付　属　資　料

１．調査日程

２．主要面談者リスト

３．ミニッツ

４．PDM 仮和訳

５．評価グリッド

月日			ブラジル側評価ﾒﾝﾊﾞｰ	日本側評価メンバー				JIRCAS
			Dr. Barros & Dr. Esteves	永代・和田	道順	佐藤	国分	篠崎・藤田
1	9月18日	火			日本発			
2	9月19日	水			ロンドリーナ着、日本人専門家と打合せ			
3	9月20日	木			Embrapa ダイズ研究所研究者インタビュー			
4	9月21日	金			Embrapa ダイズ研究所研究者インタビュー			
5	9月22日	土			資料整理			
6	9月23日	日			資料整理			
7	9月24日	月			ラボラトリー及び試験圃場視察 Embrapaダイズ研究所研究者インタビュー			
8	9月25日	火			Embrapa ダイズ研究所研究者インタビュー ダイズ栽培農家の視察・インタビュー			
9	9月26日	水		日本発	補足情報収集			
10	9月27日	木		ロンドリーナ着	補足情報収集	ロンドリーナ着		
				日本人専門家と打合せ				
11	9月28日	金		Embrapa ダイズ研究所　所長表敬、研究者インタビュー				
12	9月29日	土		レビュー報告書案作成			ロンドリーナ着	
13	9月30日	日		レビュー報告書案作成				日本発
14	10月1日	月		レビュー報告書案作成				ロンドリーナ着
			合同評価委員会（C/P からの聞き取り、中間レビューレポートについて説明）					
15	10月2日	火	合同評価委員会（日伯研究者より進捗状況報告、施設視察、中間レビューレポートについての議論） (佐藤氏　ロンドリーナ発)					
16	10月3日	水	合同評価チームによる中間レビュー報告書の内容検討、圃場視察				ロンドリーナ発	
17	10月4日	木	合同評価チームによる中間レビュー報告書の内容検討、Embrapa ダイズ研究所　所長への評価概要説明、中間レビューレポート署名					
18	10月5日	金	中間レビュー結果の関係者への説明、ミニッツ署名					
19	10月6日	土		資料整理	ロンドリーナ発			ロンドリーナ発
20	10月7日	日		資料整理	---			---
21	10月8日	月		外務省報告 日本大使館報告 JICA 事務所報告	日本着			日本着
22	10月9日	火		Embrapa 本部訪問 ブラジリア発				
23	10月10日	水		---				
24	10月11日	木		日本着				

２．主要面談者リスト

（Embrapa）
Vania Beatriz R.Castiglioni　副総裁
Antonio Nilson Roche 法務部部長
Arnoldo M. da Fonseca Jr. 知的財産所有部部長
José A.B. do Amaral 国際調整部研究員
Luciano L.Nass 国際調整部研究員

（Embrapa Soybean）
Alexandre José Cattelan 所長
Dr. Nepomuceno プロジェクトコーディネーター（アメリカ在住（Skype にて面談））
Dra. Francismar Marcelino プロジェクトコーディネーター代理
Dr. Norman Neumaier プロジェクトコーディネーター代理
Ms Silvana Marin 研究員

（ブラジル外務省科学技術課）
Ademar Seabra da Cruz Jr. 課長
Jaçanã Ribeiro 課員

（在ブラジル日本国大使館）
片平　聡　　公使参事官
福代　孝良　科学技術担当官
犬飼　武　　二等書記官
森田健太郎　二等書記官

（JICA ブラジル事務所）
室澤　智史　　所長
佐藤　一朗　　次長
竹田　パトリシア　静香　プロジェクトコーディネーター
井上　ジュリオ　　プロジェクトコーディネーター

（プロジェクト専門家）
工藤　博　　業務調整）
金森　紀仁　長期専門家

MINUTES OF MEETING
BETWEEN
JAPAN INTERNATIONAL COOPERATION AGENCY
AND
AUTHORITIES CONCERNED OF THE FEDERATIVE REPUBLIC OF BRAZIL
ON THE MID-TERM REVIEW ON
JAPANESE TECHNICAL COOPERATION (SATREPS) FOR
DEVELOPMENT OF GENETIC ENGINEERING TECHNOLOGY OF CROPS WITH STRESS TOLERANCE AGAINST DEGRADATION OF GLOBAL ENVIRONMENT

The Japanese Mid-term Review Team, organized by the Japan International Cooperation Agency (hereinafter referred to as "JICA") and headed by Dr. Narihide Nagayo, reviewed the progress of Development of Genetic Engineering Technology of Crops with Stress Tolerance against Degradation of Global Environment (hereinafter referred to as "the Project") from 19 September to 9 October, 2012 together with the Brazilian Evaluation Team in the form of joint review.

The Joint Evaluation Team (hereinafter referred to as "the Team"), which consists of five (5) members from Japanese side and two (2) members from the Brazilian side, was organized for the purpose of conducting the evaluation of the progress and for preparation of necessary recommendations to the respective governments.

After intensive study and analysis of the activities and achievements of the Project, the Team prepared the Mid-term Review Report (hereinafter referred to as "the Report"), and presented it to the persons concerned with the Project.

The persons concerned with the Project discussed the major issues pointed out in the Report, and agreed on the matters referred to in the document attached hereto.

Londrina, 5 October, 2012

Dr. Narihide Nagayo
Team Leader
Mid-term Review Team
Japan International Cooperation Agency
Japan

Dr. Alexandre José Cattelan
Head General
Brazilian Agricultural Research Corporation
(Embrapa) Soybean
Federative Republic of Brazil

Attached Document

I. Presentation of the Report

The Team presented the Report to the meeting that the persons concerned with the Project (Embrapa and JIRCAS, etc.) have participated in (for details, see Appendix 1), and they confirmed the current progress and evaluation of the Project. The Report is attached as Appendix 2.

II. Understanding of the Recommendations from the Team

After the discussion of the Report, both the Japanese and Brazilian sides understood the recommendations in the Report. The recommendations suggested by the Team are as follows;

1. Recommended Actions to be taken by the Project in the Remaining Cooperation Period

(1) Amelioration of the technology to improve transformation efficiency

The improvement of transformation efficiency by Agrobacterium method is important to achieve the Project targets. Considering the short period remaining in the Project, the continuing efforts to its improvement are needed to optimize the protocol by seeking information from external groups with recognized expertise in this area. The continuing efforts to the improvement of transformation efficiency by biolistics method are also wished.

(2) Appropriate allocation of personnel

The personnel involved in the Project are mostly properly allocated. However, if the transformation efficiency is to be improved in the future, the allocation of full-time personnel dedicated to the method will be mandatory.

(3) Communication enhancement

A communication enhancement is desirable through periodical meetings and frequent e-mail exchange and so on, in order to share, among the project researchers, the information on the progress as well as on its problems of the Project.

2. Recommended Actions to be taken by the Japanese Side

(1) Expansion of the personnel exchange

The activities of this Project include molecular biology, physiology and breeding. Therefore, the Team recommends that both sides discuss the possibilities of the Japanese side to accept trainees, as well as to dispatch the Japanese experts to Embrapa Soybean, concerning physiology and breeding, according to the progress of the Project.

(2) Dispatch of Japanese experts to Embrapa Soybean

Concerning the dispatch of the Japanese experts, the Team recommends that both sides

(5) Sustainability

Sustainability refers to the extent to which the Project can be further developed by the authorities concerned of Brazil and the extent to which the benefits generated by the Project can be sustained under national policies, technology, systems and financial state.

2. Outline of the Project

2-1 Background of the Project

Gradual warming of the earth, first caused by the increasing amount of greenhouse gases with rapid population growth and industrialization has subsequently been raising global problems such as desertification of cropland, reduction of crop yield and security of food and feedstuff. Although conventional crop breeders challenged themselves to produce crop plants tolerant to drought, results so far are not outstanding. On the other hand, recent technical progress in genetic engineering based on plant genome research has been attracting attention since crops having improved tolerance to drought by gene transfer have been developed. Under such situations research for elucidation of genes has involved for drought tolerance in crops so the utilization of the outcome of genomic research and development of genetic engineering technology utilizing these genes has become important. Technology such as genetic engineering has evolved into very significant successes in soybean, maize and cotton with herbicide and/or insect-resistance, which has led to their dominance in global trade. Development of drought-tolerant soybean plants and maize is now considered as the most important target of such technology because these plants are grown on such a large scale in areas of relatively low rainfall. Japan is a significant importer of these crops and needs to ensure a stable food supply from the world. Based on such conditions briefly mentioned here, the government of Brazil submitted a proposal on "Development of Soybean with Tolerance to Drought and Heat" to Japan.

This proposal intends to develop the genetic engineering technology of soybean that is adapted to tolerate drought and heat with the aim to stabilize soybean production in Brazil, in cooperation between Japan International Research Center for Agricultural Sciences (hereinafter referred to as "JIRCAS") and Embrapa Soybean. The research group at first took steps to isolate useful genes related to environmental stress tolerance and stress-inducible promoters in soybean plants on the basis of research of genes related to environmental stress tolerance and rapidly evolving soybean genome research carried out elsewhere. It selects candidate combinations of such genes and promoter, and then introduces them to the soybean plant. It further evaluates environmental stress tolerance of transgenic soybean plants in greenhouses and under field conditions, with continued result feedback to improve the combination of useful genes and promoters, with the eventual aim to select elite transgenic lines with improved tolerance to environmental stresses.

2-2 Summary of the Project

The framework of the project was determined on basis of the R/D signed on 28 December 2009. PDM for the Project was modified at a Joint Coordinating Committee (JCC) meeting held on March 15, 2012. The project summary described in PDM version 2 is as follows; (For more details, see Annex 2).

(1) Overall Goal

Soybeans adapted to environmental stresses are developed, which contributes to the stabilization of the soybean production in Brazil.

communicate adequately in advance in order to enhance the exchange of the technology and information.

3. Recommended Actions to be taken by the Brazilian Side

(1) Speed-up of MTA procedures
The time required for MTA procedures by Embrapa has caused delays in sending the samples exchanged between the research centers and negative impact on the achievements of the Project. The appropriate steps to speed up the procedures should be immediately taken. Embrapa should consider the possibility of establishing a comprehensive agreement that would cover all material exchanges.

(2) Employment of personnel in accordance with the M/M
The allocation of full-time personnel dedicated to the Project is mandatory to its fruitful implementation. The Team strongly recommends that Embrapa Soybean immediately consider assigning full-time personnel dedicated to the Project, in accordance with the commitments in the M/M dated August 31st, 2009.

(3) Budget provisions for the installation of the Cooling System in the Greenhouse
The efficient implementation of cultivation tests is critical to achieve goals of the Project. The installation of the Cooling System in the Greenhouse is currently secured for 2 out of 4 rooms. Considering the short period remaining in the Project, the Team recommends that Embrapa Soybean immediately provide the budget to install the other 2 rooms.

Appendix 1: Persons participated in the meeting

1. Brazilian side

1-1. Embrapa HQ:

(1) Dr. Arnoldo Medeiros da Fonseca Junior, Advisor for Technology Innovation

1-2. Embrapa Soybean:

(1) Dr. Alexandre José Cattelan, General Head
(2) Dr. José Renato Bouças Farias, Research and Development Head
(3) Dr. Francismar Correa Marcelino-Corrêa, Acting Brazilian leader of the Project
(4) Ms. Silvana Regina Rockenbach Marin, Chief of Laboratory
(5) Dr. Josirley Carvalho, Post-Doctoral Fellow

1-3. Mid-term review team member

(1) Dr. Luiz Gonzaga Esteves Vieira, Researcher, Agronomic Institute of Paraná (IAPAR)

2. Japanese side

1-1. Japanese researchers and expert

(1) Prof. Dr. Kazuko Shinozaki, Japanese leader of the Project, JIRCAS/ the University of Tokyo
(2) Dr. Hiroshi Kudo, Project Coordinator of the Project
(3) Dr. Yasunari Fujita, Senior Researcher, JIRCAS
(4) Dr. Norihito Kanamori, Senior Researcher, JIRCAS

1-2. Mid-term review team member

(1) Dr. Narihide Nagayo, Team leader, Senior Advisor, JICA
(2) Mr. Tsuyoshi Wada, Advisor, Field Crop based Farming Area Division 1, Rural Development Department, JICA
(3) Mr. Isao Dojun, Consultant, Chuo Kaihatsu Corporation
(4) Ms. Yoshiko Oyama, Interpreter (Japanese-Portuguese-English)

Appendix 2

THE JOINT MID-TERM REVIEW REPORT ON JAPANESE TECHNICAL COOPERATION (SATREPS) FOR DEVELOPMENT OF GENETIC ENGINEERING TECHNOLOGY OF CROPS WITH STRESS TOLERANCE AGAINST DEGRADATION OF GLOBAL ENVIRONMENT

Londrina, 4 October, 2012

Dr. Narihide Nagayo
Leader
Japanese Mid-term Review Team
Japan International Cooperation Agency

Dr. Luiz Gonzaga Esteves Vieira
Leader
Brazilian Mid-term Review Team
Agronomic Institute of Paraná

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Abbreviations and Acronyms

Embrapa	Brazilian Agricultural Research Corporation
FAO	Food and Agriculture Organization, United Nations
JCC	Joint Coordinating Committee
JICA	Japan International Cooperation Agency
JIRCAS	Japan International Research Center for Agricultural Sciences
JST	Japan Science and Technology Agency
MTA	Material Transfer Agreement
PDM	Project Design Matrix
PO	Plan of Operations
R/D	Record of Discussions
SATREPS	Science and Technology Research Partnership for Sustainable Development

1. Introduction

1-1 Objectives of the Mid-term Review

(1) To review the inputs with the Development of Genetic Engineering Technology of Crops with Stress Tolerance against Degradation of Global Environment (herein after referred to as "the Project"); to review the progress and achievements of project activities based on the Project Design Matrix (PDM) and the Plan of Operations (PO);, and to exchange opinions with the Brazilian authorities concerned through the visitation of the project sites,

(2) To review the Project from the viewpoints of five evaluation criteria (Relevance, Effectiveness, Efficiency, Impact and Sustainability),

(3) To formulate the Joint Mid-term Review Report and make necessary recommendations on project activities in the remaining period of the Project to both the Brazilian and Japanese sides, and to modify PDM and PO if necessary, and

(4) To explain and discuss the results of the mid-term review on the Project with the Brazilian authorities concerned and sign related Minutes of Meeting.

1-2 Member of the Joint Review Team

The Project was reviewed by the Japanese and Brazilian Joint Mid-term Review Team (hereinafter referred to as "the Team"). The Team was composed of five (5) members from the Japanese side and two (2) members from the Brazilian side.

1-2-1 Japanese Mid-term Review Team

No.	Task	Name	Organization
1	Leader	Dr. Narihide NAGAYO	Senior Advisor, Japan International Cooperation Agency (JICA)
2	SATREPS Planning and Evaluation	Dr. Makie KOKUBUN	Professor, Graduate School of Agricultural Science, Tohoku University/ Program Officer for SATREPS, JST
3	SATREPS Planning and Evaluation	Mr. Masayuki SATO	Principal Researcher, Research Partnership for Sustainable Development (SATREPS) Division, Japan Science and Technology Agency (JST)
4	Cooperation Planning	Mr. Tsuyoshi WADA	Advisor, Field Crop based Farming Area Division 1, Rural Development Department, JICA
5	Evaluation and Analysis	Mr. Isao DOJUN	Consultant, Chuo Kaihatsu Corporation

1-2-2 Brazilian Mid-term Review Team

No.	Task	Name	Organization
1	Leader	Dr. Luiz Gonzaga Esteves Vieira	Researcher, Agronomic Institute of Paraná (IAPAR)
2	Member	Dr. Everaldo G. Barros	Professor, Federal University of Viçosa

1-3 Process and schedule of Mid-term Review

The schedule is attached as Annex 1 and the mid-term review was conducted along the following process.

1-3-1 Initial Examination in Japan

The Team reviewed available documents related to the Project, interviewed some Japanese researchers involved in the Project, and prepared an evaluation grid which listed the specific review points and the data collection methods.

1-3-2 Review activities in the Federal Republic of Brazil

The Japanese Team visited Brazil for the following objectives.

- To identify to what extent the activities, the outputs and the project purpose described in the PDM (Project Design Matrix) have been implemented and/or achieved.
- To review the process and results of technology development.
- To observe the current conditions of equipment and facilities provided by the Project.
- To discuss and set the verifiable indicators of the PDM, if necessary.
- To make recommendations on the project activities for the remaining project period.

The Team visited the Brazilian Agricultural Research Corporation- National Soybean Research Center (hereinafter referred to as "Embrapa Soybean") and carried out a series of interviews and discussions with Brazilian researchers and Japanese researchers, etc.

The Team also visited laboratories and experimental fields and generally checked the equipment procured for the Project.

1-4 Methodology of the Mid-term Review

1-4-1 Method of Review

The Project was reviewed jointly by the Team based on materials showing the framework of the Project such as PDM, PO and the Record of Discussion (R/D). The review activities including analysis on reports, field surveys, and interviews with the researchers of Embrapa Soybean, JICA experts, etc. have been carried out.

