reproductive stage (ca. 10th leaf stage to water-ripe stage). As reference, four different commercial varieties were grown in all of the four fields and used for analysis.

For aboveground mass of maize, nine components were assessed: proximates components (ash, carbohydrate,

moisture, protein, and total fat), acid detergent fiber (ADF), neutral detergent fiber (NDF), calcium, and phosphorus. For grain of maize, 68 components were assessed: proximate (5 items: ash, carbohydrates, moisture, protein, and total fat), acid detergent fiber (ADF), neutral detergent fiber (NDF), total detergent fiber (TDF), minerals (9 items: calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), amino acids (18 items), fatty acids (22 items), vitamins (6 items: folic acid, niacin, vitamin B1, vitamin B2, vitamin B6, and vitamin E), antinutritional substances (2 items: phytic acid and raffinose), and secondary metabolites (3 items: ferulic acid, *p*-coumaric acid, and furfural. Among these items, measurements of fatty acids (14 items), sodium, and furfural in grains were below quantitation limits. Hence, 9 items and 52 items were analyzed for aboveground mass and grain, respectively.

In the results of analyses of the data from all fields, statistically significant differences between the transgenic maize and non-transgenic maize (control) were found in the total fat and magnesium measurements in grain under well-watered conditions ($p \leq 0.05$). Statistically significant differences between the transgenic maize and non-transgenic maize (control) were also found in total fat in aboveground biomass and 20:1 eicosenoic acid in grain ($p \leq 0.05$). However, the mean values for these components were within the 99% tolerance interval (99%T.I.) established from the commercial references grown at the same sites (Annex 6).

Based on these findings, it is unlikely that the modified CSPB and NTPII protein expressed in the transgenic maize results in production of new metabolites.

(2) Information about Vector

a. Name and origin

Plasmid vector PV-ZMAP595 used in the production of the transgenic maize was constructed based on pBR322, a vector derived from *E. coli* (Sutcliffe, 1979).

b. Characteristics

i. Number and sequence of nucleotides of the vector

The number of nucleotides in plasmid vector PV-ZMAP595 used in the production of the transgenic maize was 9,379 bp.

ii. Functions of nucleotide sequence with specific functions, if any

As a selection marker gene for constructive vector in *E. coli*, *aadA* gene derived from *E. coli* transposon Tn7, which confers tolerance to spectinomycin and streptomycin, is present in an outside region of T-DNA.

iii. Infectiousness of vector and information on the host region if infectious

There is no report to show infectiousness of the vector.

(3) Preparation of genetically modified organisms, etc.

a. Structure of the whole nucleic acid transferred into the host

The elements of the plasmid vector transferred into the host were shown in Table 1 (p.5–11). The location of elements of the donor nucleic acid in the vector and restriction enzyme cutting sites are shown in Figure 1 (p.4).

b. Transfer method of the nucleic acid into host

The T-DNA region in PV-ZMAP595 was transferred into immature embryo of maize LH59 using the agrobacterium method.

c. Development of genetically modified organisms, etc.

i. Selection method of cells transferred with nucleic acid

After callus dedifferentiated from immature embryo of an inbred maize line LH59 was co-cultured with *A. tumefaciens* ABI strain containing plasmid vector PV-ZMAP595, cell selection was carried out on tissue culture media added with carbenicillin and paromomycin. Individuals that have not been transformed by paromomycin were eliminated.

ii. Presence of agrobacterium residues, if the agrobacterium method was used for nucleic acid transfer

Agrobacterium used for transformation was eliminated by the tissue culture media added with carbenicillin. To ensure that there is no agrobacterium residue in the transgenic maize, the transgenic maize was transplanted to media free from carbenicillin. No colony of agrobacterium was developed on the media.

iii. Process of degeneration of lines (the lines on which the presence of the duplicates of the introduced nucleic acid was confirmed, the lines that were provided for isolated fieldexperiments, and the lines used for collection of information that are necessary for the biodiversity risk assessment) from the nucleic-acid transferred cells

Transformed R_0 (redifferentiated plants) was bred with an inbred maize line LH59 and then self-crossed.

The subsequent R_1 plants were screened for the presence of expression of the modified CSPB, tolerance to kanamycin, and homozygosity of the inserted gene. Only progeny of the screened plants were subjected to the analysis of the inserted genes and morphological assessments, and the results were used to select MON87460 line as the final commercial line.

The development of the transgenic maize is shown in Figure 10 (p.34). Please note that the R4 generation and all bred lines in its progeny are the subjects of this application.

[Confidential. Not disclosed]

Figure 10. Development of the transgenic maize

(4) Presence of nucleic acid transferred into cells and stability of trait expression by the nucleic acid

i. Location of the reproduced substances of the transferred nucleic acids

To determine whether the transferred gene of the transgenic maize is present in chromosome, chi-squared tests were conducted with samples taken from several generations of the transgenic maize.

Heterozygous transgenic maize plants with the modified *cspB* gene (LH59 R_0 generation) were selfed to produce LH59 R_1. Subsequently, homologous plants containing the modified *cspB* gene in the LH59 R_1 generation were selfed to produce LH59 R_2 plants. The LH59 R_4 generation was crossed with HCL301 to produce the (LH59 R_4 × HCL301) BC_3F_1 generation, and plants in the LH59 R_4 generation were crossed with HCL105 to produce the (LH59 R_4 × HCL105) BC_3F_2, (LH59 R_4 × HCL105) BC_3F_3, (LH59 R_4 × HCL105) BC_4F_1 generations, and (LH59 R_4 × HCL105) BC_5F_1 generation.

The (LH59 R_4 × HCL301) BC_3F_1 generation, (LH59 R_4 × HCL105) BC_4F_1 generation, and (LH59 R_4 × HCL105) BC_5F_1 generation, which were produced by crossing an inbred maize line with the positive heterozygous plants selected from the previous generation, was expected to segregate 1:1 (1 positive: 1 negative). The (LH59 R_4 × HCL105) BC_3F_2 generation, which was produced by selfing the positive heterozygous plants selected from the previous generation, was expected to segregate 1:2:1 (1 positive homozygous: 2 positive heterozygous: 1 negative). The (LH59 R_4 × HCL105) BC_3F_3 generation, which was produced by selfing positive homozygous plants from the previous generation, was expected to be positive homozygous plants.

The results of PCR analysis of segregation are shown in Table 9 (p.41). For the LH59 R_1 generation, (LH59 R_4 × HCL301) BC_3F_1 generation, (LH59 R_4 × HCL105) BC_3F_2 generation, (LH59 R_4 × HCL105) BC_4F_1 generation, and (LH59 R_4 × HCL105) BC_5F_1 generation, no statistically significant difference was observed between the observed and expected values in chi-squared tests (Table 9 on p.36; Table 1 on p.5 of Annex 7). For the LH59 R_2 generation and (LH59 R_4 × HCL105) BC_3F_3 generation, which were produced by self-crossing the homozygote plants in the previous generation, the modified *cspB* gene was recognized in all plants. These results indicate that the modified *cspB* gene expression cassette and *nptII* gene expression cassette in the MON87460 line follow the expected Mendelian pattern of segregation in progeny, suggesting presence of the transferred gene of the transgenic maize in the chromosome.