This mid-term review was conducted using the following Five Evaluation Criteria.

1-4-2 Evaluation Criteria (Five Evaluation Criteria)

(1) Relevance

Relevance refers to the validity of the Project Purpose and the Overall Goal in connection with the development policy of the authorities concerned of Brazil as well as the needs of the beneficiaries and the assistance policy of Japan.

(2) Effectiveness

Effectiveness refers to the extent to which the expected benefits of the Project have been achieved as planned. It also examines whether these benefits have been brought about as a result of the Project.

(3) Efficiency

Efficiency refers to the productivity of the implementation process. It examines whether the inputs of the Project have been efficiently converted into outputs.

(4) Impact

Impact refers to direct and indirect, positive and negative impacts caused by the implementation of the Project, including the extent to which the overall goal has been attained.

(2) Project Purpose

Genetic engineering technology of soybean with environmental stress tolerance is developed.

(3) Outputs

Output 1: Useful genes related to environmental stress tolerance are identified.
Output 2: Stress-responsive promoters are isolated and combinations with useful genes are optimized.
Output 3: Transgenic soybean lines containing constructs of promoters and useful genes are produced.
Output 4: Transgenic soybean lines with environmental stress tolerance are selected.

(4) Responsible organizations

Japanese side: JIRCAS with RIKEN and the University of Tokyo
Brazilian side: Embrapa Soybean

(5) Project Period

From March 4, 2010 to March 3, 2015 (5 years)

3. Achievement of the Project

3-1 Inputs

3-1-1 Japanese Side

(1) Japanese researchers involved in project activities

The number of researchers that participated in the research activities of the Project in Japan is 27 in total or at JIRCAS 7 persons, RIKEN 5 persons and the University of Tokyo 15 persons including students engaged under the master course program). For further details, refer to the Annex 3.

(2) Dispatch of Japanese experts/ researchers

A long-term expert (project coordinator) has been dispatched and 9 researchers (short-term, in the fields of Molecular Breeding Techniques and Plant Molecular Biology) have been dispatched to Brazil. For further details, refer to the Annex 4.

(3) Brazilian researchers trained in Japan

Six (6) Brazilian researchers have participated in a training program in Japan. Fields of training were "Expression Analysis of Soybean Stress-Responsive Genes", "Isolation of Promoters related to Soybean Stress-Responsive Genes", and "Agrobacterium-mediated transformation and Expression Analysis". For further details, refer to the Annex 5.

(4) Provision of Equipment

1) Equipment provided by the Japanese side for Embrapa Soybean

Vehicles, office equipment such as computers and printers, various kinds of equipment for research activities, have been provided by JICA. The amount of expenses for purchasing of the equipment is 605 thousand Reals and 38.9 million Yen (for reference, equivalent to around 798 thousand US dollars in total). For further details, refer to the Annex 6.

2) Equipment provided by Japanese side for the research institutes in Japan

Various kinds of equipment for research activities have been provided by the Japanese side for JIRCAS, RIKEN and the University of Tokyo. The amount of expenses for purchasing of this equipment is 17.9

million Yen (for reference, equivalent to around 230 thousand US dollars). For further details, refer to the Annex 7.

(5) Local Cost Allocated by Japanese Side

Local cost (in Brazil) allocated by the Japanese side for the implementation of project activities is 727 thousand Reals (for reference 358 thousand US dollars) as of June 2012. For further details, refer to the Annex 8.

3-1-2 Brazilian Side

(1) Brazilian Researchers involved in the project activities

Twenty nine (29) researchers in total have been involved in project activities. The majority of are the researchers of Embrapa. Others engaged in the Project were university students and technicians employed through the arrangement by JICA. Currently 20 researchers of Embrapa, 5 university students and others are carrying out project activities. For further details, refer to the Annex 9.

(2) Equipment provided by Brazilian side

Various kinds of equipment for research activities have been provided by the Brazilian side and installed at the new biotechnology building, the biotechnology building and the chemical analysis office located in the field of Embrapa. The amount of expenses for purchasing of equipment is 2.7 million Reals (for reference, equivalent to 1.3 million US dollars). For further details, refer to the Annex 10.

(3) Provision of Facilities

Embrapa is facilitating the following facilities for the project activities.

1) Office space for Japanese experts/ researchers
2) Biotechnology building (existing): 424m^2
3) New biotechnology building (newly constructed): 581m^2
4) Office for biological physiology: 120m^2
5) Physics and chemical analysis office: 100m^2
6) Greenhouse of Embrapa: 1,181m^2
7) Land for greenhouse that was constructed with JICA's expenses: 314m^2
8) Storage of GMO seeds: 160m^2
9) Greenhouse for GMO: 237m^2
10) Experimental field of Embrapa: 28,300m^2

(4) Project Operation Cost Allocated by Brazilian Side

Running cost (electricity and water, etc.) for the office spaces and the research activities have been shouldered by the Brazilian side.

3-2 Outputs

3-2-1 Output 1: Useful genes related to environmental stress tolerance are identified.

All of the following 3 indicators of Output 1 have achieved its target already. Therefore, it can be said that Output 1 is achieved.

Indicator 1-1: At least five (5) genes involved in regulation of stress tolerance are identified in plants such as soybean.

Seven (7) genes involved in regulation of stress tolerance have been identified by JIRCAS. The names of identified genes are AtDREB1A, AtDREB2A, AtAREB1, GmAREB1, GmAREB2, GmAREB3, and GmAREB4. The numerical target has been achieved.

Indicator 1-2: At least two (2) genes for membrane proteins involved in stress perception are identified in plants such as soybean.

Two (2) genes (GmHK1A;1 and GmHK1B;1) for membrane involved in stress perception have been identified by the University of Tokyo. The numerical target has been achieved.

Indicator 1-3: At least three (3) genes involved in regulation of stress response are identified in plants such as soybean.

Two (2) genes (GmNCED3A and GmNCED3B) involved in regulation of stress response have been identified by RIKEN and one (1) gene (GmDREB2A;2) has been identified by the University of Tokyo (in total three (3) genes). The numerical target has been achieved.

3-2-2 Output 2: Stress-responsive promoters are isolated and combinations with useful genes are optimized.

All of the following 3 indicators of Output 2 have achieved their targets. Therefore, it can be said that Output 2 is achieved.

Indicator 2-1: At least 100 stress-responsive genes are identified in soybean.

More than 100 stress-response genes have been identified by JIRCAS (4,433 drought-inducible genes have been identified as result of microarray analysis). The numerical target has been achieved.

Indicator 2-2: At least three (3) stress-responsive promoters are identified in soybean.

Six (6) stress-response promoters (GMRD1, XERO1, UN1, UN2, UN3, and EFCM) have been identified by JIRCAS. The numerical target has been achieved.

Indicator 2-3: At least five (5) constructs of useful genes and promoters are optimized.

Six (6) constructs of useful genes and promoters have been optimized in total. JIRCAS identified 5 constructs (35S:AREB1, 35S:AREB1ΔQT, 35S:GmAREB4, RD29A:DREB1A, RD29A:DREB2Aca) and the University of Tokyo identified 1 construct (RD29Apro:GmDREB2A;2). The numerical target has been achieved.

3-2-3 Output 3: Transgenic soybean lines containing constructs of promoters and useful genes are produced.

Transformation efficiency (indicator 3-1) is not achieved it target yet. Both the indicator 3-2 and 3-3 have been achieved its target.

Indicator 3-1: Genetic engineering technology with more than 1.5 % of transformation efficiency is established in soybean.

Two kinds of genetic engineering techniques have been utilized under the Project such as the biolistics method and Agrobacterium method. Transformation efficiency (at T0 generation) using the biolistics method has reached the range of 0.51 to 1.03%, considering survived plants and events that kept the inserted gene in following generations. According to Embrapa Soybean, the efficiency on introducing a construct depends primarily on the construct itself, as genes containing deletion for example are unstable and even if T0 events are obtained, the transgene is not transmitted to the next generation. Also the cultivar used and steps on tissue culture can affect the final results. Some more improvement is necessary to reach the target value (1.5%).

As for the Agrobacterium method, transformation efficiency (at T0 generation) has reached only 0.20%. The transformation efficiency is still very low and improvement of the efficiency is required. A Japanese researcher dispatched to Embrapa Soybean has been tried to optimize Agrobacterium tumefaciens transformation protocol. A researcher of Embrapa Soybean has also started to optimize Agrobacterium transformation protocol.

Significant improvement of the transformation efficiency on Agrobacterium method should be made during the remaining project period.

Indicator 3-2: At least five (5) constructs of useful genes and promoters are introduced in soybean.

Seven (7) constructs introduced using the biolistics method and 3 constructs introduced using Agrobacterium method have generated event in T0 generation in soybean plants (variety BR16) as shown in the table below. The numerical target has been achieved.

Table T0 biolistics and Agrobacterium events generated:

	Method	Construct	Number of events generated
1-1	biolistics	rd29A:AtDREB1A	11 events
1-2		rd29A:AtDREB2A	03 events
1-3		35S:AtDREB1A	28 events
1-4		35S:AtDREB2A	05 events
1-5		35S:AREB1 FL	08 events
1-6		35S:AREB1 DQT	12 events
1-7		35S:AREBM8	05 events
2-1	Agrobacterium	35S:AREB WT	15 events
2-2		35S:NCED	01 event
2-3		35S:Gols	02 events

Indicator 3-3: T1 seeds of at least three (3) lines are collected.

Six (6) constructs introduced by biolistics method and one (1) construct introduced by Agrobacterium method have generated events in T1 generation as shown in the table below. Number of line is 36 in total and the numerical target has been achieved.

Table: Events generated in T1 generation

	Method	Construct	Number of line	Name of line
1-1	biolistics	rd29A:AtDREB1A	9 lines	P58/ P1142 /P59/ P3069/ P1378 / P45/ P345/ P382/ P3075
1-2		rd29A:AtDREB2A	2 lines	P2193 and P1397 were confirmed in T1
1-3		35S:AtDREB1A	11 lines	11 identified in T1 Cb3208/ Cb3432/ Cb3489/ Cb3501/ Cb3850/ Cb4004/ Cb4128/ Cb4137 Cb4266/ Cb4351
1-4		35S:AtDREB2A	2 lines	02 identified in T1 Db2486/ Db2508
1-5		35S:AREB1 FL	6 lines	06 identified in T1 Eb24/ Eb2889/ Eb2057/ Eb2856/ Eb2992/ Eb2904
1-6		35S:AREB1 DQT	3 lines	03 identified in T1 Fb2639/ Fb2651/ Fb2654
1-7		(35S:AREBM8)	0 line	none in T1
2-1	Agrobacterium	35S:AREB WT	3 lines	03 identified in T1 Ea2939/ Ea15/ Ea2493
2-2		(35S:NCED)	0 line	none in T1
2-3		(35S:GOLS)	0 line	none in T1

3-2-4 Output 4: Transgenic soybean lines with environmental stress tolerance are selected.

There are 6 indicators for Output 4 and 2 indicators have been achieved (the indicator 4-4 and 4-5). As for other indicators, good outcomes have been producing under the Project and it is expected that all indicators will be achieved by the end of the project period if project activities progress smoothly.

Indicator 4-1: At least two (2) drought-inducible genes are identified and at least two (2) transgenic lines are selected for each construct based on gene analysis.

Number of identified drought-inducible genes is 4,433 at present. Selection of transgenic lines for each construct based on gene analysis will be carried out hereafter.

Indicator 4-2: At least two (2) heat-inducible genes are identified and at least two (2) transgenic lines are selected for each construct are selected based on gene analysis.

Number of heat-inducible genes identified is 3,317 at present. Selection of transgenic lines based on gene analysis is carrying out.

Indicator 4-3: Gene expression of at least two (2) independent lines derived from at least two (2) constructs is analyzed.

Analysis on gene expression of independent lines derived from constructs will be conducted hereafter. Delay of material transfer agreement (MTA) may affect this analysis.

Indicator 4-4: Evaluation methods of stress tolerance of soybean in greenhouse and field are established.

Experiments have been conducted in greenhouse and in field conditions to characterize physiologically and agronomically the GM lines using parameters such as Relative Water Content, Photosynthesis, Transpiration, Yield components, etc. among others explained in the Materials and Methods of the publications produced by the project up to now. For example, one of the methodologies utilized to evaluate stress tolerance is plants that survive in the water deficit of 5 days and have higher photosynthetic rates and Relative Rate of Height Growth (RRHG) during water deficit period and no leaf damage after rewatering. For further details, refer to the Annex 11. Embrapa Soybean has an established screening method on stress tolerance.

Evaluation methods of stress tolerance of soybean in greenhouse and field at Embrapa Soybean are established at a satisfactorily level.

Indicator 4-5: Stress tolerance of at least two (2) independent lines derived from at least two (2) constructs is evaluated in greenhouse.

Nine (9) lines containing 4 constructs have been evaluated for molecular and physiological responses at green-house conditions. Name of lines and constructs are as follows. The numerical target has been achieved.

	Line	construct
1	P58	rd29:DREB1
2	P1242	
3	2193	rd29:DREB2
4	A24.10	35S:AtAREB1 FL
5	A2889.12	
6	A2057.03	
7	A2639	35S:AREB1 DQT
8	A2651	
9	A2654	

Indicator 4-6: Stress tolerance of at least two (2) independent lines derived from at least two (2) constructs is evaluated in field.

Two (2) lines (P58 containing rd29:AtDREB1A construct and P2193 containing rd29:AtDREB2A construction) have been evaluated in field (Embrapa Soybean) during three consecutives soybean crop seasons. Only at the third year, there was actual drought condition in the field. In the two previous years, rain intensity exceeded annual averages for the region. In the future, Embrapa Soybean intends to test the GM events in more regions to increase the chances of testing at real drought field conditions. A crossing between the DREB1AP58 and the BR16 has been evaluated in field in the 2011/2012 cropping season.

Although DREB plants did not outperform the cultivar BR16 (the drought sensitive genotype used for transformation) in terms of yield, they showed a tendency of superiority for some yield components such as number of seeds, number of pods with seeds and total number of pods when stress was applied at the vegetative stage. For the rd29:DREB1 construct, it seems that the introduced constructs increased the plant's capacity to stand drought events by improving its water use efficiency during non-drought situations.

However, more lines need to be tested for more time and regions.

Greenhouse which has 4 separated rooms was constructed newly in Embrapa Soybean under the Project. Cooling systems for 2 rooms will be installed (soil humidity and temperature etc. can be controlled) and this greenhouse will be utilized from this cropping season for evaluation as one of field experiments.

3-3 Project Purpose

Project Purpose: Genetic engineering technology of soybean with environmental stress tolerance is developed.

The numerical target of the indicator 1, 2 and 3 has been achieved. As for the indicator 4, it was confirmed that a line has character of drought tolerance response. It is expected that several lines with environmental stress tolerance is selected by the end of the Project if project activities progress smoothly as scheduled.

As mentioned before, transformation efficiency of Agrobacterium method is still low. When the Agrobacterium method is established well with certain higher transformation efficiency, it can be said that genetic engineering technology of soybean with environmental tolerance is developed at very satisfactory level.

Indicator 1: At least 10 useful genes related to environmental stress tolerance are identified in plants such as soybean.

Twelve (12) useful genes related to environmental stress tolerance have been identified by the researchers of JIRCAS, University of Tokyo and RIKEN. The numerical target has been achieved. The following table presents the name of the genes and the number of identified genes by each institution.

Institution	Number of useful genes identified	Name of genes
JIRCAS	7	AtDREB1A, AtDREB2A, AtAREB1, GmAREB1, GmAREB2, GmAREB3, GmAREB4
University of Tokyo	3	GmDREB2A;2, GmHK1A;1, GmHK1B;1
RIKEN	2	GmNCED3A, GmNCED3B

Indicator 2: At least five (5) stress-responsive promoters are identified and combinations with useful genes are optimized.

Seven (7) stress-responsive promoters have been identified and 3 combinations with useful genes have been optimized. The numerical target has been achieved.

(a) Identified promoters

1 promoter (RD29A) from Thale Cress (Arabidopsis thaliana)

6 promoters (GMRD1, XERO1, UN1, UN2, UN3, EFCM) from the soybean plant (Norin No. 2)

(b) Optimized combinations
2 constructs (RD29A:DREB1A, RD29A:DREB2Aca) by JIRCAS
1 construct (RD29A:GmDREB2A;2) by the University of Tokyo

Indicator 3: At least five (5) constructs of useful genes and promoters are introduced in soybean and at least three transgenic lines are produced for each construct.

As mentioned before, 7 constructs of useful genes and promoters have been introduced using biolistics method and 3 constructs have been introduced using Agrobacterium method, and positive events were observed in T0 generation. Positive events in T1 generation were observed in 6 constructs (total 33 lines) with biolistics method and 1 construct (3 lines) with Agrobacterium method. Number of line of 2 kinds of construct are 2 and number of transgenic line of other 5 constructs are not less than 3. The numerical target has been achieved.

Indicator 4: At least one (1) stress-tolerant line with environmental stress tolerance is selected.