Table 9. Segregation ratios of the transferred gene in the transgenic maize[18]

Generation	Number of plants	Observed [1]		Expected		X^{2} [2]	Probability ($\alpha = 0.05$)
		+	—	+	—		
LH59 R_1	36	26	10	27	9	0.148	NS
LH59 R_2	89	89	0	89	0	Fixed	—
(LH59 R_4 × HCL301) BC_3F_1	178	84	94	89	89	0.562	NS
(LH59 R_4 × HCL105) BC_3F_2	154	124	30	115.5	38.5	2.502	NS
(LH59 R_4 × HCL105) BC_3F_3	474	474	0	474	0	Fixed	—
(LH59 R_4 × HCL105) BC_4F_1	80	44	36	40	40	0.800	NS
(LH59 R_4 × HCL105) BC_5F_1	82	44	38	41	41	0.439	NS

[1] +/—: Presence (+) or not presence (-) of the modified *cspB* gene.

[2] Chi-square value = 3.841 ($\alpha = 0.05$, degree of freedom = 1).

NS: no significant difference

ii. The number of copies of the inserted nucleic acids and stability of the copies of the transferred nucleic acids in multiple generations

A southern blot analysis of the transferred gene confirmed that one copy of the T-DNA region was present in one region of the genome of the transgenic maize (Figure 5 on p.41 of Annex 8). It was also confirmed that the T-DNA region was the only external skeletal region inserted (Figure 6 on p.42 of Annex 8).

On the other hand, analysis of the base sequence revealed that the right border region of the transferred (2,816–3,172bp of PV-ZMAP595) and the upstream 733 bp of the subsequent P-Ract 1 region (3,205–3,937bp of PV-ZMAP595) were missing (Figure 18 on p.54–55 of Annex 8).

In addition, stability of the transferred gene in the multiple generations ((LH59 R_2 × LH244) F_1, (LH59 R_3 × LH244) F_1, (LH59 R_3 × LH244) F_2, LH59 R_4, (LH59 R_4 × HCL301) F_1, (LH59 R_4 × HCL301) F_2, and (LH59 R_4 × HCL301) BC_3F_1) was confirmed by the southern blot analysis (Figure 14 on p.50 of Annex 8).

iii. Distance (either adjacent or separated) between the copies on chromosome, if multiple copies are present on chromosome

Not applicable (there is only 1 copy) (Figure 5 on p.41 of Annex 8).

iv. Stability of expression among plants and generations in natural conditions regarding the characteristics specifically shown in (6) i.

ELISA tests confirmed that the modified CSPB is stable in multiple generations of the transgenic maize ((LH59 R_2 × LH244) F_1, (LH59 R_3 × LH244) F_1, (LH59 R_3 × LH244) F_2, LH59 R_4, (LH59 R_4 × HCL301) F_1, (LH59 R_4 × HCL301) F_2, and (LH59 R_4 ×

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HCL301) BC_3F_1) (Table 1 on p.14 of Annex 9).

Using ELISA, volumes of expression of the modified CSPB and NPTII protein were analyzed in various tissues of the transgenic maize that are cultivated under well-watered and water-limited conditions (three replications for each condition) in the fields in three locations (Calera de Tango: CT, Colina: CL, and Lumbreras: LUM) in Chile in 2006–2007 (Annex 10). The test plots designated for the well-watered conditions received proper levels of irrigation, while irrigation in the test plots designated for water-limited conditions was limited in the period from the later vegetative stage to the early reproductive stage (ca. 10th leaf stage (V10) to blister stage (R2)).

The expression volumes of the modified CSPB and NPTII protein in each tissue under well-watered and water-limited conditions are shown in Table 10 and Table 11 (p.38–39) (Table 1–5 on p.27–32 of Annex 10). The modified CSPB was expressed in each tissue under both well-watered and water-limited conditions. It was also shown that the volume of the modified CSPB decreases with plant growth.

Table 10. Modified CSPB levels in different tissues in the transgenic maize under well-watered and water-limited conditions (2006–2007, Chile)[19]

Tissue type[1]	Well-watered condition Modified CSPB (SD)[2] Range[3] (μg/g dwt)[4]	(μg/g dwt)[5]	Water-limited condition Modified CSPB (SD) Range (μg/g fwt)	(μg/g dwt)	LOQ / LOD (μg/g fwt)
Leaf (OSL-1)	0.50 (0.19) 0.28 - 0.80	2.8 (1.0) 1.7 - 4.5	0.50 (0.20) 0.26 - 0.80	2.8 (0.95) 1.7 - 4.2	0.015/0.0069
Leaf (OSL-2)	0.48 (0.18) 0.21 - 0.69	2.6 (1.2) 0.96 - 3.8	0.47 (0.15) 0.23 - 0.62	2.6 (1.0) 1.1 - 3.6	0.015/0.0069
Leaf (OSL-3)	0.13 (0.10) 0.023 - 0.33	0.56 (0.48) 0.10 - 1.5	0.11 (0.073) 0.023 - 0.25	0.45 (0.32) 0.086 - 1.1	0.015/0.0069
Leaf (OSL-4)	0.10 (0.041) 0.040 - 0.14	0.39 (0.13) 0.18 - 0.58	0.11 (0.054) 0.050 - 0.20	0.44 (0.17) 0.22 - 0.69	0.015/0.0069
Root (OSR-1)	0.13 (0.029) 0.079 - 0.18	1.3 (0.29) 0.79 - 1.8	0.14 (0.034) 0.10 - 0.20	1.5 (0.43) 0.95 - 2.2	0.0020/0.0018
Root (OSR-2)	0.086 (0.025) 0.070 - 0.13	0.86 (0.25) 0.70 - 1.4	0.10 (0.015) 0.082 - 0.12	0.82 (0.092) 0.74 - 0.95	0.0020/0.0018
Root (OSR-3)	0.061 (0.012) 0.035 - 0.075	0.49 (0.12) 0.27 - 0.62	0.054 (0.012) 0.036 - 0.076	0.41 (0.13) 0.24 - 0.63	0.0020/0.0018
Root (OSR-4)	0.045 (0.012) 0.032 - 0.067	0.31 (0.076) 0.22 - 0.45	0.058 (0.016) 0.036 - 0.084	0.40 (0.087) 0.28 - 0.52	0.0020/0.0018
Aboveground (OSWP-1)	0.32 (0.11) 0.18 - 0.52	3.2 (0.98) 1.8 - 4.8	0.30 (0.092) 0.20 - 0.42	2.9 (0.84) 1.8 - 3.8	0.0045/0.0043
Aboveground (OSWP-2)	0.19 (0.036) 0.12 - 0.24	2.3 (0.54) 1.4 - 3.0	0.18 (0.046) 0.12 - 0.25	2.2 (0.61) 1.4 - 3.1	0.0045/0.0043
Aboveground (OSWP-3)	0.10 (0.042) 0.065 - 0.17	0.89 (0.34) 0.59 - 1.4	0.091 (0.032) 0.067 - 0.15	0.71 (0.25) 0.44 - 1.1	0.0045/0.0043
Aboveground (OSWP-4)	0.11 (0.026) 0.076 - 0.17	0.67 (0.16) 0.48 - 0.98	0.13 (0.037) 0.10 - 0.20	0.70 (0.16) 0.55 - 1.0	0.0045/0.0043
Root (Early Dent stage)	0.0052 (0.0018) 0.0026 - 0.0088	0.039 (0.015) 0.017 - 0.068	0.011 (0.0039) 0.0056 - 0.016	0.076 (0.029) 0.035 - 0.12	0.0020/0.0018
Root (Immediately after harvest)	0.0040 (0.0017) 0.0026 - 0.0073	0.031 (0.015) 0.020 - 0.061	0.0067 (0.0051) 0.0026 - 0.017	0.052 (0.040) 0.019 - 0.14	0.0020/0.0018
Forage (Early Dent stage)	0.026 (0.0041) 0.018 - 0.034	0.11 (0.018) 0.077 - 0.14	0.035 (0.0078) 0.022 - 0.047	0.15 (0.040) 0.087 - 0.22	0.0045/0.0043
Stover (Immediately after harvest)	0.011 (0.0023) 0.0071 - 0.014	0.033 (0.0070) 0.018 - 0.040	0.021 (0.010) 0.011 - 0.036	0.072 (0.033) 0.035 - 0.12	0.0045/0.0043
Silk (Flowering stage)	0.073 (0.019) 0.050 - 0.12	0.82 (0.28) 0.50 - 1.5	0.13 (0.048) 0.054 - 0.22	1.1 (0.38) 0.49 - 1.8	0.0075/0.0047
Pollen (Flowering stage)	18 (5.6) 7.0 - 24	25 (7.4) 8.9 - 33	18 (6.5) 12 - 31	27 (10) 18 - 48	0.050/0.045
Grain (Maturing stage)	0.041 (0.012) 0.028 - 0.065	0.048 (0.014) 0.033 - 0.075	0.033 (0.0067) 0.021 - 0.045	0.038 (0.0079) 0.024 - 0.053	0.0038/0.0017

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[19]All the rights pertinent to the information in the table above and the responsibility for the contents rest upon Monsanto Japan Limited.

[1]OSL, OSR, OSWP-1: 2–4 leaf stage; OSL, OSR, OSWP-2: 6–8 leaf stage; OSL, OSR, OSWP-3: 10–12 leaf stage; OSL, OSR, OSWP-4: –tasseling stage

[2] The arithmetic mean and standard deviation (SD) were calculated for each tissue type across all sites (n = 9 for well-watered and n = 9 for water-limited, except OSR-2 where n = 6 for under both well-watered and water-limited conditions and root after harvest where n = 6 for well-watered).

[3] Minimum and maximum values were determined for each tissue type across all sites.

[4] Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt) basis (µg/g fwt).

[5] Protein levels are expressed as "µg/g" of tissue on a dry weight (dwt) basis (µg/g dwt). The dry weight values were calculated by dividing the fresh weight values by the dry weight conversion factors obtained from moisture analysis data.

Table 11. NPTII protein levels expressed in tissues collected from the transgenic maize produced in well-watered and water-limited condition (2006–2007, Chile)[20]

Tissue type[1]	Well-watered condition		Water-limited condition		LOQ/LOD
	NPTII protein (SD)[2] Range[3]		NPTII protein (SD) Range		
	(µg/g fwt)[4]	(µg/g dwt)[5]	(µg/g fwt)	(µg/g dwt)	(µg/g fwt)
Leaf (OSL-1)	0.42 (0.23) 0.15 - 0.85	2.4 (1.3) 0.84 - 5.0	0.46 (0.18) 0.16 - 0.68	2.6 (0.98) 0.98 - 4.0	0.047/0.0090
Root (OSR-1)	0.051 (0.0083) 0.041 - 0.064	0.51 (0.083) 0.41 - 0.64	0.046 (0.0075) 0.035 - 0.057	0.48 (0.097) 0.39 - 0.64	0.0075/0.0043
Foliage (Early Dent stage)	0.037 (0.0041) 0.031 - 0.044	0.16 (0.020) 0.13 - 0.19	0.039 (0.0048) 0.034 - 0.048	0.17 (0.028) 0.14 - 0.22	0.0056/0.0024
Grain (Maturing stage)	<LOQ(N/A[6]) <LOD-0.0057	N/A (N/A) N/A	<LOQ (N/A) <LOD-0.0051	N/A (N/A) N/A	0.0047/0.0024

[1]OSL, OSR-1: 2–4 leaf stage

[2]The mean and standard deviation (SD) were calculated for each tissue type across sites (n = 9 for well-watered and n = 9 for water-limited, except OSL-1 where n = 8 for water-limited).

[3] Minimum and maximum values were determined for each tissue type across all sites.

[4] Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt) basis (µg/g fwt).

[5] Protein levels are expressed as "µg/g" of tissue on a dry weight (dwt) basis (µg/g dwt). The dry weight values were calculated by dividing the fresh weight values by the dry weight conversion factors obtained from moisture analysis data.

[6]N/A: Not applicable.

[20]All the rights pertinent to the information in the table above and the responsibility for the contents rest upon Monsanto Japan Limited.

v. Presence or absence, and if present, degree of transmission of nucleic acid transferred through virus infection and/or other routes to wild animals and wild plants

Regarding the plasmid vector PV-ZMAP595, the region of recipient organism, which allows autonomous replication, is limited to gram-negative bacteria such as *E. coli* and *A. tumefaciens*. Therefore, there is no possibility that the plasmids might be transmitted to any wild animals and wild plants under natural environment.

(5) Methods of detection and identification of living modified organisms and their sensitivity and reliability

Detection is available by using the PCR method (Annex 11). The method can detect genetically transgenic maize if at least 1 seed of genetically transgenic maize is present in 150 seeds. The reliability of this method was verified by an experiment with 87 sample groups of 150 seeds containing 1 seed of the transgenic maize and 88 sample groups of 150 seeds containing no seeds of the transgenic maize (Annex 11).

(6) Difference from the host organism or the species to which the host organism belongs

i. Specific physiological or ecological characteristics that were conferred by the expression of replication products of transferred nucleic acid

As shown in I-2-(1)-b-ii-(ii), the modified *cspB* gene has been reported to show tolerance to various environmental stresses (Castiglioni *et al.*, 2008). Therefore, it is likely that the gene contributes to reduction of yield loss or shows other kinds of tolerance under non-drought environmental stresses. To investigate such possibilities, studies were carried out in environment-controlled chambers for tolerance to low-temperature stress, high-temperature stress, and salt stress. The results from the drought tolerance experiments were included in the study as positive control. The environmental stress treatments were carried out at the period from the later vegetative stage (V10) to early reproductive stage (R3), the period where reduction of yield loss by the transgenic maize was confirmed in field studies. In general, collecting reliable samples in regard with yield components from experiments in environment-controlled chambers is difficult (Ainsworth *et al.*, 2002; Long *et al.*, 2006). However, since the transgenic maize has been known to improve physiological functions under drought stress, physiological functions are used as stress tolerance index in the study.

The experiments were conducted in 2010 in greenhouse and environment-controlled chambers at Monsanto Company (USA). To make the cultivation conditions and stress conditions consistent to all tests, plants were grown in a greenhouse and moved to the environment-controlled chamber at ca. 8-leaf stage (V8). The stress treatments were applied from ca. 10-leaf stage (V10) to milk stage (R3). Three levels of stress treatments, including the optimal condition, were given.

(i) Drought tolerance assessment (Annex 12)

Drought tolerance was compared between the transgenic maize and non-transgenic maize (control) using the plants from ca. 10-leaf stage (10V) to milk stage (R3) under three levels of drought treatment (Total weight of the pot, soil, plant, and water (moisture ratio to the well-watered condition). Optimal condition: 9.5 kg (100%); Mild drought treatment: 7.7 kg (67%); Severe drought treatment: 5.9 kg (33%)). Morphological characteristics

(plant height, growth stage, fresh weight and dry weight of foliage, fresh weight and dry weight of ears, grain count, and anthesis-silking interval) and physiological characteristics (photosynthesis rate, stomatal conductance, and quantum efficiency at photosystem II) were measured before the treatment, during the treatment, and at the time of harvest.