Considering events generated and transmitted to T1 generation, experiments in greenhouse and field, and also crossing with other genotypes using P58 (rd29:DREB1A) and P2193 (rd29:DREB2) lines, that were generated in the laboratory of Embrapa Soybean at the beginning stage of the Project, were focused. It was confirmed that P58 line has character of drought tolerance response.
More lines are being explored in terms of experiments to confirm drought tolerance responses and breeding with other genotypes.

4. Results of Review

4-1 Relevance

The relevance of the Project is high.

(1) Need to respond to global climate change

It is said that global warming is in progress due to an increase of greenhouse gas emissions which is related to the rapid population increase and global industrialization. Global warming has become a problem on a global scale. In many parts of the world, disasters caused by extreme weather events such as drought, torrential rains, typhoons, and hurricanes are increasingly occurring. Due to global warming, damages by drought are also increasing being reported in many parts of the world. Desertification of crop cultivated lands has been progressing, which is producing decreased yields, and this becomes a significant problem for the production of food and animal feed.

The FAO has estimated in a recent report (World agriculture: towards 2015/2030, An FAO Perspective) that world agriculture in the years between 2015-2030 will significantly decline due to climate changes, particularly temperature rise, change of distribution of annual rainfall, reduced precipitation, and soil moisture decline. The report stressed the necessity of development of foodstuff varieties with a drought, high temperature and salt-tolerances as countermeasures.

In recent years, crop genome research has rapidly progressed and it is expected that molecular breeding

technology can be used effectively for developing drought tolerant varieties. Therefore, there is a need to develop genetic engineering technology that identifies genes related to drought tolerance utilizing results of crop genome researches.

(2) Need for the development of drought and heat tolerance soybean varieties in Brazil

Commercial cultivation of soybeans started in the southern part of Brazil in the 1960s. Brazil is now the second largest soybean producer, the United States being the largest, and thereby has quite an important position in the supply of soybeans worldwide. Because of the increase of soybean cultivation area in Brazil, cultivation has been expanding into the mid-western part of Brazil, an erratic precipitation region of the country, and frequent drought and shortage of water resources have negatively affected soybean production in recent years.

In this situation, breeding of drought tolerant soybean plant varieties becomes one of the important research themes and research on drought tolerant soybeans using transgenic technology are therefore progressing in Brazil.

As mentioned above, the need of development of a drought and heat tolerant soybean plant variety is quite important not only in Brazil but also as one of measures to deal with global climate change. Therefore this project is relevant to the needs of the target area and global society as a whole.

(3) Needs of the target group

Embrapa Soybean started research on a transgenic soybean plant in 1996. Embrapa Soybean and JIRCAS have jointly carried out research on the "Agro-Pastoral System" and "Soybeans in South America" having a joint research agreement in place since 1995. Joint search activities have been carried out to develop a heat tolerant soybean plant using transgenic technology since 2003. Although, soybean gene transfer technology has been developed in Brazil as mentioned, there is the necessity to have genes for drought tolerance as well. Specifically, there remains a strong desire to succeed in the development of drought tolerant soybean plant varieties, which are genetically modified and can be cultivated in the farmers' field.

In this way, Embrapa Soybean has made research achievements on the soybean plant transgenic and the need for the development of a drought tolerant soybean plant. Therefore, this project is consistent with the needs of the target group (Embrapa Soybean).

(4) Relevance to the national development plan of Brazil

For Brazil, the soybean plant is one of the very important crops in terms of gross domestic product, exports, employment and is regarded also by the Federal Government of Brazil as one of important production sectors. Under the "Agricultural and Livestock Plan 2012/2013" one of the roles of Embrapa is to contribute to the reduction of the effects of global warming on agricultural production. In other words, Embrapa Soybean will contribute to Embrapa's agricultural research on the provision of crops useful for food security and renewable energy. Therefore, this project is aiming at the development of drought and heat tolerant soybean plant varieties and is consistent with the development policy of the Federative Republic of Brazil.

(5) Conformity to assistance policy of Japan to Brazil

One of the priority areas of Japanese cooperation with Brazil is agriculture, and cooperation for reducing effects of climate change is also regarded as important because agriculture is an important sector in Brazil. This project has the objective to adapt to global climate change. in particular, adapt to climate change

through developing drought and heat tolerant soybean plant varieties. Therefore, this project is consistent with the Japanese cooperation policy.

4-2 Effectiveness

It is expected that the effectiveness of the Project becomes high at the end of the project period.

As mentioned before, it is expected that several lines with environmental stress tolerance is selected by the end of the Project if project activities progress as scheduled. A lot of useful works have been done to identify genes which will increase the chance to achieve the project objectives.

One of the important issues is improvement of transformation efficiency of Agrobacterium method. When the Agrobacterium method is established well with higher transformation efficiency, it can be said that genetic engineering technology of soybean with environmental tolerance would be achieved at very satisfactory level and effectiveness of the Project is high.

4-3 Efficiency

The efficiency of the Project is at a satisfactory level so far, despite longer time necessary to obtain agreement on Material Transfer Agreement (MTA), low transformation efficiency using Agrobacterium method, and delay of construction of the new biotechnology building of Embrapa Soybean, etc.

4-3-1 Inputs by Japanese Side

Relatively large number and highly qualified Japanese researchers have involved in the research activities of the Project in Japan (JIRCAS, RIKEN and the University of Tokyo) and they are producing outputs more than the targets.

Nine (9) Japanese researchers have been dispatched to Brazil in the fields of molecular breeding techniques and plant molecular biology. Most of their stays at Embrapa Soybean were less than 10 days. There are opinions that it would be better for technical cooperation if Japanese researchers can stay longer time.

As for training program in Japan for Brazilian researchers, 6 persons in the field of biotechnology have participated in. It seems that the trainings in Japan have good effect for improving researchers' capability further and smooth progress of project activities. The related technical fields with project activities are not only biotechnology (molecular biology), therefore, it may better to send Brazilian researchers of other fields (plant physiology and plant breeding, etc.) in accordance with needs.

It seems that machinery, equipment and facilities that were provided by Japanese side for Embrapa Soybean have been utilized effectively for project activities and supporting smooth progress of activities.

4-3-2 Inputs by Brazilian Side

Brazilian side also relatively large number and highly qualified researchers/ university students have involved in the research activities of the Project at Embrapa Soybean. In the first half of the project period, project activities in the field of molecular biology have been focused and its importance is continued. For the remaining project period, project activities in the fields of physiology and breeding etc. become more important to have stress tolerant lines of soybean. It is importance to increase degree of involvement of

researchers/ technicians etc. of Embrapa Soybean in these fields.

Based on the agreement[1] (the Minutes of Meeting signed by the Japanese and Brazilian sides on August 31, 2009), the Japanese side employed three (3) technicians and contract period of them was ended in August 2012. According to the agreement, expenses on contract with post-doctoral researchers and technicians for remaining project period will be covered by the Brazilian side. However, Embrapa Soybean has difficulty to employ these personnel and it is affecting progress of project activities especially in the activities related with Agrobacterium method.

With procurement of various kinds of equipment and facilities, construction of a new biotechnology building, and utilization of existing facilities of Embrapa Soybean have been brought good effect for smooth progress of project activities. Although, there was delay of construction of the new biotechnology building, it is reported that there was no significant negative effect on project activities. A new greenhouse, which has 4 separated rooms, was constructed with the expenses of Japanese side. A cooling system for 2 rooms of the greenhouse is going to be installed within several months with the expenses of Japanese side. However, financial arrangement is not done for procuring cooling system for the remaining 2 rooms with expenses by Brazilian side.

4-3-3 Technical development

Efforts for establishment or optimization of Agrobacterium method have been made from the year 2008 mainly with initiative of Japanese side. Agrobacterium method is one of the effective methods for gene transformation, because only 1 or 2 copies can be transformed and it is easy to forward subsequent process. However, its transformation efficiency is still low and improvement of efficiency is required for obtaining stress tolerant lines further.

4-3-4 Material Transfer Agreement (MTA)

In order to ship constructs from Japan to Embrapa Soybean and to ship soybean seeds from Brazil to Japan, MTA is necessary between Embrapa and institution in Japan every time. Usually a lot of time on examination of document on MTA is required in Embrapa and affecting smooth progress of project activities.

4-4 Impact

4-4-1 Prospect for Achieving the Overall Goal

Overall Goal: Soybeans adapted to environmental stresses are developed, which contributes to the stabilization of the soybean production in Brazil.

The Overall Goal of the Project might not achieved by 2019 and it may take some more years. When commercial line is developed, its impact will be very significant not only in Brazil but also in the world.

Indicator: Soybeans adapted to environmental stresses are developed before 2019.

[1] Expenses of contract with two (2) post-doctoral researchers (or one post-doctoral and one post-master researcher) and two (2) technicians (one is student of the master course, and the other of the doctoral course) to improve the implementation structure of the Project is covered by JICA until two (2) years and half after the project begins. The cost for remaining period of the Project, however, will be covered by Brazilian side, considering that self-sustaining development of the Project in Brazil is required.

According to Embrapa Soybean, usually it takes around 12 years for developing a new commercial GM crops from laboratory level to the field. And for development of stress tolerant varieties such as drought tolerant, it might take more time.

One of the project objectives is to select at least one (1) stress-tolerant line by the end of the project period with environmental stress tolerance is selected. After the selection of line, biosecurity test and soil management experiment are required. Around 5 years are necessary for these examinations respectively. These tests can be conducted in parallel.

This project ends on March 2015 and there are 4 or 5 years for the year 2019. Therefore, it might difficult to develop soybean adapted to environmental stresses as commercial line.

4-4-2 Other Impacts

As mentioned above, it will take many years to obtain commercial line. When commercial line is developed, its impact will be very significant not only in Brazil but also in the world.

Institutions in Brazil recognize that DREB gene is very important gene for plant in order to have drought tolerance and there are many requests for provision. Japanese side (JIRCAS) has provided DREB genes to other research centers of Embrapa for crops such as cotton, sugarcane, coffee and common bean etc.

4-5 Sustainability

Sustainability of the Project will be secured in terms of policy, organizational, financial, and technical aspects.

(1) Policy Aspect

As mentioned in the item of the relevance, for Brazil, the soybean plant is one of the very important crops in terms of gross domestic product, exports, employment and is regarded also by the Federal Government of Brazil as one of important production sectors. One of the important roles of Embrapa Soybean is to contribute to the reduction of the effects of global warming on agricultural production. Therefore, sustainability policy of the Project will be secured.

(2) Organizational Aspect

Embrapa Soybean has necessary research sections and organizational setup for developing genetically modified soybeans such as biotechnology, physiology and breeding etc. with highly qualified researchers, technicians and university students (having scholarship from the Government of Brazil). Embrapa Soybean has successful experiences in developing genetically modified soybeans (herbicide tolerant) jointly with private sector. Therefore, organizational sustainability for continuing development of stress tolerant soybean is secured.

(3) Financial Aspect

As mentioned in the article of the inputs, Embrapa invested significant amount of budget for procuring machinery, equipment and construction of facilities such as the new biotechnology building. Therefore, financial sustainability for continuing development of stress tolerant soybean is secured.

(4) Technical Aspect

As mentioned before, Embrapa Soybean has successful experiences in developing genetically modified soybeans (herbicide tolerant). In addition, Embrapa Soybean has conducted researches on stress tolerant soybean jointly with JIRCAS from 2003. Therefore, Embrapa Soybean has quite high expertise in this field. One of the technical aspects necessary for improvement is the Agrobacterium transformation method and technicians of Embrapa Soybean started to optimize the protocol. When protocol is optimized transformation efficiency can be sustained at a desirable rate. It seems that technical sustainability for continuing development of stress tolerant soybean efficiently and effectively can be secured.

4-6 Conclusions

The progress of project activities is mostly as planned by producing results more than planned in most cases. There are some risks that may affect smooth implementation of project activities in the remaining two and half years. Recommendations for reducing such risks and obtaining better outcomes are described in the recommendation section.

5. General comments

The research groups are to be complimented for the establishment of the Japan-Brazil partnership for using the DREB/AREB technology in soybean. The Team notes that this is the only cooperative project between Japanese and other research institutions in the world for development of soybean plants tolerant to drought and heat. Hence, it is a unique arrangement with unique opportunities to produce GM soybean lines tolerant to abiotic stresses for using in breeding programs elsewhere and to generate new commercial cultivars. However, the Team would like to stress that with unique opportunity also comes unique responsibility. Hence, the collective group of investigators should set their goals high, applying the highest standards of scientific rigor while seeking unique and innovative solutions to the challenges that presently limit the yield in soybean crop under drought and heat stresses.

A very positive aspect of the project is the focus on manpower development. It is clear that the specific groups vary in their level of experience and expertise. However, collectively, working together there is the opportunity to significantly raise the level of scientific excellence. The training of young scientists as part of the Japan-Brazil cooperation is clearly a very worthy goal and one that the group appears to be committed to.

Overall the Team was pleased by the work on the search of genes and regulatory sequences involved in plant response to drought and heat stresses by the Japanese side. In particular the research on these topics is considered to be cutting-edge science and likely to deliver high level international quality research. The Team was also pleased with results and huge amount of work done by the Brazilian side on the transformation experiments and field tests.

The achievements are remarkable when taken in the context of the challenges that hinder some aspects of the collaboration. These include difficulties of importation of equipment, delays in ordering and delivery of consumables, issues related to adding key personnel and the red tape involved in exchange of genetic material.

As the Project moves forward, there will be real opportunities to expand the integration and cooperation

among the individual groups, which will serve to raise the level of scientific excellence and better assure the success of the individual objectives.

The Team compliments Japanese and Brazilian researchers and the project coordinators for their effective leadership for achieving most of the planned goals established in the Project. The positive interactions between the groups, developing collaborations and improved integration of efforts reflect this leadership and were viewed very positively by the Team.

6. Recommendations

6-1 Recommended Actions to be taken by the Project in the Remaining Cooperation Period

(1) Amelioration of the technology to improve transformation efficiency

The improvement of transformation efficiency by Agrobacterium method is important to achieve the Project targets. Considering the short period remaining in the Project, the continuing efforts to its improvement are needed to optimize the protocol by seeking information from external groups with recognized expertise in this area. The continuing efforts to the improvement of transformation efficiency by biolistics method are also wished.

(2) Appropriate allocation of personnel

The personnel involved in the Project are mostly properly allocated. However, if the transformation efficiency is to be improved in the future, the allocation of full-time personnel dedicated to the method will be mandatory.

(3) Communication enhancement

A communication enhancement is desirable through periodical meetings and frequent e-mail exchange and so on, in order to share, among the project researchers, the information on the progress as well as on its problems of the Project.

6-2 Recommended Actions to be taken by the Japanese side

(1) Expansion of the personnel exchange

The activities of this Project include molecular biology, physiology and breeding. Therefore, the Team recommends that both sides discuss the possibilities of the Japanese side to accept trainees, as well as to dispatch the Japanese experts to Embrapa Soybean, concerning physiology and breeding, according to the progress of the Project.

(2) Dispatch of Japanese experts to Embrapa Soybean

Concerning the dispatch of the Japanese experts, the Team recommends that both sides communicate adequately in advance in order to enhance the exchange of the technology and information.

6-3 Recommended Actions to be taken by the Brazilian side

(1) Speed-up of MTA procedures

The time required for MTA procedures by Embrapa has caused delays in sending the samples exchanged between the research centers and negative impact on the achievements of the Project. The appropriate steps

to speed up the procedures should be immediately taken. Embrapa should consider the possibility of establishing a comprehensive agreement that would cover all material exchanges.

(2) Employment of personnel in accordance with the M/M

The allocation of full-time personnel dedicated to the Project is mandatory to its fruitful implementation. The Team strongly recommends that Embrapa Soybean immediately consider to assign full-time personnel dedicated to the Project, in accordance with the commitments in the M/M dated August 31st, 2009.

(3) Budget provisions for the installation of the Cooling System in the Greenhouse

The efficient implementation of cultivation tests is critical to achieve goals of the Project. The installation of the Cooling System in the Greenhouse is currently secured for 2 out of 4 rooms. Considering the short period remaining in the Project, the Team recommends that Embrapa Soybean immediately provide the budget to install the other 2 rooms.