In the results of assessment, a statistically significant difference between the transgenic maize and non-transgenic maize (control) was observed in the anthesis-silking interval under well-watered condition ($p \leq 0.05$) (Table 2 on p.10 of Annex 12). The mean anthesis-silking intervals for the transgenic maize and non-transgenic maize (control), where statistically significant difference was observed, were 0.1 day and -0.3 day, respectively.

With the mild drought treatment, statistically significant differences between the transgenic maize and non-transgenic maize (control) were observed in mean values of stomatal conductance and quantum efficiency at photosystem II during the treatment period (from 7th to 36th days) ($p \leq 0.05$) (Table 3 on p.11 of Annex 12). The mean values for stomatal conductance and quantum efficiency at photosystem II during the treatment period (when statistically significant differences were observed) were 0.13 mol $CO_2/m^2/s$ and 0.40, respectively, for the transgenic maize, and 0.11 mol $CO_2/m^2/s$ and 0.37, respectively, for the non-transgenic maize (control). For both stomatal conductance and quantum efficiency at photosystem II, the mean was higher for the transgenic maize (the higher the value of stomatal conductance or quantum efficiency at photosystem II, the higher the activity). The physiological characteristics tended to be higher for the transgenic maize than for the non-transgenic maize (control) (Table 3 on p.11 of Annex 12 and Figure 1–3 on p.12–14).

With the severe drought treatment, statistically differences between the transgenic maize and non-transgenic maize (control) were not observed for all measurements. However, the physiological characteristics tended to be higher for the transgenic maize than for the non-transgenic maize (control) (Table 3 on p.11 of Annex 12 and Figure 1–3 on p.12–14).

These findings suggest that the expression of the modified CSPB is responsible for the reduction of the physiological function declines in the transgenic maize under the conditions used for the tests.

(ii) Low-temperature tolerance assessment (Annex 13)

Low-temperature tolerance was compared between the transgenic maize and non-transgenic maize (control) using the plants from ca. 10-leaf stage (10V) to milk stage (R3) under three levels of low-temperature treatment (day/night temperatures. Optimal temperature treatment: 30°C/22°C; Mild cold treatment: 20°C/15°C; Severe cold treatment: 12°C/5°C (15°C /12°C for the first 11 days)). Morphological characteristics (plant height, growth stage, fresh weight and dry weight of foliage, fresh weight and dry weight of ears, grain count, and anthesis-silking interval) and physiological characteristics (photosynthesis rate, stomatal conductance, and quantum efficiency at photosystem II) were measured before the treatment, during the treatment, and at the time of harvest.

Under the optimal temperature conditions, statistically significant difference between the transgenic maize and non-transgenic

maize (control) was not observed for all measurements.

Under the mild cold treatment conditions, significant differences between the transgenic maize and non-transgenic maize (control) were observed in photosynthesis rate, stomatal conductance, and quantum efficiency at Photosystem II ($p \leq 0.05$) (Table 3 on p.13–14 of Annex 13). The mean values of the photosynthesis rate, stomatal conductance, and quantum efficiency at Photosystem II on the 28th day (when statistically significant differences were observed) were 26.20 mol $CO_2/m^2/s$, 0.14 mol $H_2O/m^2/s$, and 0.35, respectively, for the transgenic maize, and 31.70 mol $CO_2/m^2/s$, 0.19 mol $H_2O/m^2/s$, and 0.40, respectively, for the non-transgenic maize (control). All of these measurements were lower for the transgenic maize (the higher the value of photosynthesis rate, stomatal conductance or quantum efficiency at photosystem II, the higher the plant activity.) The physiological characteristics did not suggest low-temperature tolerance acquired by the transgenic maize (Table 3 on p.13–14 of Annex 13).

Under the severe low-temperature treatment, significant differences between the transgenic maize and non-transgenic maize (control) were observed in the plant height on the 21st, 28th and 35th days ($p \leq 0.05$) (Table 2 on p.11–12 of Annex 13). The mean values of plant heights for the 21st, 28th, and 35th days (when the statistically significant differences were observed) were 162.9 cm, 164.8 cm, and 167.6 cm, respectively, for the transgenic maize, and 158.3 cm, 160.1 cm, and 162.7 cm, respectively for the non-transgenic maize (control). For these days, the values were higher for the transgenic maize. On the 42nd day (the time of the harvest), no statistically significant difference was observed. On the 21st, 28th, and 35th days (statistically significant differences were observed for the plant height), statistically significant difference was not observed for physiological characteristics, and the transgenic maize tended to show lower values (Table 3 on p.13–14 of Annex 13). The physiological characteristics did not suggest the transgenic maize acquired low-temperature tolerance (Table 3 on p.13–14 of Annex 13).

These findings did not suggest that the transgenic maize has low-temperature tolerance under the test conditions.

iii. High-temperature assessment (Annex 14)

High-temperature tolerance was compared between the transgenic maize and non-transgenic maize (control) using the plants from ca. 10-leaf stage (10V) to milk stage (R3) under three levels of high-temperature treatment (day/night temperatures. Optimal temperature treatment: 30°C/22°C; Mild heat treatment: 35°C/35°C; Severe heat treatment: 40°C/40°C). Morphological characteristics (plant height, growth stage, fresh weight and dry weight of foliage, fresh weight and dry weight of ears, grain count, and anthesis-silking interval) and physiological characteristics (photosynthesis rate, stomatal conductance, and quantum efficiency at photosystem II) were measured before the treatment, during the treatment, and at the time of harvest.

Under the optimal temperature conditions, the results did not show statistically significant difference between the transgenic maize and the non-transgenic maize (control).

Under the mild heat treatment, a statistically significant difference between the transgenic maize and non-transgenic maize (control) was observed in the stomatal conductance on the 14th day ($p \leq 0.05$) (Table 3 on p.12–13 of Annex 14). The mean values of the stomatal conductance on the 14th day (where the statistically significant difference was observed) were 0.16 mol $H_2O/m^2/s$ for the transgenic maize and 0.21 mol $H_2O/m^2/s$ for the non-transgenic maize (control). The stomatal conductance was lower for the transgenic maize (the higher the value of stomatal conductance, the higher the activity). Thus, the physiological characteristics did not suggest that the transgenic maize acquired high-temperature tolerance.

Under the severe heat treatment, a statistically significant difference between the transgenic maize and non-transgenic maize

(control) was observed in the quantum efficiency at photosystem II on the 35th day ($p \leq 0.05$) (Table 3 on p.12–13 of Annex 14). The mean values for the quantum efficiency at photosystem II on the 35th day (when the statistically significant differences were observed) were 0.13 for the transgenic maize and 0.24 for the non-transgenic maize (control). The quantum efficiency at photosystem II was lower for the transgenic maize (the higher the value of the quantum efficiency at photosystem II, the higher the activity). Thus, the physiological characteristics did not suggest that the transgenic maize obtained high-temperature tolerance.