Annex 1 Schedule of the Mid-term Review

Date			Brazilian Evaluation Members	Japanese Evaluation Members				JIRCAS
			Dr. Barros Dr. Esteves	Dr. Nagayo and Mr. Wada	Mr. Dojun	Mr. Sato	Dr. Kokubun	Dr. Shinozaki and Dr. Fujita
1	18-Sep	Tue			Leave Japan			
2	19- Sep	Wed			Arrival at Londrina Meeting with Japanese Expert			
3	20- Sep	Thu			Interview with researchers of Embrapa Soybean			
4	21- Sep	Fri			Interview with researchers of Embrapa Soybean			
5	22- Sep	Sat			Documentation			
6	23- Sep	Sun			Documentation			
7	24- Sep	Mon			Observation of laboratories and experimental field of Embrapa Interview to researchers of Embrapa Soybean			
8	25- Sep	Tue			Interview to researchers of Embrapa Soybean Visit and interview to soybean producing farmer			
9	26- Sep	Wed		Leave Japan	Collection of additional information			
10	27- Sep	Thu		Arrival at Londrina	Collection of additional information	Arrival at Londrina		
				Meeting with Japanese Expert				
11	28- Sep	Fri		Courtesy call to Head General of Embrapa Soybean, Interview with researchers of Embrapa Soybean				
12	29- Sep	Sat		Documentation			Arrival at Londrina	
13	30- Sep	Sun		Documentation				Leave Japan
14	1-Oct	Mon		Documentation				Arrival at Londrina
			Presentation of the progress of project activities by the researchers of Japanese and Brazilian sides					
15	2- Oct	Tue	Interview with researchers of Embrapa Soybean			(Mr. Sato leave Londrina)		
16	3- Oct	Wed	Discussion on Mid-term review report and M/M by Joint Review Team				Leave Londrina	
17	4- Oct	Thu	Discussion on Mid-term review report and M/M by Joint Review Team					
18	5- Oct	Fri	Presentation of results of Mid-term Review, Sign on M/M (Minutes of Meeting)					
19	6- Oct	Sat		Documentation	Leave Londrina			Leave Londrina
20	7- Oct	Sun		Documentation	(via Atlanta)			---
21	8-Oct	Mon		Report to the Ministry of External Relations Report to the Embassy of Japan and JICA Brazil office	Arrival Japan			Arrival Japan
22	9- Oct	Tue		Visit to Embrapa Headquarters Leave Brazilia				
23	10- Oct	Wed		(via Atlanta)				
24	11-Oct	Thu		Arrive Japan				

Annex 2 Project Design Matrix (version 1 and 2)

(1) PDM Version 1

Site of the Project: Londrina City, the State of Parana, Brazil
Direct beneficiary: Embrapa Soybean
Period of collaboration: From Mar 2010 to Mar 2015

Brief of project	Objectively Verifiable Indicator	Means of Verification	Important Assumption
[Overall Goal] Soybeans adapted to environmental stresses are developed, which contributes to the stabilization of the soybean production in Brazil.	Soybeans adapted to environmental stresses are developed before 2019.	—	—
[Project purpose] Genetic engineering technology of soybean with environmental stress tolerance is developed.	1. At least 10 useful genes related to environmental stress tolerance are identified in plants such as soybean. 2. At least five (5) stress-responsive promoters are identified and combinations with useful genes are optimized. 3. At least five (5) constructs of useful genes and promoters are introduced in soybean and at least three transgenic lines are produced for each construct. 4. At least one (1) stress-tolerant line with environmental stress tolerance is selected.	—	—
[Outputs] 1. Useful genes related to environmental stress tolerance are identified.	1-1 At least five (5) genes involved in regulation of stress tolerance are identified in plants such as soybean. 1-2 At least two (2) genes for membrane proteins involved in stress perception are identified in plants such as soybean. 1-3 At least three (3) genes involved in regulation of stress response are identified in plants such as soybean.	---	---
2. Stress-responsive promoters are isolated and combinations with useful genes are optimized.	2-1 At least 100 stress-responsive genes are identified in soybean. 2-2 At least three (3) stress-responsive promoters are identified in soybean. 2-3 At least five (5) constructs of useful genes and promoters are optimized.	---	
3. Transgenic soybean lines containing constructs of promoters and useful genes are produced.	3-1 Genetic engineering technology with more than 2 % of transformation efficiency is established in soybean. 3-2 At least five (5) constructs of useful genes and promoters are introduced in soybean. 3-3 T1 seeds of at least three (3) lines are collected.	---	
4. Transgenic soybean lines with environmental stress tolerance are selected.	4-1 At least two (2) drought-inducible genes are identified and at least two (2) transgenic lines are selected for each construct based on gene analysis. 4-2 At least two (2) heat-inducible genes are identified and at least two (2) transgenic lines are selected for each construct are selected based on gene analysis. 4-3 Gene expression of at least two (2) independent lines derived from at least two (2) constructs is analyzed. 4-4 Evaluation methods of stress tolerance of soybean in greenhouse and field are established. 4-5 Stress tolerance of at least two (2) independent lines derived from at least two (2) constructs is evaluated in greenhouse. 4-6 Stress tolerance of at least two (2) independent lines derived from at least two (2) constructs is evaluated in field.	---	

[Activities]	[Inputs]		
	<Japanese side>	<Brazilian side>	—
1-1 Genes involved in regulation of stress tolerance are identified in plants such as soybean. 1-2 Genes involved in stress perception are identified in plants such as soybean. 1-3 Genes involved in regulation of stress response are identified in plants such as soybean. 2-1 Stress-responsive genes are searched in soybean. 2-2 Stress-responsive promoters are identified in soybean. 2-3 Constructs of useful genes and promoters are optimized. 3-1 Genetic engineering technology is established in soybean. 3-2 Constructs of useful genes and promoters are introduced in soybean. 3-3 T1 seeds of transgenic lines are collected. 4-1 Drought-inducible genes are identified and transgenic lines are selected based on gene analysis. 4-2 Heat-inducible genes are identified and transgenic lines are selected based on gene analysis. 4-3 Gene expression of transgenic plants is analyzed. 4-4 Evaluation methods of stress tolerance of soybean are established. 4-5 Stress tolerance of transgenic soybean lines is evaluated in greenhouse. 4-6 Stress tolerance of transgenic soybean lines is evaluated in field.	・the Project Coordinator ・Short-term researchers ・Invitation of Brazilian researchers to Japan ・the Equipment ・Partial contribution for expenses of contract with two post-doctoral researchers (or one post-doctoral and one post-master researcher) and two technicians. ・Cost for project and others	・Arrangement of researchers and technicians ・Partial contribution for expenses of contract with two post-doctoral researchers (or one post-doctoral and one post-master researcher) and two technicians. ・Offer of facilities and project office ・the Equipment and running cost ・Securement of cost for project	[Pre-Conditions] —

(2) PDM Version 2

Site of the Project: Londrina City, the State of Parana, Brazil
Direct beneficiary: Embrapa Soybean
Period of collaboration: From Mar 2010 to Mar 2015 Version 20120215

Brief of project	Objectively Verifiable Indicator	Means of Verification	Important Assumption
[Overall Goal] Soybeans adapted to environmental stresses are developed, which contributes to the stabilization of the soybean production in Brazil.	Soybeans adapted to environmental stresses are developed before 2019.	---	---
[Project purpose] Genetic engineering technology of soybean with environmental stress tolerance is developed.	1. At least 10 useful genes related to environmental stress tolerance are identified in plants such as soybean. 2. At least five (5) stress-responsive promoters are identified and combinations with useful genes are optimized. 3. At least five (5) constructs of useful genes and promoters are introduced in soybean and at least three transgenic lines are produced for each construct. 4. At least one (1) stress-tolerant line with environmental stress tolerance is selected.	---	---
[Outputs] 1. Useful genes related to environmental stress tolerance are identified.	1-1 At least five (5) genes involved in regulation of stress tolerance are identified in plants such as soybean. 1-2 At least two (2) genes for membrane proteins involved in stress perception are identified in plants such as soybean. 1-3 At least three (3) genes involved in regulation of stress response are identified in plants such as soybean.	---	---
2. Stress-responsive promoters are isolated and combinations with useful genes are optimized.	2-1 At least 100 stress-responsive genes are identified in soybean. 2-2 At least three (3) stress-responsive promoters are identified in soybean. 2-3 At least five (5) constructs of useful genes and promoters are optimized.	---	
3. Transgenic soybean lines containing constructs of promoters and useful genes are produced.	3-1 Genetic engineering technology with more than 1.5 % of transformation efficiency is established in soybean. 3-2 At least five (5) constructs of useful genes and promoters are introduced in soybean. 3-3 T1 seeds of at least three (3) lines are collected.	---	
4. Transgenic soybean lines with environmental stress tolerance are selected.	4-1 At least two (2) drought-inducible genes are identified and at least two (2) transgenic lines are selected for each construct based on gene analysis. 4-2 At least two (2) heat-inducible genes are identified and at least two (2) transgenic lines are selected for each construct are selected based on gene analysis. 4-3 Gene expression of at least two (2) independent lines derived from at least two (2) constructs is analyzed. 4-4 Evaluation methods of stress tolerance of soybean in greenhouse and field are established. 4-5 Stress tolerance of at least two (2) independent lines derived from at least two (2) constructs is evaluated in greenhouse. 4-6 Stress tolerance of at least two (2) independent lines derived from at least two (2) constructs is evaluated in field.	---	

[Activities]	[Inputs]		
1-1 Genes involved in regulation of stress tolerance are identified in plants such as soybean. 1-2 Genes involved in stress perception are identified in plants such as soybean. 1-3 Genes involved in regulation of stress response are identified in plants such as soybean. 2-1 Stress-responsive genes are searched in soybean. 2-2 Stress-responsive promoters are identified in soybean. 2-3 Constructs of useful genes and promoters are optimized. 3-1 Genetic engineering technology is established in soybean. 3-2 Constructs of useful genes and promoters are introduced in soybean. 3-3 T1 seeds of transgenic lines are collected. 4-1 Drought-inducible genes are identified and transgenic lines are selected based on gene analysis. 4-2 Heat-inducible genes are identified and transgenic lines are selected based on gene analysis. 4-3 Gene expression of transgenic plants is analyzed. 4-4 Evaluation methods of stress tolerance of soybean are established. 4-5 Stress tolerance of transgenic soybean lines is evaluated in greenhouse. 4-6 Stress tolerance of transgenic soybean lines is evaluated in field.	**<Japanese side>** ・the Project Coordinator ・Short-term researchers ・Invitation of Brazilian researchers to Japan ・the Equipment ・Partial contribution for expenses of contract with two post-doctoral researchers (or one post-doctoral and one post-master researcher) and two technicians. ・Cost for project and others	**<Brazilian side>** ・Arrangement of researchers and technicians ・Partial contribution for expenses of contract with two post-doctoral researchers (or one post-doctoral and one post-master researcher) and two technicians. ・Offer of facilities and project office ・the Equipment and running cost ・Securement of cost for project	— **[Pre-Conditions]** ---

Annex 3 List of Japanese researchers involved in the project activities

(1) Group 1 (Biological Resources and Post-harvest Division, JIRCAS)

No.	Name	Position	Organization	Period of participation into research activities	
				From	To
1	Kazuko Yamagichi-Shinozaki	Chief Researcher, Biological Resources and Post-harvest Division	JIRCAS	Mar. 2010	At present
2	Kazuo Nakajima	Senior Researcher	JIRCAS	Mar. 2010	At present
3	Yasunari Fujita	Senior Researcher	JIRCAS	Mar. 2010	At present
4	Kyonoshin Maruyama	Researcher	JIRCAS	Mar. 2010	At present
5	Daisuke Todaka	Special Researcher	JIRCAS	Apr. 2010	Mar. 2011
6	Norihito Kanamori	Senior Researcher	JIRCAS	Apr. 2010	At present
7	Kensuke Kodaira	Special Researcher	JIRCAS	Nov. 2011	Mar. 2011

(2) Group 2 (Plant Science Center, RIKEN)

No.	Name	Position	Organization	Period of participation into research activities	
				From	To
1	Kazuo Shinozaki	Director, Plant Science Center (PSC)	RIKEN	Mar. 2010	At present
2	Yasushi Umezawa	Research Scientist, PSC	RIKEN	Mar. 2010	Jun. 2011
3	Hironori Takasaki	Special Research Scientist, PSC	RIKEN	Apr. 2011	At present
4	Tetsuya Sakurai	Unit Leader, Integrated Genome Informatics Research Unit, PSC	RIKEN	Mar. 2010	At present
5	Kaoru Urano	Research Scientist, PSC	RIKEN	Jul. 2010	At present

(2) Group 3 (Graduate School of Agricultural and Life Sciences, the University of Tokyo)

No.	Name	Position	Organization	Period of participation into research activities	
				From	To
1	Kazuko Yamagichi-Shinozaki	Professor, Laboratory of Plant Modecular Physiology	University of Tokyo	Mar. 2010	At present
2	Yuriko Osakabe	Lecturer	University of Tokyo	Mar. 2010	Jul. 2011
3	Daisuke Todaka	Project Research Fellow	University of Tokyo	Mar. 2010	Mar. 2010
4	Junya Mizoi	Project Assistant Professor	University of Tokyo	Apr. 2010	At present
5	Satoshi Kidokoro	Assistant Professor	University of Tokyo	Apr. 2010	At present
6	Takuya Yoshida	Project Assistant Professor	University of Tokyo	Jul. 2011	At present
7	Naohiko Ohama	M2	University of Tokyo	Oct. 2010	Mar. 2012
8	Teppei Ohori	M2	University of Tokyo	Oct. 2010	Mar. 2012
9	Teru Sato	M2	University of Tokyo	Oct. 2010	Mar. 2012
10	Aya Tanaka	M2	University of Tokyo	Oct. 2010	Mar. 2012
11	Keita Nagamachi	M2	University of Tokyo	Oct. 2010	Mar. 2012
12	Keitaro Watanabe	M2	University of Tokyo	Oct. 2010	Mar. 2013
13	Haruka OHIRAKI	M2	University of Tokyo	Apr. 2012	Mar. 2013
14	Midori Abekura	M1	University of Tokyo	Apr. 2012	Mar. 2013
15	Shinya Koizumi	M1	University of Tokyo	Apr. 2012	Mar. 2013

Annex 4 Dispatch of Japanese experts/ researchers

No	Name	Field	Position and Organization	Period of Dispatch		
				From	To	Days
1	Dr. Hiroshi Kudo	Project Coordinator	JICA	04/mar/10	at present	
2	Dr. Norihito Kanamori	Molecular Breeding Techniques	Senior Researcher, JIRCAS	23/mai/10	12/ago/10	82
				21/set/10	20/dez/10	91
				22/jan/11	31/mar/11	69
				15/mai/11	19/ago/11	97
				20/set/11	20/dez/11	92
				21/jan/12	12/abr/12	83
				14/mai/12	28/ago/12	107
3	Dr. Daisuke Todaka	Plant Molecular Biology	Special Researcher, JIRCAS	17/out/10	28/out/10	12
4	Dr. Yasushi Umezawa	Plant Molecular Biology	Researcher, RIKEN	17/out/10	28/out/10	12
5	Dr. Kyonoshin Maruyama	Plant Molecular Biology	Senior Researcher, JIRCAS	22/out/10	29/out/10	8
6	Dr. Satoshi Kidokoro	Plant Molecular Biology	Special Researcher, University of Tokyo	22/out/10	29/out/10	8
7	Dr. Hironori Takasaki	Plant Molecular Biology	Special Researcher, RIKEN	21/jan/12	02/fev/12	13
8	Dr. Takuya Yoshida	Plant Molecular Biology	Special Assistant Professor, University of Tokyo	21/jan/12	02/fev/12	13
9	Dr. Kazuko Yamaguchi-Shinozaki	Plant Molecular Biology	Chief Researcher, JIRCAS	03/mar/12	11/mar/12	9
10	Dr. Kazuo Nakajima	Plant Molecular Biology	Senior Researcher, JIRCAS	03/mar/12	11/mar/12	9

Annex 5 Brazilian researchers trained in Japan

No.	Name	Position	Organization	Venue of training	Theme of Training	Training period		
						From	To	Days
1	Ms. Amanda Alves de Paiva Rolla	Student of Master course	University of Londrina/ Embrapa Soybean	JIRCAS	Technical Learning of Expression Analysis of Soybean Stress-Responsive Genes	04/jun/10	15/jan/11	226
2	Mr. Alexandre Lima Nepomuceno	Senior Researcher	Embrapa Soybean	JIRCAS	Participation in the Kick-off Meeting of the Project	16/jul/10	22/jul/10	7
3	Ms. Silvana Regina Rockenbach Marin	Chief of Laboratory	Embrapa Soybean	JIRCAS	Technical Learning of Molecular Analysis of Soybean Stress-Responsive Genes	22/ago/10	11/nov/10	82
4	Ms. Maria Cecilia do Amaral Soldera	Technician	Embrapa Soybean	JIRCAS	Technical Training on Isolation of Promoters related to Soybean Stress-Responsive Genes	15/out/11	18/dez/11	65
5	Ms. Cibelle Engels	Student of Doctor course	Embrapa Soybean	JIRCAS	Technical Learning of Expression Analysis of Soybean Stress-Responsive Genes	27/nov/11	06/set/12	285
6	Ms. Maria Cecilia do Amaral Soldera	Technician	Embrapa Soybean	JIRCAS	Technical Training on Agrobacterium-mediated transformation and Expression Analysis	17/jul/12	30/ago/12	45

Annex 6 Equipment provided by Japanese side for Brazilian Implementing Agency (Embrapa Soybean)

	Place of procurement	Name of equipment	Maker	Model	Main Specification	Quantity	Unit Price (Yen)	Amount (Yen)	Price (Real)	Location of use	Date of arrival
1	Brazil	Vehicle	Mitsubishi	Pajero TR4	4WD, Engine (2.0 liters, 16V, 100HP)	1			R$ 78.000,00		20100319
2	Brazil	Air conditioner	AAC conditioned	Split	22.000/18.000BTUS	2			R$ 8.790,00	Biotechnology	20100329
3	Brazil	Hygrometer	Gehaka	G-800	Range of temperature: 5 ~50℃ Humidity accuracy: +/- 0.1%	1			R$ 6.000,00	Biotechnology	20100318
4	Brazil	Refrigerator	Eletrolux	DC48	462L, 220V, 70.2 x 186.5 x 73.3cm	2			R$ 3.538,00	Biotechnology	20100326
5	Brazil	Freezer	Consul	300	246L, 110V, 61.6 x 170 x 69.1	2			R$ 3.198,00	Biotechnology	20100326
6	Brazil	Computer	Dell	Inspiron 580 BCC	With monitor and software	6			R$ 20.472,66	Biotechnology	20100810
7	Brazil	Printer	HP	HPCP 1515N	Color with scanner	2			R$ 1.940,00	Biotechnology	20100810
8	Brazil	Computer (Note type)	Dell	Vostro 13	13 inches, Intel Core 2 Duo T8100	2			R$ 6.922,54	Biotechnology	20100810
9	Brazil	Weight scale	Balmak	Be Mod MP-25	25kg, 37 x 37 x 12cm, LED display	2			R$ 1.088,01	Biotechnology	20100326
10	Brazil	Low temperature seed storage	Von Stein Refrigeração	Von Stein Refrigeração	2 units of digital timer, 2 compressors, 2 evaporators, 3.5m x 2.5m x 2.9m	1			R$ 21.855,00	Biotechnology	20100731
11	Brazil	GPS	Garmin	Map60 CXS	Micro SD card, High sensitivity GPS receiver, color TFT, altimeter/ atmospheric pressure/ altitude meter	2			R$ 2.943,00	Field	20110105
12	Brazil	pH meter	SPLabor	FX-2,00	16.00PH	2			R$ 620,00	Biotechnology	20110111
13	Brazil	Tablet computer	Apple	IPAD 1	Wi-Fi 646 3G	2			R$ 5.395,00	Biotechnology	20110211
14	Brazil	Meteorological observation equipment	J C da Silva	J C da Silva	Sonder and data logger	2			R$ 30.521,58	Field	20110221
15	Brazil	Magnetic stirrer	Fisatom	753A	120 - 1800 RPM, capacity 10L	3			R$ 3.060,00	Biotechnology	20110223
16	Brazil	Field alarm system	Alarme System	Alarme System	2 channels video server, dome camera, protection box, telephone cable, radio 5750APSG, 5700MPSG	1			R$ 19.361,00	Field	20110310
17	Brazil	Data logger	Hobo	U10,U23,U4, U14	Temperature: -40 ~70℃, humidity: 0~100%	14			R$ 9.913,00	Ecological physiology	20110325
18	Brazil	Microscope with camera	Motic	SMZ168	Zoom 1:6.7, scope: 0.75X-5X, max 320X, Illumination control: 12v/10W	2			R$ 10.598,00	Biotechnology	20110329
19	Brazil	Oximeter	Lutron	DO-5519	Humidity: Max. 80%, Range of temperature: 0°C - 50°C, DC 6.2mA	2			R$ 1.915,98	Ecological physiology	20110404
20	Brazil	Data logger	HOBO	U14-002	Memory 1MB, 36,000 scan	1			R$ 4.353,00	Ecological physiology	20110614

	Place of procurement	Name of equipment	Maker	Model	Main Specification	Quantity	Unit Price (Yen)	Amount (Yen)	Price (Real)	Location of use	Date of arrival
21	Brazil	Nobreaks	NHS	Premium GII 3000Va	18Ah	2			R$ 4.397,10	Biotechnology	20110711
22	Brazil	Analytical Balance	Shimadzu	SHI-AY 220	Max measurement: 220g, minimum display: 0.1mg, response time: 3 seconds	2			R$ 5.220,00	Biotechnology	20110713
23	Brazil	Oven	Cienlab	CE-220/480	800 x 600 x 1000mm, 900W, 480L	1			R$ 7.986,00	Biotechnology	20110714
24	Brazil	Nobreaks	SMS	Net Winner Expert	1800Va	2			R$ 1.482,36	Biotechnology	20110720
25	Brazil	Electrophoresis system (vertical)	Loccus	LCH-192	GEL tray: 24 x 26cm, buffer capacity: 1500ml, weight: 1500g, voltage: 300V (max), current: 360mA (max), electric power: 108W (max), temperature control range: 55℃	2			R$ 5.524,00	Biotechnology	20110721
26	Brazil	Electrophoresis system (horizontal)	Loccus	LCV-20x20	EVM-E/EVM-XX, buffer capacity: lower part 500ml and upper part 650ml, size: 31cm x 16cm x 20cm, capacity: max 56 samples	2			R$ 11.490,00	Biotechnology	20110721
27	Brazil	Shaking incubator	Cienlab	CE-320	Temperature range: 7°C - 70°C, Rotation range: 50 - 250 rpm	1			R$ 6.900,00	Biotechnology	20110726
28	Brazil	Ice machine	Termall	EGE300M	EDG-180, 600KG/24 hours	1			R$ 11.667,00	Biotechnology	20110729
29	Brazil	Refrigerator	Termall		2 doors, 337L	1			R$ 1.420,00	Biotechnology	20110729
30	Brazil	Freezer	Termall	Consul	246L	1			R$ 1.126,00	Biotechnology	20110729
31	Brazil	Air conditioner	Termall	Eletrolux	30.000BTUS	2			R$ 6.270,00	Biotechnology/ Ecological physiology	20110729
32	Brazil	Multimeter	MINIPA	ET-2042D	Sampling rate: 3x/ second, temperature range: 0℃ - 40℃, altitude range: 2000m	2			R$ 230,00	Ecological physiology	20110815
33	Brazil	BOD incubator	Splabor	Sp-225	334L , 60 x 110 x 450cm	1			R$ 5.698,00	Biotechnology	20110826
34	Brazil	Clean bench	Vecoflow	Biosafe Plus 12CI II	A2 (B3) 110/120v, ISO class (class 100), size of work area: 623mm x 1,184mm x 605mm, external dimensions: 780mm x 1.270mm x 2.100mm	2			R$ 32.760,00	Biotechnology	20110829
35	Brazil	Guelph Permeameter	FUNDAG	IAC	20cm x 60cm, PVC	2			R$ 19.000,00	Ecological physiology	20110830
36	Japan	Seesaw shaker	Cole-Parmer	Adjustable Rocker	120V, 4A,60HZ	2	¥ 210.000	¥ 420.000		Biotechnology	20110725
37	Japan	Ultrasonic washing machine (large)	Cole-Parmer	8893-21	Capacity: 2.5 gallons, heater, digital timer, temperature monitor	1	¥ 147.000	¥ 147.000		Biotechnology	20110725

	Place of procurement	Name of equipment	Maker	Model	Main Specification	Quantity	Unit Price (Yen)	Amount (Yen)	Price (Real)	Location of use	Date of arrival
38	Japan	Micro-Volume Spectrophotometer	Nanodrop	ND2000	Measurement time: less than 5 seconds, minimum sample required: 0.5μL, measurement range up to 15,000 ng/μL (dsDNA conversion), without the need for sample dilution of high concentration	1	¥ 1.386.000	¥ 1.386.000		Biotechnology	20110801
39	Japan	Water potential measurement device	DECAGON	PROACHK	Measuring range: 0～-300MPa, accuracy: 0～5MPa/±0.05MP a-5～-300MPa/±1%, resolution: 0.01MPa, sample volume: 7～20ml	2	¥ 89.000	¥ 178.000		Ecological physiology	20110829
40	Japan	Centrifugal Concentrator	Eppendorf	5305C	with PTFE Vacuum pump, micro tube (1.5/2.0ml) max. 96 tubes, revolution: 1,400rpm, temperature setup range: Off/30 /45 /60°C	1	¥ 1.094.027	¥ 1.094.027		Biotechnology	20110831
41	Japan	Fluorescence scanner	GE Healthcare	Typhoon 7000	High speed scan: 5 minutes for sample size of 20 x 25cm, compact size suitable on experimental table, analytical software: Image Quant TL	1	¥ 9.660.000	¥ 9.660.000		Biotechnology	20110907
42	Japan	Thermal cycler	Thermal	Veriti 96-well	Correspond applications (standard PCR and fast PCR), VeriFlex block, independent 6 temperature setting zone	2	¥ 956.970	¥ 1.913.940		Biotechnology	20110908
43	Japan	Ultra-low temperature freezer	Revco	Ult1786-10dd-Btype	Max temperature: -86°C, inside capacity: 705L, temperature recorder, battery for automatic support cooling, remote alarm device	1	¥ 1.972.425	¥ 1.972.425		Biotechnology	20111125
44	Brazil	Sterilizer	Marconi	1202/CT	Temperature control: 350°C in 20 minutes, stainless steel, 500W, 250V, microprocessor temperature control, digital display	1			R$ 1.540,00	Biotechnology	20110930
45	Brazil	Autoclave	Phoenix Luferco	AH-39206/200	Horizontal type, stainless steel, microprocessor control, outside dimensions: 60 x 135 x 1,400cm, inside dimensions: 40 x 40 x 1200cm, pressure steam sterilization	1			R$ 42.500,00	Biotechnology	20111101
46	Brazil	Security monitoring system	SCCR Controle de Ponto de Acesso	Prisma Acesso	Registered finger number: large finger 1000, verification search fingers: 200 fingers	3			R$ 8.510,00	Biotechnology	20111017
47	Brazil	Thermometer	HANNA	HI－99301	Measuring range: EC 0.00～20.00mS/cm, TDS 0.00～10.00ppt, temperature: 0.0～60.0℃	2			R$ 1.555,00	Ecological physiology	20111103
48	Brazil	Shaking incubator	Tecnal	TE-421	0～ +60°C, 800/550/400mm	1			R$ 12.800,00	Biotechnology	20111104
49	Brazil	Security monitoring system	SCCR Controle de Ponto de Acesso	Prisma Acesso	Registered finger number: large finger 1000, verification search fingers: 200 fingers	1			R$ 2,836,66	Storage of genetically modified	20111202

	Place of procurement	Name of equipment	Maker	Model	Main Specification	Quantity	Unit Price (Yen)	Amount (Yen)	Price (Real)	Location of use	Date of arrival
50	Brazil	Furniture for laboratory in newly constructed biotechnology building (first floor)	IMPERIAL	IMPERIAL	shelves, experimental table (water proof), cabinet, and storage unit, etc.	1			R$ 79.990,00	Biotechnology	20120103
51	Brazil	Ultrasonic cleaner	Alfa Mare	USC-1600A	with heater	1			R$ 2.055,00	Biotechnology	20120111
52	Brazil	Mixer, stirring and shaking	Splabor	AP-56 Vortex	14,5x13x16cm 110/220v	7			R$ 4.550,00	Biotechnology	20120112
53	Brazil	pH meter	Mettler-Toledo	PH-S20-K	"3-In-1" pH electrode , buffer sachets (2 each ; 4.01, 7.00 and 9.21),	2			R$ 7.550,00	Biotechnology	20120126
54	Brazil	Green house	Londriestufa	Londriestufa	4 unit and corridor= 25.6m x 9.82m x 35m, 1 unit= 6.4m x 7.32m	1			R$ 73.095,00	Biotechnology	20120213
55	Brazil	Mini centrifuge	Alfa Mare	Mini Star	110V, 6 micro tubes	10			R$ 7.220,00	Biotechnology	20120224
56	Japan	Cooled centrifuge	Sorvall	Legend XTR	230v 50/60Hz, max revolution: 15,200rpm, max centrifugal force: 25,314g, temperature setting range: -10～ 40°C	1	¥ 1.000.000	¥ 1.000.000		Chemical analysis office	20120217
57	Japan	Fixed angle rotor for cooled centrifuge	Sorvall	F14-S6x250LE F15-8x50c	for above cooled centrifuge of Sorvall	2	¥ 199.500	¥ 199.500		Biotechnology	20120217
58	Japan	Cooled centrifuge	Sorvall	Legend XIR	230V, 50/60Hz	4	¥ 455.140	¥ 1.820.560		Biotechnology	20120217
59	Japan	Fixed angle rotor for cooled centrifuge	Sorvall	F15-6x100	for above cooled centrifuge of Sorvall	4	¥ 50.000	¥ 200.000		Biotechnology	20120217
60	Japan	Bucket for Swing rotor	Sorvall	TX-200	for above cooled centrifuge of Sorvall	4	¥ 49.200	¥ 196.800		Biotechnology	20120217
61	Japan	Potable measuring device for photosynthesis and transpiration	ADC	LCPro	XI Serials	2	¥ 4.125.000	¥ 8.270.000		Ecological physiology	20120217
62	Japan	Open type measuring system for photosynthesis and transpiration	Licor	Li-6400XT	SD main unit、leaf chamber、LED	1	¥ 100.000	¥ 100.000		Ecological physiology	20120217
63	Japan	Desktop type lead area meter	Licor	Li3100C	Measuring range: CO2 0～3,000μmol/mol, H2O 0～75mmol/mol	1	¥ 2.169.000	¥ 2.169.000		Ecological physiology	20120217
64	Japan	Water potential measurement device	Wescor	Psypro	Operation temperature: +55℃ ～ +15, Storage Temperature: +65 ～ -20℃	1	¥ 4.019.048	¥ 4.019.048		Ecological physiology	20120217
65	Japan	Beads type multi Cell Disruption device	Stainless Beads	3-Dimentional High Thoughqut Bead Type Homoginzer	V-30-SF	1	¥ 2.753.500	¥ 2.753.500		Biotechnology	20120217
66	Japan	Centrifugal Concentrator	Decagon	WP4C	2.4mm, 5.0mm, 3.0mm, measuring accuracy: ±0.05MPa, measuring range: 0 ～ -300MPa, Temperature setting: 15～40℃	1	¥ 1.422.750	¥ 1.422.750		Ecological physiology	20120217
							Total	¥ 38.922.550	R$ 605.856,89		

Annex 7 Equipment procured for the research institutes in Japan (JIRCAS, RIKEN and the University of Tokyo)

	Name of Equipment	Maker	Model	Main Specification	Quantity	Unit Price (Yen)	Amount (Yen)	Location of use	Date of arrival
1	PCR equipment	Applied Biosystems	Thermal Cycler 2720	Range of sample capacity: 5～100μL, 0.2mL, size 21cm x 36cm x 22cm, weight 6.1kg	1	499.590	499.590	JIRCAS	20/05/2010
2	Frame Side Experimental Table	Dulton	MW-61AC-12001	Size: 120cm x 60cm x 80cm, with 2 units of movable cabinet	1	207.270	207.270	JIRCAS	03/06/2010
3	High Speed Refrigerated Micro Centrifuge	Hitachi Koki	CF15RXII	Max revolving speed: 15,000rpm, max centrifugal acceleration: 22,200Xg, revolution control range: 300～15,000rpm, timer: 5-55seconds/ 1-99minutes/ HOLD, temperature setup range: -9～40℃, size: 37cm x 52cm x 84cm, weight: 82kg, rotor: a unit (T15AP31)	1	730.800	730.800	JIRCAS	09/06/2010
4	Computer	Hewlett-Packard Japan	Pavilion Desktop PC HPE-390jp/CT	Windows® 7 Home Premium （64bit）, Intel Core i7-930 processor, memory: 6GB （2GB×3）, HDD 500GB Serial ATA 3Gb/s （7,200rpm）, DVD supermulti drive	1	256.410	256.410	JIRCAS	29/10/2010
5	Artificial climate chambers	Nippon Medical & Chemical Instruments Co.,Ltd	LH-350S	with manual lighting adjusting function, controlled operation of temperature and illuminance, illuminance: 0～27,000lx, chamber capacity: 350L, size: 88cm x 81cm x 188cm, weight: 285kg	1	1.502.550	1.502.550	JIRCAS	30/11/2010
6	Multilabel Reader	Perkin Elmer	ARVO X3 system	Measurable items: absorbance, fluorescence and emission, scanning, fluorescence bottom reading, stirring plate, temperature control, size: 50cm x 60cm x 36cm, weight: 46kg	1	3.617.250	3.617.250	JIRCAS	22/12/2010
7	Multifunctional shaking incubator	THOMAS KAGAKU Co.,Ltd.	AT-24R	Rotary method, temperature range: 5 - 60℃, shaking number: 20-200times/minute, shaking width: 70mm, size: 128cm x 69cm x 93cm, weight: 230kg	1	1.690.500	1.690.500	JIRCAS	10/11/2011
8	Artificial climate chambers	Nippon Medical & Chemical Instruments Co.,Ltd	LH-410S	with manual lighting adjusting function, controlled operation of temperature and illuminance, illuminance: 0 - 31,000lx, chamber capacity: 410L, size: 88cm x 81cm x 188cm, weight: 285kg	1	1.606.500	1.606.500	JIRCAS	20/12/2011
9	Double Shaker	TAITEC CORPORATION	NR-30	Round trip/ turning switching, shaking speed: 20～200r/mm, shaking width: 10～40mm, size: 45cm x 41cm x 16cm, weight: 17kg	1	274.050	274.050	JIRCAS	07/03/2012
10	Analytical Balance	Mettler Toledo	MS304S/02	Max measurement: 320g, Minimum display: 0.1mg, size of plate: diameter 90mm, size: 21cm x 35cm x 35cm, weight: 6.5kg	1	233.845	233.845	JIRCAS	25/04/2012
11	Artificial climate chambers	Panasonic	MIR-254-PJ	Additional LED unit, inside temperature: -10～60℃, chamber capacity: 254L, size: 70cm x 58cm x 162cm, weight: 108kg	1	2.305.800	2.305.800	JIRCAS	19/07/2012
12	Ultra-low temperature freezer	Nihon Freezer Co., Ltd.	CLM-31UW	Capacity: more than 324L, inside humidity: -80℃～-85℃	1	1.473.150	1.473.150	RIKEN	09/12/2010
13	Artificial climate chambers	Nippon Medical & Chemical Instruments Co.,Ltd	LPH-350SP	Fixed value operation, Switching operation day and night, illumination: 5-sided irradiation, 3-position control function on temperature and humidity	1	1.874.250	1.874.250	RIKEN	08/02/2011
14	High Speed Micro Centrifuge	Hitachi Koki	CF15RXII	Max revolution speed: 15,000rpm, max centrifugal acceleration: 22,200Xg (T15A41), revolution control range: 300～15,000rpm	1	683.550	683.550	University of Tokyo	24/05/2010
15	Shaking incubator	THOMAS KAGAKU Co.,Ltd.	AT-12R	Temperature range: 60℃ ～ +5℃, high accuracy on temperature control, shaking and temperature distribution in incubator, automatic operation of freezer	1	996.030	996.030	University of Tokyo	28/06/2010
						Total	17.951.545		