These findings did not suggest that the transgenic maize has high-temperature tolerance under the test conditions.

iv. Salt tolerance assessment (Annex 15)

Salt tolerance was compared between the transgenic maize and non-transgenic maize (control) using the plants from ca. 10-leaf stage (10V) to milk stage (R3) under three kinds of salt treatment (concentration in mole of the salt solution applied to the potted plants. Optimal salt condition: 0 M, Mild salt treatment: 0.2 M; Severe salt treatment: 0.4 M). Morphological characteristics (plant height, growth stage, fresh weight and dry weight of foliage, fresh weight and dry weight of ears, grain count, and anthesis-silking interval) and physiological characteristics (photosynthesis rate, stomatal conductance, and quantum efficiency at photosystem II) were measured before the treatment, during the treatment, and at the time of harvest.

Under the optimal salt condition, statistically significant differences between the transgenic maize and non-transgenic maize (control) were observed in the ear fresh weight and ear dry weight ($p \leq 0.05$) (Table 2 on p.10 of Annex 15). The mean values of the fresh weight and dry weight of ears were 596.4 g and 254.1 g, respectively, for the transgenic maize and 510.1 g and 218.5 g, respectively, for the non-transgenic maize (control). The fresh and dry weights of ears were heavier for the transgenic maize.

Under the mild salt treatment, a statistically significant difference between the transgenic maize and non-transgenic maize (control) was observed in the quantum efficiency at photosystem II on the 21st day ($p \leq 0.05$) (Table 3 on p.11 of Annex 15). The mean values of the quantum efficiency at photosystem II on the 21st day (when the significant difference was observed) were 0.25 for the transgenic maize and 0.32 for the non-transgenic maize (control). The quantum efficiency at photosystem II was lower for the transgenic maize (the higher the value of quantum efficiency at photosystem II, the higher the activity). Thus, the physiological characteristics did not suggest that the transgenic maize acquired salt tolerance.

Under the severe salt treatment, statistically significant differences between the transgenic maize and the non-transgenic maize (control) were not observed for all measured items. The physiological characteristics did not suggest that the transgenic maize acquired salt tolerance.

These findings did not suggest that the transgenic maize has salt tolerance under the test conditions.

The results of these assessments together conclude that the transgenic maize obtains drought tolerance through expression of the modified CSPB but does not have tolerance to low temperatures, high temperatures, and salt.

ii. Differences, if any, and their degrees in the physiological or ecological characteristics listed below between genetically modified crops and the taxonomical species to which the host organism belongs[21]

[21] All the rights pertinent to the information in this section and the following sections ((i)-(a) to (i)-(g), (ii)-(a) to (ii)-(b), (iii)-(a) to

In order to conduct biological diversity risk assessment of the transgenic maize in Japan, three experiments (typical cultivation conditions with irrigation, typical cultivation conditions without irrigation, and cultivation without agronomic management) were carried out.

(i) Experiment with typical cultivation with irrigation

The experiment with typical cultivation with irrigation was conducted in 2010 in an isolated field located at Kawachi Research Farm of Monsanto Japan Limited. The (LH59 R_5 × HCL301) F_1 generation of the transgenic maize was subjected to the experiment (Figure 10 on p.34). As control, LH59 × HCL301 (a non-transgenic maize that shares the same genetic background with the transgenic maize) was used. The low-temperature tolerance tests were conducted by Monsanto Company (USA) using (LH59 R_4 × HCL301) F_1 generation of the transgenic maize.

(a) Morphological and growing characteristics

To assess morphological and growing characteristics, the transgenic maize and non-transgenic maize (control) were evaluated regarding the morphological and growing items (14 items: the uniformity of germination (date and month), germination rate (%), time of tasseling (date and month), time of silking (date and month), start of flowering (date and month), time of flowering (date and month), culm length (cm), height of ear (cm), tiller number, plant shape or plant type, maturation time (date and month), weight of aboveground biomass at harvesting time (kg), grain shape, and grain color). The assessment was conducted following the Test Guidelines for the Plant Variety Protection by the Ministry of the Agriculture, Forestry and Fisheries of Japan, a criteria set for registration of seeds and seedlings.

For the items for which statistical treatments were applied (germination rate, culm length, height of ear, tiller number, and weight of aboveground biomass at harvesting time), statistically significant difference was not observed between the transgenic maize and non-transgenic maize (control) in any of the characteristics (Table 3 on p.12 Annex 16). For the items for which statistical treatments were not applied (uniformity of germination, time of tasseling, time of silking, start of flowering, time of flowering, plant shape or plant type, maturation time, grain shape, and grain color), statistically significant difference was not observed between the transgenic maize and non-transgenic maize (control) in any of the characteristics except for the time of flowering (Table 3 on p.12 Annex 16). The time of flowering was one day earlier for the transgenic maize (July 30 for the transgenic maize and July 31 for the non-transgenic maize (control)).

(b) Low temperatures or high temperatures at the early stage of growth

Low-temperature tolerance at the early stage of growth was evaluated by experiments at an environment-controlled chamber at Monsanto Company (USA) (Annex 17). Plants of the transgenic maize and non-transgenic maize (control) at the 3-leaf stage were grown under four different temperature conditions

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(iii)-(b)) and the responsibility for the contents rest upon Monsanto Japan Limited.

(optimal temperature: 30°C/22°C (day/night); mild low-temperature treatment: 20°C/15°C; moderate low-temperature treatment: 15°C/10°C; severe low-temperature treatment: 4°C/4°C) for 8 days. Plant height, growing stage, chlorophyll content, and plant vigor, were measured before the treatment as well as 4 days and 8 days after treatment. Fresh and dry weights were measured 8 days after treatment.

Under the optimal temperatures, statistically significant difference was observed between the transgenic maize and non-transgenic maize (control) in the growth stage on the 4th day after moving to the environment-controlled chamber and in the growth stage and dry weight on the 8th day after moving to the environment-controlled chamber ($p \leq 0.05$) (Table 2 on p.17 Annex 17). However, statistically significant difference was not observed between the transgenic maize and non-transgenic maize (control) on the lower-temperature treatments applied in this experiment (Table 2 on p.17 Annex 17).

On the 4th day after moving to the environment-controlled chamber (when the statistically significant difference was observed), the growth stage was 3.6-leaf stage for the transgenic maize and 3.3-leaf stage for non-transgenic maize (control). On the 8th day after moving to the environment-controlled chamber (when the statistically significant difference was observed), the growth stage was 4.5-leaf stage and 4.1-leaf stage, respectively, and the dry weight was 2.8 g and 2.4 g, respectively, for the transgenic maize and the non-transgenic maize (control).

(c) Wintering ability and summer survival of the matured plant

Maize is a summer type annual plant, and after ripening it usually dies out in winter, and it does not regrow and propagate vegetatively, or produce seeds. To experimentally verify this wintering ability, matured plants of the transgenic maize and non-transgenic maize (control) were left on an isolated field to keep growing and sample plants were inspected on November 8, 2010. Withering and death were observed both for the plants of transgenic maize and the non-transgenic maize (control) plants (Figure 5 p.14 of Annex 16).

(d) Fertility and size of the pollen

The transgenic maize and non-transgenic maize (control) both exhibited high fertility of the pollen, and no significant difference was observed between the two lines. No difference was observed also in the shape and size of pollen (Figure 6 on p.15 of Annex 16).

(e) Production, shedding habit, dormancy, and germination rate of the seed

To evaluate production of seed, characteristics regarding seed production (total number of effective ears, ear length (cm), ear diameter (cm), row number per ear, grain number per ear, 100-kernel weight (g)) were compared between the transgenic maize and the non-transgenic maize (control).