Annex 8 Local Cost Allocated by Japanese Side

Items	Contents	Local Expenses (Real)				Total
		From April 2010 to March 2011	From April to September 2011	From October 2011 to April 2012	From April 2012 to June 2012	
1) General expenses		103.815,29	40.160,31	22.568,46	8.494,22	175.038,28
(breakdown)						
Civil works		-	-	-	-	*0,00*
Expenses for facility operation and maintenance		-	-	-	-	*0,00*
Expenses for equipment operation and maintenance	Car insurance and maintenance, Maintenance of PC, highway expenses	*5.813,13*	*8.728,47*	*2.120,00*	*1.776,00*	*18.437,60*
Expenses for procurement of equipment	reagent, ultrasonic cleaner, cabinet, monitor, camera, etc.	*62.841,58*	*11.642,56*	*7.791,17*	*869,00*	*83.144,31*
Consumables	Office supplies and laboratory supplies, etc.	*24.210,49*	*16.024,66*	*7.796,69*	*1.938,86*	*49.970,70*
Transportation	Taxi expenses, etc.	*4.410,11*	*722,76*	*602,63*	-	*5.735,50*
Communication and mail	Expenses for mail, fax and telephone	*4010,28*	*2237,46*	*2.675,47*	*1.070,36*	*9.993,57*
Document preparation	Poster, translation of document (English to Portuguese)	*480*	*63*	*1.060,00*	-	*1.603,00*
Rental fee		-	-	-	-	*0,00*
Other expenses		*2049,7*	*741,4*	*522,50*	*2.840,00*	*6.153,60*
2) Remuneration (other than staff)	Expenses for scholarship and salaries	172.870,17	130.753,00	141.790,33	64.188,43	509.601,93
3) Business contract		-	-	-	-	0,00
4) Business contract (local consultants)	Expenses for lawyer	2.700	-	4.500,00	-	7.200,00
5) Business contract (local NGO)		-	-	-	-	0,00
6) Expenses for air tickets	Air tickets	10.278,78	2.700,66	4.791,74	3.821,29	21.592,47
7) Travel expenses (other than air tickets)	Expenses for hotels and per diem	1.077,10	4.152,00	4.553,89	3.909,62	13.692,61
8) Meeting expenses		-	-	-	-	0,00
Total		290.741,34	177.765,97	178.204,42	80.413,56	727.125,29

Annex 9 List of Brazilian Researchers involved in the project activities

No.	Name	Position	Organization	Period of participation into research activities		Field of specialty
				From	To	
1	Dr. Alexandre Nepomuceno	Senior Researcher/Project Leader	Embrapa Soybean	Mar. 2010	At present	Molecular Biology/ Plant Physiology
2	Dr. Norman Lima Neumaier	Senior Researcher	Embrapa Soybean	Mar. 2010	At present	Plant Physiology
3	Dr. José Renato B. Farias	Director of Research Development	Embrapa Soybean	Mar. 2010	At present	Agrometheorology
4	Dr. Amelio Dall Agnol	Director of Technology Transfer	Embrapa Soybean	Mar. 2010.	At present	
5	Dr. Carlos Alberto Arrabal Arris	Senior Researcher	Embrapa Soybean	Mar. 2010	At present	Breeding
6	Dr. Antonio Eduardo Pipolo	Senior Researcher	Embrapa Soybean	Mar. 2012	At present	Breeding
7	Dr. Renata Fuganti	Post-Doctoral Fellow	Embrapa Soybean	Mar. 2010	At present	Transformation
8	Dr. Ricardo Vilela Abdelnoor	Senior Researcher/ Acting Project Leader (after October 2012)	Embrapa Soybean	Mar. 2010	At present	Molecular Biology
9	Dr. Francismar Correia Marcelino	Researcher/ Acting project Leader Until October, 2012)	Embrapa Soybean	Mar. 2010	At present	Molecular Biology
10	Dr. Maria Cristina Neves de Oliveira	Researcher	Embrapa Soybean	Mar. 2010	At present	Statistics
11	Dr. Clara Beatriz Hoffman-Campo	Senior Researcher	Embrapa Soybean	Mar. 2010	At present	Metabolomics
12	Dr. Júlio Franchini dos Santos	Senior Researcher	Embrapa Soybean	Mar. 2010	At present	Crop Management
13	Gedi Jorge Sfredo	Senior Researcher	Embrapa Soybean	Mar. 2012	Sep. 2012	Plant Nutrition
14	Silvana Marin	Chief of Lab./ 2 grade in master	Embrapa Soybean/ University of Londrina	Mar. 2010	At present	Molecular Biology
15	Dr. Henrique Debiasi	Crop Management	Embrapa Soybean	Mar. 2010	At present	Crop Management
16	Dr. Adilson de Oliveira Junior	Soil Science	Embrapa Soybean	Mar. 2010	At present	Plant Physiology
17	Cesar Augusto Silveira	Sub-chief of Lab.	Embrapa Soybean	Mar. 2010	At present	Molecular Biology
18	Marcia Kamogae Kuwahara	Technician	Embrapa Soybean	Mar. 2010	At present	Molecular Biology
19	Rubson Sibaldeli	Technician	Embrapa Soybean	Mar. 2010	At present	Ecophysiology
20	Claudinei de Freitas Toledo	Technician	Embrapa Soybean	Mar. 2010	At present	Ecophysiology
21	Bianca Peral Pereira	Technician	Embrapa Soybean	Mar. 2010	At present	Ecophysiology
22	Dr. Josirley Carvalho	Post-Doctoral Fellow	CNPQ	Dec. 2010	At present	Plant Physiology
23	Amanda Paiva	3 grade in doctor	University of Londrina	Mar. 2012	At present	Molecular Biology
24	Cibelle Engels	3 grade in doctor	University of Londrina	Mar. 2012	At present	Molecular Biology
25	Mayla D. C. Molinari	2 grade of University	UNIFIL University (Londrina)	Mar. 2011	Jul. 2012	Molecular Biology
26	Elton Gargioni Grisoste Barbosa	1 grade in doctor	University of Londrina	Mar. 2010	Aug. 2012	Molecular Biology
27	Juliana Paula Leite	1 grade in doctor	University of Paulista	Mar. 2010	Mar. 2012	Molecular Biology
28	Juliane Marinho	1 grade in master	University of North Parana	May. 2011	At present	Molecular Biology
29	Patricia Honna	5 grade of University	University of Bandeirante	May. 2011	At present	Molecular Biology
30	Larissa Girotto	Technician	Employed by JICA	Mar. 2010	Aug. 2012	Molecular Biology
31	Maria Cecilia Amaral Soldera	Technician	Employed by JICA	Oct. 2010	Aug. 2012	Molecular Biology
32	Gislaine Vasquez	Technician	Employed by JICA	Oct. 2010	Jun. 2012	Molecular Biology

Annex 10 Equipment procured and facilities constructed by Brazilian side

(1) Year 2010

Name of equipment	Location of use	Date of arrival	Price (Real)	Remarks
Capillary electrophoresis apparatus	New Biotechnology Building (NBB)	Year 2010	86.000	Agrofuture project
Refrigerator	NBB	25 Oct. 2010	1.799	446L, MABE
Autoclave (vertical type)	NBB	26 Oct. 2010	12.150	Phoenix
Thermocycler	NBB	10 Nov. 2010	24.372	Veriti 96Well, Applied Biosystems
Freeezer (vertical type)	NBB	18 Nov. 2010	1.670	296L, Consul
Sample crusher	NBB	20 Nov. 2010	41.296	Geno/Grinder
Ice machine	NBB	26 Nov. 2010	12.469	EGE300, TERMALL
Refrigerator	NBB	3 Dec. 2010	1.500	362L, Consul
Magnetic stirrers with heating	NBB	17 Dec. 2010	1.050	Nova Etica
No brake	Ground Floor, NBB	Year 2010	6.000	10KVA
No brake battery	Ground Floor, NBB	Year 2010	4.000	
Micropipette	Ground Floor, NBB	Year 2010	3.500	Eppendorf
Micropipette (6 units)	Ground Floor, NBB	Year 2010	4.600	
Freezer	Ground Floor, NBB	Year 2010	2.000	Eletrolux
Genotyper	Ground Floor, NBB	Year 2010	19.000	Beadxpress, ILLUMINA
			221.406	

(2) Year 2011

Name of equipment	Location of use	Date of arrival	Price (Real)	Remarks
New Biotechnology Building	adjacent to Biotechnology Building	Construction completed 30 Jun. 2011	722.400	including air conditioner, growth chamber, etc., PAC project
Power supply for electrophoresis	NBB	19 Jan. 2011	900	LSP 300V, Loccus
Desktop computers	NBB and Biotechnology Building	23 Mar. 2011	15.856	8units, Elite 8100, HP
Thermocycler	NBB	19 Apr. 2011	24.372	Veriti 96Well, Applied Biosystems
SNPs Genetic Analysis System	NBB	4 May. 2011	199.546	BX113
Cube electrophoresis apparatus	NBB	17 May. 2011	4.206	3units, Thermo
Thermocycler	NBB and Biotechnology Building	17 May. 2011	41.111	3units, Veriti 384 Well, Applied Biosystem
No brake	NBB	5 Aug. 2011	11.994	2units, 10KVA, Maxxi Mono, HDS
Thermostatic bath	NBB	5 Aug. 2011	2.992	2units, Marconi
Realtime PCR	NBB	Year 2011	230.000	Applied Biosystems

Name of equipment	Location of use	Date of arrival	Price (Real)	Remarks
No brake	NBB	16 Aug. 2011	3.552	SP100/336A, SP Labor
DNA workstation	NBB	30 Aug. 2011	5.600	LWS-01-110V
Test tube stirrer	Biotechnology Building	6 Sep. 2011	800	KMC-1300V
Liquid chromatography	Chemical Analysis office	1 Dec. 2011	100.000	
Stainless steel pipe for gas chromatography	Chemical Analysis office	1 Dec. 2011	10.000	
Computers (7 Units)	Ecological Physiology	Year 2011	21.000	
Renovation of Eco-Physiology Lab.	Ecological Physiology	1 Dec. 2011	40.000	
GMO Seed storage building	Field	Year 2011	319.798	
Green house for GMO	Field	Year 2011	300.000	
Autoclave	Ground Floor, NBB	Year 2011	4.000	Phoenex Luferco
Oven	Ground Floor, NBB	Year 2011	3.300	
Balance	Ground Floor, NBB	Year 2011	7.000	Shimadzu
Centrifugation	Ground Floor, NBB	May. 2011	20.387	Thermo Scientific X1R
Ice Machine	Ground Floor, NBB	Year 2011	7.459	Scotsman
Shaker	Ground Floor, NBB	Year 2011	2.000	ILLUMINA
Freezer	Ground Floor, NBB	Year 2011	1.200	Consul
Thermostatic bath (2 units)	Ground Floor, NBB	Year 2011	2.400	Marconi
Laminar flow	Ground Floor, NBB	Year 2011	5.500	Amazonlab
Cart (2 units)	Ground Floor, NBB	Year 2011	2.400	
Autoclave	Ground Floor, NBB	Year 2011	5.880	Prismatec
			2.115.653	R$ 1.392.273

(3) Year 2012

Name of equipment	Location of use	Date of arrival	Price (Real)	Remarks
Chairs (14 units)	NBB	1 Jan. 2012	4.200	
Furniture for laboratory (ground floor)	NBB	Feb. 2012	50.000	
Washing table (2 units)	NBB	1 Jan. 2012	2.700	
Automatic workstation	Ground Floor, NBB	Year 2012	300.000	Perkin Elmer
DNA workstation	NBB	Year 2012	6.000	Loccus
		Total	362.900	
		Grand total	2.699.959	

Annex 11 Initial screening of plants for drought tolerance

Soybean seeds from the transformed lines and WT are germinated on filter paper for four days in growth chamber where temperature and relative humidity is set to 25 ± 1 °C and 100%, respectively. Seedlings are cultivated into pots containing a mixture of soil:sand:manure (3:3:3 26% holding capacity) in well-watered conditions (80% of the holding capacity) until the V2 developmental stage (Fehr et al. 1971). Completely randomized experimental design with 10 repetitions may be used if conditions in the glasshouse are uniform; otherwise completely randomized block should be preferred. Conditions in the greenhouse are set to natural photoperiod of approximately 12/12 h light/dark cycle, temperature of 30 ± 5 °C and 60 ± 10% relative humidity (RH). When the plants reach the V2 stage, growth is measured (day 0-control condition), the pot is saturated with water and watering is suspended thereafter. Another measurement is performed five days after suspension of irrigation (day 5-stress condition) with the plant at the V3 stage. The characteristics analyzed in both conditions are plant height, leaf number, number of nodes and mean internode length (at least height should be measured). Stomatal conductance (gs) and photosynthetic rate (A) are also evaluated using a LI-6400 Portable Photosynthesis System (LiCor, Inc.). Measurements are taken on the middle leaflet of the youngest soybean leaf, fully expanded, under photon flux density of 1.000 µmol m-2 s-1. Following, the plants are rewatered and three days later, the percentage of foliar damage is evaluated. The index of stress tolerance is estimated by the relative rate of height growth (RRHG) calculated according to the formula - RRHG = (height at day 5 - height at day 0) / height at day 0 x 100%.

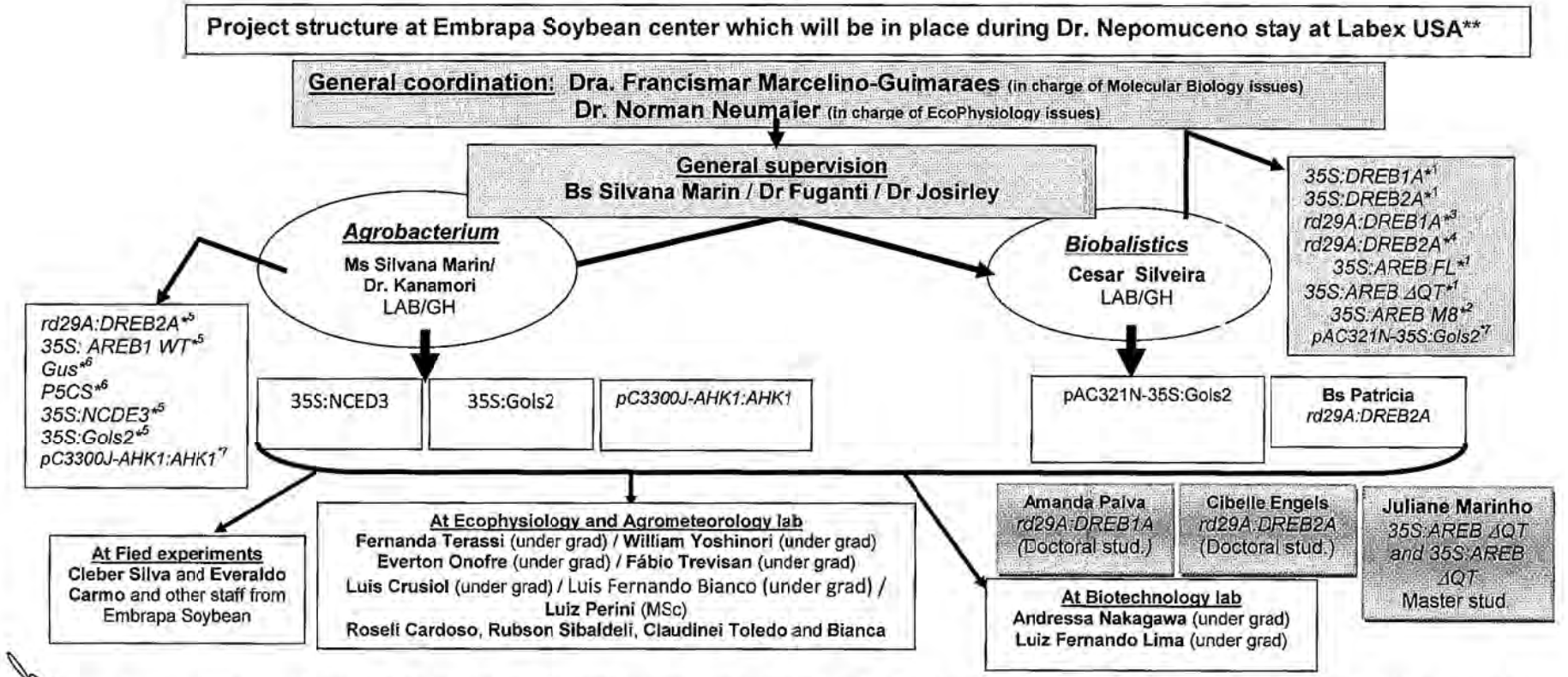
Tolerant plants are: Plants that survive the water deficit of 5 days and have higher photosynthetic rates and Relative Rate of Height Growth (RRHG) during water deficit period and no leaf damage after rewatering

Annex 12: Plan of Operations (comparison of progress with planned schedule)

			Main responsible institutions					FY 2009	FY 2010				FY 2011				FY 2012				FY 2013				FY 2014		
			JIRCAS	University of Tokyo	RIKEN	Embrapa Soybean		IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III
1. Useful genes related to environmental stress tolerance are identified.	1-1 Genes involved in regulation of stress tolerance are identified in plants such as soybean.	Search of genes involved in regulation of stress tolerance in model plants.					Planned																				
							Actual																				
		Search of genes involved in regulation of stress tolerance in soybean.					Planned																				
							Actual																				
		Functional analysis of genes involved in regulation of stress tolerance using protoplasts.					Planned																				
							Actual																				
		Functional analysis of genes involved in regulation of stress tolerance using transgenic plants.					Planned																				
							Actual																				
	1-2 Genes involved in stress perception are identified in plants such as soybean.	Search of genes involved in stress perception in model plants.					Planned																				
							Actual																				
		Search of genes involved in stress perception in soybean.					Planned																				
							Actual																				
		Functional analysis of genes involved in stress perception using transgenic plants.					Planned																				
							Actual																				
	1-3 Genes involved in regulation of stress response are identified in plants such as soybean.	Search of genes involved in regulation of stress response in model plants.					Planned																				
							Actual																				
		Search of genes involved in regulation of stress response in soybean.					Planned																				
							Actual																				
		Functional analysis of genes involved in regulation of stress response using transgenic plants.					Planned																				
							Actual																				
2. Stress-responsive promoters are isolated and combinations with useful genes are optimized.	2-1 Stress-responsive genes are searched in soybean.	Making of oligo-array of soybean.					Planned																				
							Actual																				
		Search and identification of stress-responsive genes using oligo-array.					Planned																				
							Actual																				
	2-2 Stress-responsive promoters are identified in soybean.	Identification of stress-responsive genes using cDNA array of soybean.					Planned																				
							Actual																				
		Isolation of stress-responsive promoters of soybean.					Planned																				
							Actual																				
	2-3 Constructs of useful genes and promoters are optimized.	Collection of soybean full-length cDNA and identification of promoter sequences.					Planned																				
							Actual																				
		Optimization of combinations of promoters and useful genes.					Planned																				
							Actual																				
3. Transgenic soybean lines containing constructs of promoters and useful genes are produced.	3-1 Genetic engineering technology is established in soybean.	Establishment of high-efficient gene transfer technology for soybean.					Planned																				
							Actual																				
	3-2 Constructs of useful genes and promoters are introduced in soybean.	Introduction of constructs containing useful genes.					Planned																				
							Actual																				
		Check of existence of transgenes.					Planned																				
							Actual																				
	3-3 T1 seeds of transgenic lines are collected.	Collection of next generation seeds.					Planned																				
							Actual																				
4. Transgenic soybean lines with environmental stress tolerance are selected.	4-1 Drought-inducible genes are identified and transgenic lines are selected based on gene analysis.	Creation of gene expression database of soybean.					Planned																				
							Actual																				
		Selection of transgenic soybean lines using gene analysis.					Planned																				
							Actual																				
	4-2 Heat-inducible genes are identified and transgenic lines are selected based on gene analysis.	Creation of gene expression database of soybean.					Planned																				
							Actual																				
		Selection of transgenic soybean lines using gene analysis.					Planned																				
							Actual																				
	4-3 Gene expression of transgenic plants is analyzed.	Gene expression analysis of transgenic soybean plants using oligo-array.					Planned																				
							Actual																				
	4-4 Evaluation methods of stress tolerance of soybean are established.	Establishment of evaluation methods of stress tolerance of soybean.					Planned																				
							Actual																				
		Selection of transgenic soybean using PCR.					Planned																				
							Actual																				
	4-5 Stress tolerance of transgenic soybean lines is evaluated in greenhouse.	Stress tolerance test of transgenic soybean plants in greenhouse.					Planned																				
							Actual																				
	4-6 Stress tolerance of transgenic soybean lines is evaluated in field.	Stress tolerance test of transgenic soybean plants in field.					Planned																				
							Actual																				

FY : Fiscal Year in Japan (I :Apr.to Jun. II :Jul.to Sep. III :Oct.to Dec. IV:Jan. to Mar.)

Annex 13 Project Structure at Embrapa Soybean



- *1 - Transformation using *35S:AREB ΔQT and 35S:AREB FL* genes were concluded with 03 and 06 positive lines, respectively, obtained and identified in T1. For *35S:DREB1A* and *35S:DREB2A*, 10 and 02 positive lines, respectively, were identified in T1.
- *2 – For *AREB M8* construct, a few T0 positive lines were identified, and no transfer to the next generation was observed after at least 8.000 tries. Transformations stopped with this construction.
- *3 – Six positive lines were already obtained characterized and will be tested in field experiments soon. Seeds from 5 lines are in Japan already.
- *4 – Transformation restarted to obtain more positive lines.
- *5 – Keep transforming with these constructs – For *rd29A:DREB2A*, 05 positive lines were positive but they died.
- *6 – Transformations stopped with these genes.
- *7 – Constructs just received, ready to be used in transformation.
- ** **Dr. Nepomuceno will be weekly informed about project progress and at least monthly, the working group will prepare and send him a report about all obtained results and future experiments/procedures.**

→ Arrows direction indicates the hierarchical chain of command in the projects leadership.

PDM 仮和版

PDM Version 2

プロジェクト名： （科学技術）地球環境劣化に対応した環境ストレス耐性作物の作出技術の開発
プロジェクトサイト： パラナ州ロンドリーナ
相手国機関名： ブラジル農牧研究公社ダイズ研究所
日本側協力機関名： 独立行政法人　国際農林水産業研究センター(JIRCAS) (*代表研究機関)、国立大学法人 東京大学、独立行政法人 理化学研究所(RIKEN)
協力期間： 2010 年 3 月 4 日～2015 年 3 月 3 日（5 年間）**作成日：** 2012 年 3 月 15 日の JCC で承認

プロジェクトの要約	指標	指標入手手段	外部条件
[上位目標] 環境ストレスに適応したダイズが開発され、ブラジルのダイズ生産の安定化に資する。	2019 年までに環境劣化に対応したダイズが開発される。	---	---
[プロジェクト目標] 環境ストレス耐性ダイズの作出技術が開発される。	1. ダイズ等の環境ストレスに対する耐性獲得に関与する有用遺伝子が少なくとも 10 種類同定される。 2. ダイズのストレス応答性プロモーターが少なくとも 5 種類単離され、有用遺伝子との組合せの最適化が行われる。 3. プロモーターと有用遺伝子の組合せが少なくとも 5 種類ダイズへ導入され、各組合せから少なくとも 3 系統の組換え体を得る。 4. 少なくとも 1 種類の環境ストレス耐性系統を選抜される。	---	---
[アウトプット] 1. 環境ストレスに対する耐性獲得に関与する有用遺伝子が同定される。	1-1 ダイズ等のストレス耐性制御遺伝子が 5 種類以上同定される。 1-2 ダイズ等のストレス受容に関与する膜タンパク質遺伝子が 2 種類以上同定される。 1-3 ダイズ等のストレス応答制御遺伝子が 3 種類以上同定される。	---	---
2. ストレス応答性プロモーターの単離と有用遺伝子との組合せの最適化が行われる。	2-1 ダイズのストレス応答性遺伝子が少なくとも 100 種類同定される。 2-2 ダイズのストレス応答性プロモーターが少なくとも 5 種類同定される。 2-3 少なくとも 5 種類のプロモーターと有用遺伝子の組合わせの最適化が試みられる。	---	
3. プロモーターと有用遺伝子の組合せが導入されたダイズ系統が得られる。	3-1 ダイズへの形質転換効率が 1.5 %以上の遺伝子組換え技術が確立される。 3-2 プロモーターと有用遺伝子の組み合わせが少なくとも 5 種類ダイズに導入される。 3-3 少なくとも 3 系統の T1 世代種子が増殖される。	---	
4. 環境ストレス耐性を示す組換えダイズ系統が選抜される。	4-1 乾燥応答性遺伝子を少なくとも 2 種類同定され、遺伝子解析が行われ、組換えダイズが少なくとも 2 系統選抜される。 4-2 高温応答性遺伝子を少なくとも 2 種類同定され、遺伝子解析が行われ、組換えダイズが少なくとも 2 つ系統選抜される。 4-3 少なくとも 2 種類の遺伝子とプロモーターの組合せに由来する独立な系統から、少なくとも 2 系統の遺伝子発現が解析される。	---	

	4-4 温室、圃場でのダイズのストレス耐性試験手法が確立する。 4-5 温室で 2 種類以上（各 2 ライン以上）の組換えダイズのストレス耐性評価が行われる。 4-6 圃場で 2 種類以上（各 2 ライン以上）の組換えダイズのストレス耐性評価が行われる。		

[活動]	**[投入]**		
	＜**日本側**＞	＜**ブラジル側**＞	---
1-1 ダイズ等のストレス耐性制御遺伝子の同定を行う。 1-2 ダイズ等のストレス受容に関与する遺伝子の同定を行う。 1-3 ダイズ等のストレス応答制御遺伝子の同定を行う。 2-1 ダイズのストレス応答性遺伝子の探索を行う。 2-2 ダイズのストレス誘導応答性プロモーターの同定を行う。 2-3 プロモーターと有用遺伝子の組合せの最適化を行う。 3-1 ダイズへの遺伝子組換え技術を確立する。 3-2 プロモーターと有用遺伝子の組合せをダイズに導入する。 3-3 遺伝子を導入したダイズの T1 世代種子を増殖する。 4-1 乾燥応答性遺伝子の同定と、遺伝子解析を行って、組換えダイズ系統の選抜を行う。 4-2 高温応答性遺伝子の同定と、遺伝子解析を行って、組換えダイズ系統の選抜を行う。 4-3 組換えダイズの遺伝子発現解析を行う。 4-4 ダイズの乾燥ストレス耐性評価手法を確立する。 4-5 温室での組換えダイズのストレス耐性評価を行う。 4-6 圃場での組換えダイズのストレス耐性評価を行う。	・ 業務調整員 ・ 在外研究員（短期） ・ ブラジル人研究者の日本への招聘 ・ 機材 ・ ポスドク 2 名（あるいはポスドク 1 名と修士研究員 1 名）及び研究補助者 2 名の雇用費用の一部負担 ・ プロジェクトに必要なその他の経費等	・ 研究者・技術者の配置 ・ ポスドク 2 名（あるいはポスドク 1 名と修士研究員 1 名）及び研究補助者 2 名の雇用費用の一部負担 ・ 施設、プロジェクト事務所提供 ・ 資機材、ランニングコスト ・ プロジェクトに必要な経費の確保	**[前提条件]** ---

評価グリッド

5項目 その他	評価設問 大項目	評価設問 小項目	調査結果
妥当性	プロジェクト目標及び上位目標は、対象地域・社会のニーズに合致しているか。	ブラジル国における耐乾性･耐暑性のダイズ品種開発の必要性	(1)　地球規模における気候変動への対応の必要性 急激な人口増加と世界的な工業化進展による温室効果ガスの増加に伴って、地球の温暖化が進んでいるとされ、地球規模での問題となっている。世界各地で、干ばつ、集中豪雨、大型の台風やハリケーンの多発といった異常気象による災害が頻繁に発生している。温暖化に伴い、世界各地で頻繁に干魃被害が報告されているとともに、作物耕作地での乾燥が進み、干魃による作物の収量の減少が生じ、食料や飼料などの生産にとって大きな問題となっている。 国連食糧農業機関（FAO）の2015-2030年の世界農業予測では、気候変動、特に気温上昇、降雨の年間分布の変化、降水量低下、土壌水分低下が上げられている。その対策として、干ばつに強い品種、高温耐性品種、耐塩性品種の開発が上げられている。 なお、従来の育種方法を用いて、干魃に強い系統の選抜と育種への利用が試みられているが、時間がかかりあまり目覚ましい成果は上げられていないのが現状である。近年急激に作物のゲノム研究が進展しており、旱魃に強い作物の開発のためには、これらの成果を利用した分子育種技術に期待がかけられている。そのため、ゲノム研究の成果を利用し作物の乾燥耐性に関わる遺伝子を明らかにして､それらの遺伝子を利用した遺伝子組換え技術を開発することが求められている。 (2)　ブラジル国における耐乾性・耐暑性のダイズ品種開発の必要性 ブラジルでは、ダイズの商業栽培が1960年代から南部地域で始まり、現在ではアメリカに次いでダイズ生産量第2位であり、ダイズ供給において重要な地位を占めている。ダイズ生産の増加に伴う耕作地の拡大によって、現在では乾燥地帯である中西部でも栽培が行われるようになり、近年、頻発する旱魃と水資源の枯渇懸念によりダイズの生産に大きな影響が出ている。このような状況で、ブラジルでは干魃に強いダイズの育種は、重要な研究開発目標となっており、遺伝子組換え技術を用いて乾燥に強いダイズの研究に取り組んでいる。 以上のとおり､耐乾性･耐暑性のダイズ品種開発の必要性は､ブラジル国において大変で重要であるだけでなく､地球規模の気候変動への対応策の一つとして、重要であり、対象地域・社会のニーズに合致していると言える。
	ターゲット・グループのニーズに合致しているか。	（PDM上の裨益者：Embrapaダイズ研究所）	Embrapaダイズ研究所は、1996年から遺伝子組み換えダイズの研究を始めている（除草剤耐性遺伝子の組み入れ)。また、Embrapaダイズ研究所とJIRCASは、1995年に共同研究合意書を締結し､「農牧輪換」や「南米ダイズ」プロジェクトを通じて研究協力が行われてきた。2003年度からは、遺伝子組換え技術を利用して、干ばつあるいは高温耐性ダイズを作出することを目的に共同研究が実施されている。このように、ブラジルでは、ダイズへの遺伝子導入技術の開発が進んできており、乾燥耐性を付与するために利用できる遺伝子を求めていた。しかし、分子生物学的研究や遺伝子研究はブラジルではまだ始まったばかりであり､我が国との協力でこれらの研究を発展させることが望まれていた。具体的には、圃場で利用可能な乾燥耐性の遺伝子組換えダイズの開発を成功させたいと強く期待していた。 このように、ダイズ研究所は、遺伝子組み換えダイズの研究実績を持ち、また、乾燥耐性遺伝子を持つダイズの開発ニーズを持っていたことから、ターゲット・グループのニーズに合致したプロジェクトであると言える。