In all measurements above, there was no statistically significant difference between the transgenic maize and the non-transgenic maize (control) (Table 4 on p.17 of Annex 16).

Shedding habits of the transgenic maize and non-transgenic maize (control) were evaluated by visually determining whether the ears were covered by bracts, whether grains were missing after removing the bracts, and the degree of missing grains, if any.

For both the transgenic maize and non-transgenic maize (control), grains were covered with bracts at the time of harvesting, and no shedding was observed under the natural conditions. When bracts were removed, seeds did not easily shed from the ears for both lines, suggesting no difference between the shedding habit between the transgenic maize and non-transgenic maize (control) (Table 4 on p.17 of Annex 16).

To evaluate the seed dormancy and germination rate, seeds on the 15th day after harvest were statically cultivated at 25°C on a petri dish set in a humidity-controlled chamber. The numbers of germinated seeds were measured with time.

Both the transgenic maize and non-transgenic maize (control) showed high germination rates, and statistically significant difference was not observed in between the germination rates of these lines (Table 4 and 5 on p.17 of Annex 16). No dormancy was observed for both the transgenic maize and non-transgenic maize (control).

(f) Crossability

A crossability test was not performed, since there are no wild relatives that can be cross-grown in Japan.

(g) Productivity of harmful substances

To determine whether the transgenic maize produces substances that are harmful to the surrounding plants or microorganisms in soil, soil microflora tests, plow-in tests, and succeeding crop tests were conducted. Statistically significant difference was not observed in the numbers of soil microorganisms and germination rates and dry weight of radishes (Table 6–8 on p.19 of Annex 16).

(ii) Typical cultivation conditions without irrigation,

In order to evaluate the drought tolerance of the transgenic maize in the environment of Japan, plants of the transgenic maize and the non-transgenic maize (control) were grown under typical cultivation conditions without irrigation, and (a) morphological and growing characteristics and (b) productivity and shedding habits of seeds were measured. The experiments were conducted in an isolated field located at Kawachi Research Farm of Monsanto Japan Limited. The (LH59 R_5 × HCL301) F_1 generation of the transgenic maize was subjected to the experiment (Figure 10 on p.39). As control, LH59 × HCL301 (a non-transgenic maize that shares the same genetic background with the transgenic maize) was used. The total precipitation of July and August at the field was 237.5 mm for the mean between 1979 and 2000, while it was 65.0 mm in 2010 (Appendix 1 of Annex 16).

(a) Morphological and growing characteristics

To assess morphological and growing characteristics, the transgenic maize and non-transgenic maize (control) were evaluated regarding the morphological and growing items (14 items: the uniformity of germination (date and month), germination rate (%), time of tasseling (date and month), time of silking (date and month), start of flowering (date and month), time of flowering (date and month), culm length (cm), height of ear (cm), tiller number, plant shape or plant type, maturation time (date and month), weight of aboveground biomass at harvesting time (kg), grain shape, and grain color). The assessment was conducted following the Test Guidelines for the Plant Variety Protection by the Ministry of the Agriculture, Forestry and Fisheries of Japan, a criteria set for registration of seeds and seedlings.

Among the items for which statistical treatments were applied (germination rate, culm length, height of ear, tiller number, and weight of aboveground biomass at harvesting time), a statistically significant difference was observed between the transgenic maize and non-transgenic maize (control) in the height of ear (Table 9 on p.25 Annex 16). Among the items for which statistical treatments were not applied (uniformity of germination, time of tasseling, time of silking, start of flowering, time of flowering, plant shape or plant type, maturation time, grain shape, and grain color), a numeric difference was observed between the transgenic maize and non-transgenic maize (control) in the start of flowering (Table 9 on p.25 Annex 16). The mean heights of ear (the item for which a statistically significant difference was observed) for the transgenic maize and the non-transgenic maize (control) were 99.2 cm and 91.8 cm, respectively. The mean height of ear was larger for the transgenic maize. The start of flowering was one day earlier for the transgenic maize (July 30 for the transgenic maize and July 31 for the non-transgenic maize (control)).

(b) Production and shedding habit of the seed

To evaluate production of seed, characteristics regarding seed production (total number of effective ears, ear length (cm), ear diameter (cm), row number per ear, grain number per ear, and 100-kernel weight (g)) were compared between the transgenic maize and the non-transgenic maize (control).

In the results of the measurements, statistically significant differences between the transgenic maize and the non-transgenic maize (control) were observed for the total number of effective ears, ear length (cm), and grain number per ear (Table 10 on p.28 of Annex 16). The mean of the total number of effective ears was significantly larger for the transgenic maize (9.00 for the transgenic maize and 6.50 for the non-transgenic maize (control)). The mean ear length was significantly larger for the transgenic maize (16.46 cm for the transgenic maize and 15.11 cm for the non-transgenic maize (control)). The mean number of grains per ear was significantly larger for the transgenic maize (249.85 for the transgenic maize and 159.88 for the non-transgenic maize (control)).

Shedding habits of the transgenic maize and non-transgenic maize (control) were evaluated by visually determining whether the ears were covered by bracts, whether grains were missing after removing the bracts, and the degree of missing grains, if any.

For both the transgenic maize and non-transgenic maize (control), grains were covered with bracts at the time of harvesting, and no shedding was observed under the natural conditions. When bracts were removed, seeds did not easily shed from the ears for both lines, suggesting no difference in the shedding habit between the transgenic maize and non-transgenic maize (control) (Table 10 on p.28 of Annex 16).

(iii) Cultivation without agronomic management

Self sustainability of the transgenic maize was assessed by measuring (a) morphological and growing characteristics and (b) production and shedding habit of seeds of the transgenic maize and non-transgenic maize (control) that were grown under conditions where agronomic management (e.g., irrigation, fertilization, pest control, and weed control) were not applied. The experiments were conducted in an isolated field located at Kawachi Research Farm of Monsanto Japan Limited. The (LH59 R_5 × HCL301) F_1 generation of the transgenic maize was subjected to the experiment (Figure 10 on p.39). As control, LH59 × HCL301 (a non-transgenic line that shares the same genetic background with the transgenic maize) was used.

In response to the stresses imposed by abundance of weeds, nutrient deficiency due to the lack of fertilization, drought, and feeding damage by pest insect, 18 out of 33 the transgenic maize plants tested and 26 out of 33 non-transgenic maize (control) plants withered and died. The number of surviving plants was extremely low for the non-transgenic maize (control) (Table 13 on p.35 of Annex 16). While the total number of effective ears was 9 out of the 33 plants tested for the transgenic maize, the number of the non-transgenic maize (control) plants was a mere 2 (Table 13 on p.35 of Annex 16). Accordingly, the test measurements were not statistically processed.

(a) Morphological and growing characteristics

To assess morphological and growing characteristics, the transgenic maize and non-transgenic maize (control) were evaluated regarding the morphological and growing items (5 items: the uniformity of germination (date and month), germination rate (%), culm length (cm), maturation time (date and month), and weight of aboveground biomass at harvesting time (kg)). The assessment was conducted following the Test Guidelines for the Plant Variety Protection by the Ministry of the Agriculture, Forestry and Fisheries of Japan, a criteria set for registration of seeds and seedlings.

The measurements suggested no difference between the transgenic maize and the non-transgenic maize (control). (Table 14 on p.36 of Annex 16).

(b) Production and shedding habit of the seed

To evaluate production of seed, characteristics regarding seed production (total number of effective ears, ear length (cm), ear diameter (cm), row number per ear, and grain number per ear) were compared between the transgenic maize and the non-transgenic maize (control).

In the results of the measurements, numeric differences between the transgenic maize and the non-transgenic maize (control) were observed for the total number of effective ears and grain number per ear (Table 15 on p.38 of Annex 16). The mean of the total number of effective ears was larger for the transgenic maize (3.00 for the transgenic maize and 0.67 for the non-transgenic maize (control)). The mean number of grains per ear was significantly larger for the transgenic maize (38.46 for the transgenic maize and 19.13 for the non-transgenic maize (control)).

Shedding habits of the transgenic maize and non-transgenic maize (control) were evaluated by visually determining whether the ears were covered by bracts, whether grains were missing after removing the bracts, and the degree of missing grains, if any.

For both the transgenic maize and non-transgenic maize (control), grains were covered with bracts at the time of harvesting, and no shedding was observed under the natural conditions. When bracts were removed, seeds did not easily shed from the ears for both lines, suggesting no difference between the shedding habit between the transgenic maize and non-transgenic maize (control) (Table 15 on p.38 of Annex 16).

Self-sustainability of the transgenic maize was assessed at four fields in the U.S. (Illinois, Indiana, Louisiana, and Nebraska States). The transgenic maize and non-transgenic maize (control) were grown under conditions where agronomic management (e.g., irrigation, fertilization, pest control, and weed control) were not applied, and the number of seedlings, growing stage, plant vigor, height of plant, time of tasseling, time of silking, final number of plants left, number of ears, and grain count were measured. F_2 seeds harvested from F_1 seeds were used for the tests to respond to the phenomenon since self-seeding usually occurs after harvesting.

Statistically significant difference was not observed in the results obtained from all of the four fields. No ear or grain was obtained from the fields except for the one in Indiana (Table 5 on p.21–22 of Annex 18). To test germination of the F_3 seeds obtained from the Indiana field, none of the seeds both from the transgenic maize and non-transgenic (control) maize plants germinated. Thus, production of seed with germination capacity was not observed for both the transgenic maize and non-transgenic (control) maize (Table 10 on p.26 of Annex 18).

II. Results of the discussion by the experts on the biological diversity risk assessment

Name of the Living Modified Organism: Drought tolerant maize (Modified *cspB, Zea mays* subsp. *mays* (L.) Iltis) (MON87460, OECD UI: MON-8746Ø-4)

Content of the Type 1 Use of Living Modified Organism: Provision as food, provision as feed, cultivation, processing, storage, transportation, and disposal, and acts incidental to them

Applicant: Monsanto Japan Limited

(1) Results of the biological diversity risk assessment

The transgenic maize is developed by transferring the T-DNA region of the plasmid vector PV-ZMAP595 constructed based on pBR322 (a vector derived from *E. coli*), etc. by using the agrobacterium method.

It has been confirmed that 1 copy of T-DNA containing modified cspB gene that encode CSPB protein (modified low-temperature shock protein B) derived from *Bacillus subtilis*, and nptII genes that encode NPTII protein (neomycin phosphotransferase type II) derived from *E. coli* transposon Tn5. The presence of these genes and their stabile transferring for multiple generations have been verified by gene segregation patterns and southern blot analyses. The stability of the genes for multiple generations has been verified by ELISA analysis.

A. Competitiveness

Maize (*Zea mays* subsp. *mays* (L.) Iltis) has been long used in Japan, though there is no report that it has become self-seeding in a natural environment in Japan.

The modified cspB gene is known to confer tolerance to various kinds of environmental stresses. In 2010, a study was carried out in greenhouse and environment-controlled chambers to determine whether the transgenic maize shows tolerance to drought stress and other environmental stresses. Based on the results of this study, the transgenic maize was considered to possess tolerance to drought stress but not likely to have tolerance to low-temperature, high-temperature, and salt stresses.

In a different study conducted in 2010 in an isolated field in Japan to evaluate various characteristics regarding competitiveness, the transgenic maize and a non-transgenic maize (control) were cultivated under typical cultivation conditions with or without irrigation. In addition, plants were grown without agronomical management to verify the self-sustainability.

(1) Results of the experiment under typical cultivation conditions with irrigation

While the time of flowering was different for the transgenic maize and non-transgenic maize (control) (July 30 and July 31, respectively), the difference (1 day) was considered too small to make the transgenic maize more competitive.

On the 4th day after moving to the environment-controlled chamber, the growth stage was 3.6-leaf stage for the transgenic maize and 3.3-leaf stage for non-transgenic maize (control). On the 8th day after moving to the environment-controlled chamber, the growth stage was 4.5-leaf stage and 4.1-leaf stage, respectively, and the dry weight was 2.8 g and 2.4 g, respectively, for the transgenic maize and the non-transgenic maize (control). Although statistically significant, these differences were small and other measurements in low-temperature tolerance tests conducted with the early stage of plants at the same period of time did not show statistically significant differences, suggesting that the difference observed does not improve competitiveness.

ii. Results of the experiment under typical cultivation conditions without irrigation

There was a statistically significant difference in the mean heights of ear between the transgenic maize (99.2 cm) and the non-transgenic maize (91.8 cm). However, the observed difference was considered too small to improve the competitiveness of the transgenic line.

The start of flowering was one day earlier for the transgenic maize (July 30 for the transgenic maize and July 31 for the non-transgenic maize (control)). The difference was also considered too small to improve the competitiveness of the transgenic line.

In the measurements for seed production, statistically significant differences between the transgenic maize and the non-transgenic maize (control) were observed in the total number of effective ears (9.00 and 6.50, respectively), ear length (16.5 cm and 15.1 cm, respectively), and the mean number of grains per ear (250 for the transgenic maize and 160, respectively).

iii. Results of experiment with no agronomical treatment

In response to abundance of weeds, nutrient deficiency due to the lack of fertilization, drought stress, feeding damage by pest insect, and other types of causes, 18 out of 33 transgenic maize plants tested withered and died. The total number of effective ears was only 9. For the non-transgenic maize (control), 26 out of 33 plants withered and died. The total number of effective ears was only 2. Because of the low values, the test measurements were not statistically processed.

Among the measurements regarding seed production, there were differences between the transgenic maize and the non-transgenic maize (control) in the means of the total number of effective ears (3.00 and 0.67, respectively) and mean of the number of grains per ear (38.5 and 19.1, respectively).

In the results of ii. Results of the experiment under typical cultivation conditions without irrigation, the volume of seed production was higher for the transgenic maize than the non-transgenic maize (control). This was considered to be due to the low precipitation and high temperature of the test year compared with the normal year, especially the decreased rainfall in the period from the later vegetative stage to harvesting time, which imposed drought stress to the test plants and the trait inferred to the transgenic maize. The transgenic maize showed higher seed production than the non-transgenic maize as a result of the inferred trait also under the conditions lacking agronomical management (iii).

In comparison, the survival capacity of the transgenic maize, as well as the non-transgenic control, was considerably lower under the conditions without agronomical management (iii) than the conditions with agronomical management (i and ii). It was also verified that the transgenic maize, as well as the non-transgenic control, does not possess tolerance to non-drought stresses (i.e., low-temperature, high-temperature, and salt stresses). No difference was observed in the wintering capability, seed shedding habit, and dormancy between the transgenic maize and the non-transgenic maize (control).

These results together indicate that the transgenic maize has the same tolerance to the non-drought stresses with the conventional maize lines, and the self-sustainability of the transgenic maize in natural conditions of Japan is no higher than that of the conventional maize lines.

Therefore, it is not likely that the transgenic maize will survive for multiple generations or exterminate other species in the natural conditions of Japan. The competitiveness of the transgenic maize is determined to be no higher than the conventional maize lines.

For the reasons described above, the conclusion by the applicant (no wild organisms that might be adversely affected by the transgenic maize were identified, and therefore there is no risk of adverse effects on biological diversity) was determined feasible.

B. Productivity of harmful substances

Maize (*Zea mays* subsp. *mays* (L.) Iltis), the species the host organisms belong to, has been long used in Japan, and there is no report of production of harmful substances by this plant.

Both of the modified CSPB protein and NPTII protein produced by the transgenic maize have been verified to have no sequence structurally similar to known allergens.

The modified CSPB protein under drought and other kind of stress are suggested to disassociate the double strand formed on the RNA, and thus stabilize the RNA to help cells to maintain normal functions. Therefore, it is not likely to alter the metabolic system of the host or produce harmful substances in the metabolic system of the host. There is no report to identify NPTII protein as a harmful substance.

The modes of action differ between the modified CSPB and NPTII protein. Also, NPTII protein has high substance specificity, and the modified CSPB protein does not possess an aminoglycoside structure that allows it to act as a substrate to NPTII protein. Thus, these proteins are considered to act independently and are unlikely to interact in plants. This is also confirmed by the experiments conducted in 2006 and 2007 in three fields in Chile. In these experiments, the transgenic maize and a non-transgenic maize (control) were grown under typical moisture conditions and drought stress. Although structural analysis of the plants and harvested seeds indicated statistically significant differences in some measured items, they were within the range of the differences obtained for 12 conventional commercial lines that were tested at the same time. These findings suggest that the modified CSPB and NPTII proteins transferred into the transgenic maize do not produce new metabolites.

In an isolated field in Japan, soil microflora tests, plow-in tests, and succeeding crop tests were conducted to determine whether the transgenic maize produces harmful substances (substances that are secreted from the roots and adversely affect the surrounding plants or microorganisms in soil, as well as substances the plant maintains in its body and affect the surrounding plants after the death of the plant) in 2010. Statistically significant difference was not observed between the transgenic maize and the non-transgenic maize (control).

For the reasons described above, the conclusion by the applicant (no wild organisms that might be adversely affected by the transgenic maize were identified, and therefore there is no risk of adverse effects on biological diversity) was determined feasible.

C. Crossability

In the Japanese natural environment, maize has not been established as a wild plant, and there has been no report of wildly growing teosinte, a wild species that is genetically close and compatible with maize. Therefore, it was judged that there are no specific wild organisms that are possibly affected by the crossability of the transgenic maize in their biodiversity.

For the reasons described above, the conclusion by the applicant (there is no risk of adverse effects on biological diversity due to the crossability of the transgenic maize) was determined feasible.

(2) Conclusion based on the Biological Diversity Risk Assessment Report

Based on the above understanding, the Biological Diversity Risk Assessment Report concluded that there is no risk that the use of this recombinant maize in accordance with Type 1 Use Regulation causes Adverse Effect on Biological Diversity. It was judged that the conclusion made above by the applicant is reasonable.

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Annex: Drought tolerant maize (modified *cspB, Zea mays* subsp. *mays* (L.) Iltis)
(MON87460, OECD UI: MON-8746Ø-4)

Annex 1	Phenotypic Evaluation and Ecological Interactions of Drought Tolerant Corn MON87460 Under Well-Watered and Water-Limited Conditions in Chilean Field Trials During 2006-2007 (MSL0021857) (Confidential: Not made available or disclosed to unauthorized person)
Annex 2	Amended Report for MSL0021590: Cold Shock Protein B (CSPB) Interation with RNA and Expression in MON 87460 (MSL0021728) (Confidential: Not made available or disclosed to unauthorized person)
Annex 3	Physiological Responses of MON87460 Compared to the Control Under Water-Limited Conditions in the Greenhouse (MSL0021719) (Confidential: Not made available or disclosed to unauthorized person)
Annex 4	Amended Report for MSL0023062 Efficacy, Mode of Action, and Physiological Assessments of MON87460 Under Water-Limited Conditions in U.S. Field Trials in 2009 US 2009 (MSL0023227) (Confidential: Not made available or disclosed to unauthorized person)
Annex 5	Amino acid sequence of the modified CSPB estimated from the modified *cspB* gene used for production of the transgenic maize (Confidential: Not made available or disclosed to unauthorized person)
Annex 6	Amended Report for MSL0021180: Compositional Analyses of Forage and Grain Collected from Drought Tolerant Corn MON 87460 Grown in a 2006/2007 Chile Field Production (MSL0021754) (Confidential: Not made available or disclosed to unauthorized person)
Annex 7	Assessment of Insert Segregation for MON87460 (07-RA-B3-01) (Confidential: Not made available or disclosed to unauthorized person)
Annex 8	Amended Report for MSL0020487: Molecular Analysis of Corn MON87460 (MSL0022131) (Confidential: Not made available or disclosed to unauthorized person)
Annex 9	Assessment of the Presence of CSPB Protein in Grain Samples from Multiple Generations of MON 87460 by Enzyme-Linked Immunosorbent Assay (MSL0021623) (Confidential: Not made available or disclosed to unauthorized person)
Annex 10	Amended Report for MSL0021185: Assessment of the CSPB and NPTII Protein Levels in Tissues of Drought Tolerant Corn MON 87460 Produced in a 2006-2007 Chilean Field Trial under Well-Watered and Water-Limited Conditions (MSL0021731) (Confidential: Not made available or disclosed to unauthorized person)

Annex 11 Corn Mon87460 EndPoint TaqMan PCR for 150 seed pools (BQ-QC-10719-01) (Confidential: Not made available or disclosed to unauthorized person)

Annex 12 Assessment of the Effect of Drought Stress on the Drought Tolerant Corn MON87460 under Growth Chamber Conditions (Study # PLC-09-607) (Confidential: Not made available or disclosed to unauthorized person)

Annex 13 Assessment of the Effect of Cold Stress on the Drought Tolerant Corn MON87460 under Growth Chamber Conditions (Study # PLC-10-332) (Confidential: Not made available or disclosed to unauthorized person)

Annex 14 Assessment of the Effect of Heat Stress on the Drought Tolerant Corn MON87460 under Growth Chamber Conditions (Study # PLC-10-184) (Confidential: Not made available or disclosed to unauthorized person)

Annex 15 Assessment of the Effect of Salt Stress on the Drought Tolerant Corn MON87460 under Growth Chamber Conditions (Study # PLC-10-043) (Confidential: Not made available or disclosed to unauthorized person)

Annex 16 Report of the Results of Biological Diversity Assessment Tests in Isolated Fields for Drought Tolerant Maize (Modified *cspB, Zea mays* subsp. *mays* (L.) Iltis) (MON87460, OECD UI: MON-8746Ø-4) (Confidential: Not made available or disclosed to unauthorized person)

Annex 17 An Assessment of the Effect of Cold Stress on Drought Tolerant Corn MON87460 Under Growth Chamber Conditions in 2008 (MSL0021509) (Confidential: Not made available or disclosed to unauthorized person)

Annex 18 Assessment of Survival of Drought Tolerant Corn MON87460 in Unmanaged Environments During 2008 (MSL0021997) (Confidential: Not made available or disclosed to unauthorized person)