	本プロジェクトが目指す効果は、ブラジルの開発政策に合致しているか。	国家計画等で耐乾性・耐暑性のダイズ品種開発が優先課題として位置付けられているか。	ブラジル国において大豆は、国内総生産、輸出、雇用の面で大変重要な穀物であり、ブラジル政府が重要視している生産分野である。農務省作成（Ministério da Agricultura, Pecuária e Abastecimento）（Ministry of Agriculture, Livestock and Supply）の「農業牧畜計画 2012/2013」（Agricultural and Livestock Plan 2012/2013）では、Embrapa に期待する役割として以下の点を挙げている。「地球規模の温暖化が農業生産に与える影響にどう対処するかについて貢献すること、すなわち、Embrapa が、食料安全保障および再生可能エネルギーのための他の作物の提供といった面で、農業研究が貢献することが求められている。このように、本プロジェクトが目指している「耐乾性・耐暑性のダイズ品種開発」は、ブラジル国の開発政策との整合性があると言える。
	日本の援助政策・JICA の援助実施方針との整合性はあるか。	対ブラジル国援助方針との整合性はあるか。	日本国の対ブラジル国の協力重点分野の一つは農業であり、また、ブラジル国で農業が重要に位置を占め、気候変動の影響に適応する取り組みを支援する方針を有する。本プロジェクトは、地球規模の気候変動、具体的には、「耐乾性・耐暑性のダイズ品種開発」を通じて、気候変動に適応することを目的に持つものであり、日本国の協力方針に合致している。
	手段としての適切性	プロジェクトのアプローチ、対象地域の選択は適切であったか。	本プロジェクトは、環境ストレス耐性ダイズの作出技術の開発を目標に、成果1「有用遺伝子の同定」、成果 2「プロモーターの単離と有用遺伝子との組み合わせの最適化」、成果3「プロモーターと有用遺伝子の組み合わせが導入されたダイズ系統の選抜」、成果4「」環境ストレス耐性を示す組み替えダイズ系統の選抜」に関する研究が進められている。成果 1 と成果 2 については、JIRCAS、東京大学、理化学研究所が、これまでに進めてきたシロイヌナズナやイネの乾燥ストレス応答や耐性獲得に関する研究の成果・知見をダイズに応用することを通じて、乾燥ストレス耐性遺伝子やプロモーター探索し、最適な組み合わせを見つけ出す。一方、Embrapa は主として、組み合わせの最適化が行われた導入遺伝子を用いて、これをブラジル国で栽培されているダイズ品種に導入し、圃場試験を行うことで乾燥耐性を示す、組み替えダイズ系統の選抜を進める。Embrapa は、1990 年代から遺伝子組み換えダイズに関わる研究を進めてきた実績を持ち、また、パーティクルガン法（particle gun method）と呼ばれる形質転換技術を持つ。このように、日本側およびブラジル側の研究機関が持つ優れた技術を持ち寄り、それぞれ役割を分担して、共同研究を進めることで、環境ストレス耐性ダイズの作出技術開発を進めることは、適切なアプローチであると言える。
		日本の技術の優位性はあるか。	JIRCAS、東京大学、理化学研究所ともに、乾燥耐性作物の基盤技術となる有用遺伝子やプロモーターの解析に優れた技術を有し、特に、JIRCAS は、DREB と呼ばれる環境ストレス耐性遺伝子についての特許を持つ。また、日本側は、アグロバクテリウム法（Agrobacterium）という、形質転換をより効率的に行うことが可能な技術を持ち、この技術を Embrapa に移転することができる。このように、日本側研究機関は、共同研究を行うにあたって有用な技術を有しており、技術的優位性があるといえる。

5 項目	評価設問		調査結果
	大項目	小項目	
有効性	プロジェクト目標は、達成される見通しか？「環境ストレス耐性ダイズの作出技術が開発される。」		指標 1、指標 2、指標 3 については、その数値目標を達成している。指標 4 については、乾燥耐性応答の特徴を有する系統が一つあることが確認されている。今後、プロジェクト活動が計画通り順調に進捗すれば、プロジェクト終了時までに環境ストレス耐性を持つ複数のダイズの系統が選定されることが期待される。 すでに述べたように、アグロバクテリウム法の形質転換効率がまだ低い。形質転換効率がある程度高くできた場合には、環境ストレス耐性ダイズの作出技術が十分満足できる水準で開発されたと評価できるであろう。

	プロジェクトのアウトプットはプロジェクト目標の達成に貢献しているか。	アウトプットは、プロジェクト目標を達成するために十分であったかどうか。「アウトプットがすべて達成されればプロジェクト目標は達成されるだろう」という論理に無理はなかったか。	プロジェクト目標を達成する上で必要なアウトプットが設定されており、論理上適切であると考えられる。
	外部条件の影響	（設定なし）	外部条件は設定されていない。なお、プロジェクト目標の達成上、特に影響を与えそうな外部要因は無いと考える。
	プロジェクト以外に貢献した要因はあるか。		特になし。
	プロジェクト目標達成を阻害する要因はあるか。		これまでの所、大きなマイナスの影響は生じていないが、材料移転契約（MTA）手続きの遅延が、プロジェクト活動の進捗を遅らせ、プロジェクト成果発現に影響を与える懸念がある。手続き上の遅延は、日本側研究機関側の問題と Embrapa 側の問題の両方があるが、特に Embrapa の法務部門での処理に時間を要している。 2010 年 10 月頃から 2012 年 8 月末まで、JICA 雇用によるテクニシャン（フルタイムでプロジェクト活動に従事）が 3 名いたが、契約期間が満了した。プロジェクト開始前に署名されたミニッツでは、プロジェクト後半の 2 年半は、ブラジル側が要員を雇用することになっていたが、法律的面からEmbrapa が、期間雇用であっても、新規の人員を雇用することは困難であるため、後任が不在となっている。このため、特に、アグロバクテリウム法を用いた形質転換作業が円滑に進展しない状況にある。

5 項目	評価設問		調査結果
	大項目	小項目	
効率性	アウトプットは、達成される見込みであるか。		アウトプットは、4 つ設定されている。各アウトプットの達成状況については、本文を参照のこと。
	達成されたアウトプットからみて、投入の質・量・タイミングは適切か。	日本人専門家派遣の人数、専門分野・能力、派遣のタイミング・期間は適切か。	(1)　プロジェクト活動に参加した日本の研究者 これまでに本プロジェクトの研究活動に参加した JIRCAS、理化学研究所、東京大学の研究者は、合計27 名である。研究機関別では、JIRCAS が 7 名、理化学研究所が 5 名、東京大学が 15 名（修士コースの学生を含む）である。詳細は、ミニッツの Annex 3 を参照のこと。 日本の研究機関（JIRCAS、理化学研究所、東京大学）の比較的多くの人数で、かつ高い資格を有する日本人研究者が本プロジェクトの研究活動に従事し、目標値以上の成果を生み出している。 (2)日本人研究者及び JICA 専門家のブラジル国への派遣 業務調整専門家（長期専門家）が 1 名派遣されている。また、短期派遣として、これまでに 9 名の研究者がブラジル国に派遣された。分野は、分子育種技術および植物分子生物学である。詳細は、ミニッツの　Annex 4 を参照のこと。 分子育種技術および植物分子生態学の分野の日本人研究者 9 名がブラジルに派遣された。派遣時の現地滞在期間は、大半が 10 日以下である。日本人研究者がもっと長い期間滞在できれば、技術協力においてより望ましいとの意見がある。

	研修員受け入れの人数、内容、時期などは適切か（本邦研修）	6 人のブラジル側研究者が日本（JIRCAS）での研修を受けた。研修分野は、「ダイズのストレス誘導性遺伝子の発現解析技術」、「ダイズの耐乾性遺伝子のプロモーターの分離技術」、「アグロバクテリウム法 を用いた形質転換と遺伝子発現解析技術」である。詳細は、ミニッツの Annex 5 を参照のこと。 ブラジル人研究者の日本での研修参加者は、これまでにバイオテクノロジー分野で 6 名である。日本での研修は、研究者の能力のさらなる向上に良い効果があり、また、プロジェクト活動の円滑な進捗に貢献していると思われる。プロジェクト活動に関連する分野は、バイオテクノロジー（分子生態学）だけではないので、必要に応じて、植物生態や植物育種などの分野のブラジル人研究者を日本に受け入れることも良いかも知れない。
	供与機材の種類、量、供与時期は適切か。	1) Embrapa ダイズ研究所への機材供与 JICA は、車輌、コンピュータやプリンターなどの事務用機器、研究活動用の各種機器を供与した。機器購入額は、60 万レアルと 3,890 万円である（参考： ドル換算値で合計が約 79.8 万ドル）。詳細は、ミニッツの Annex 6 参照のこと。 日本側が Embrapa ダイズ研究所に供与した機器類・施設は、プロジェクト活動のために効果的に使用され、プロジェクト活動の円滑な進捗に貢献していると思われる。 2) 日本側研究機関への機材供与 研究活動のための各種機器が JIRCAS、理化学研究所、東京大学のために調達された。調達された機器の購入額は、合計 1,790 万円である（参考： ドル換算値で約 23 万ドル）。詳細は、ミニッツの Annex 7 参照のこと。
	日本側の費用負担	日本側がブラジル現地での活動経費として支出した金額は、72.7 万レアル（参考： ドル換算値で約 35.8 万ドル）である。詳細は、ミニッツの Annex 8 参照のこと。
	カウンターパートの人数、配置のタイミング、能力は適切か。	＜プロジェクト活動に参加したブラジル国人研究者＞ プロジェクト活動に参加した研究員等は合計 29 名である。その大半は、Embrapa ダイズ研究所の研究者で、一部、大学の学生や JICA が雇用したテクニシャンが含まれる。中間レビュー時点では、20 名の Embrapa ダイズ研究の研究者、5 名の学生等がプロジェクト活動に従事している。詳細は、ミニッツの Annex 9 参照のこと。 ブラジル側も同様に、比較的多くの人数で、かつ高い資格を有する研究者及び学生が、Embrapa ダイズ研究所において、本プロジェクトの研究活動に従事している。プロジェクト期間の前半では、分子生物学分野のプロジェクト活動に焦点が置かれ、この分野の重要性は今後も継続する。残りのプロジェクト期間では、ストレス耐性のダイズの系統を選抜するには、生態学および育種の分野も重要になってくる。したがって、Embrapa ダイズ研究所のこれら分野の研究員・テクニシャンの参加度がさらに増加することが重要である。 Embrapa ダイズ研究所の常勤の研究者の本プロジェクトへの参加は、他の研究業務の抱えていることから、基本的にパートタイムでの従事であり、人数的には、それほど多くない。ラボでの作業は、その多くを、政府から奨学金得ている学生（博士課程、修士課程、学部学生）が担っている。基本的に、ブラジル国では、実験計画と実験から上がってくるデータの分析を研究者が行うという欧米のスタイルであり、研究者自身がラボでの作業を行うことはあまりない。 合意（2009 年 8 月 31 日に日本側とブラジル側とが署名したミニッツ）に基づき、日本側は、日本側は 3 名のテクニシャンを、2012 年 8 月末まで雇用した。合意書によれば、残りのプロジェクト期間においては、ポスドク（Post-Doctoral Fellow）の研究員・テクニシャンの雇用に関する費用は、ブラジル側が負担することになっている。しかし、Embrapa ダイズ研究所はこれら要員を雇用することが困難であり、プロジェクト活動、特にアグロバクテリウム法に関わる活動の進捗に影響を与えている。

		ブラジル側の機材調達	ブラジル側によって、プロジェクト活動のために各種の機器類が調達され、Embrapa ダイズ研究所の敷地にある新しいバイオテクノロジー・ビルあるいは化学分析棟などに配置された。機器類調達費用は、270 万レアルである（参考： ドル換算値で約 130 万ドル）。詳細は、ミニッツの Annex 10 参照のこと。 相当高額の分析機器等も調達されており、プロジェクト活動実施に必要な機器類の多くが整備されている。 各種の機器・施設が調達され、また新規のバイオテクノロジー・ビルが建設され、既存のEmbrapa ダイズ研究所の施設の利用もあり、これらの投入は、プロジェクト活動の円滑な進捗に良い影響を与えている。新規のバイオテクノロジー・ビルの建設に遅れが生じたものの、プロジェクト活動に大きな影響を与えることはなかった。日本側の費用を用いて、4 つの仕切られた部屋を有する温室が建設され、その内、2 部屋については、日本側費用により、今後数ヶ月以内に冷房施設が設置される予定である。しかしながら、残り 2 室分の冷房施設の調達については、まだブラジル側による資金手当が行われていない。
		事務室等の規模､利便性は適切か。	プロジェクト活動のために Embrapa ダイズ研究所が提供している施設は次のとおりである。 1) 日本人専門家及び研究者の執務室 2) 既存のバイオテクノロジー・ビル： 424m^2 3) 新規のバイオテクノロジー・ビル（新規にブラジル側が建設）： 581m^2 4) 生態生理学オフィス： 120m^2 5) 生態物理・化学分析室： 100m^2 6) Embrapa ダイズ研究所の温室： 1,181m^2 7) JICA 費用で建設した温室の用地提供： 314m^2 8) 遺伝子組み換え（GMO）種子の保管庫： 160m^2 9) GMO 用の温室： 237m^2 10) Embrapa ダイズ研究所の圃場試験用地： 28,300m^2（2.8ha） かなり多くの施設が、本プロジェクトの活動に使用されており、その利便性も高い。
		ブラジル国側のプロジェクト予算は適切な規模か。	ブラジル側は、事務スペースや研究活動のための光熱費等を負担した。 なお、上記のとおり、ブラジル側は、機材調達・ラボの建物建設に約 1 億円の資金を支出し、また、専門家執務室、研究室、温室、圃場での活動経費（光熱費や消耗品等）に相当の予算を支出している。予算規模は、かなり大きいと言える。
		研究試料提供契約書(MTA)	コンストラクトを日本から Embrapa ダイズ研究所に送る場合、また、Embrapa ダイズ研究所から日本にダイズ種子を送る場合、Embrapaダイズ研究所と日本の当該研究所間で、毎回、材料移転契約（MTA）が必要となる。通常、Embrapa 内で MTA 書類の審査に多くの時間を要しており、プロジェクト活動の円滑な進捗に影響を与えている。
	投入は十分活用されているか	供与機材等は有効に利用されているか	有効に活用されている。
	効率性を阻害した要因はあるか。	C/Ps の定着度は、良好か。	ブラジル側で本プロジェクトに参加している研究者のうち、Embrapa ダイズ研究所の職員については、継続性が高い。一方、学生の場合は、ラボでの作業を担当することが主な役割であり、プロジェクト期間中での交替は当然生じる。 なお、本邦研修に参加したブラジル研究者のうちの数人については、本プロジェクトとの関係が無くなっているケースがある。本邦研修者を選定する場合、少なくともプロジェクト期間中は、本プロジェクトに継続的に従事する研究者を受け入れすべきであると考える。
		その他の要因はあるか。	特に効率性に大きなマイナスの影響を与える要因は、これまでのところなかった。

5項目	評価設問		調査結果
	大項目	小項目	
インパクト	上位目標「環境ストレスに適応したダイズが開発され、ブラジルのダイズ生産の安定化に資する。」		上位目標が目標年である2019年までに達成されることは難しく、さらに数年を要すると考えられる。市場に流通するダイズの系統が開発されれば、ブラジル国内だけでなく、世界的にも大きなインパクトをもたらすと考えられる。
	上位目標を達成するために必要な方策が考えられているか。		本プロジェクトでは、環境ストレス耐性を持つダイズの系統を少なくとも1種類、選抜するところまでが目標である。それ以降、商品化できるダイズの品種の開発には、安全性確認試験や土壌管理試験を経る必要があるが、これら試験については、Embrapaダイズ研究所が実施してきた経験と能力を有することから、特に、新たな方策を講じる必要性は少ない。 なお、今回の中間レビュー調査時にEmbrapaダイズ研究所からは、一般農家に広く普及可能なダイズ品種を開発するには、乾燥耐性だけでなく、それに加えて、除草耐性の遺伝子も組み入れることが必要であり、その活動を民間企業を組み込んで、本プロジェクトとともに実施したいとの提案があった。JICA調査団としては、プロジェクトの枠組み内で、そのような活動を組み入れることは、本プロジェクトにマイナスの影響を与えかねないとの判断し、この提案に対してNoとの回答を返した。ただし、現状では、ブラジル国で生産されているダイズの85%は、除草耐性を持つ遺伝子組み換えダイズが栽培されていること、世界的にも、除草耐性の遺伝子組み換えダイズが栽培されている現状を考慮すれば、今後、除草耐性も併せ持つダイズの品種を開発することが、実際に農家に普及する品種開発につながるものであると考える。
	上位目標達成のための外部条件が影響する可能性	（設定なし）	特になし。
	ターゲット・グループ以外に波及した影響はあるか	これまでのプロジェクト活動を通じて、ターゲット・グループ以外へ波及したインパクトの事例があるか。	本プロジェクトとは、直接関係ないが、ブラジル国内の研究機関は、植物が乾燥耐性を持つうえで、DREB遺伝子が大変重要であるとの認識を持っており、DREB遺伝子提供についての要望が多くある。JIRCASは、要望を受けて、綿花、サトウキビ、コーヒー、インゲン豆に関する研究を行っているEmbrapa研究所に、DREB遺伝子を提供した。将来的には、他の作物でも乾燥耐性を有する作物が開発されるかも知れない。
	その他の正負のインパクト	その他のインパクト	特になし。

5項目	評価設問		調査結果
	大項目	小項目	
持続性（見込み）	今後も、国家開発計画や農業セクター戦略等の関連政策において、耐乾性・耐暑性のダイズ品種開発の重要性が継続するかどうか（見込み）。		妥当性の項で述べたように、ブラジルではダイズは、国内総生産、輸出、雇用の面において極めて重要な作物の一つであり、連邦政府は、重要な生産セクターの一つであると位置付けている。Embrapaダイズ研究所の重要な役割の一つは、地球温暖化が農業生産面に与える影響を少なくすることに貢献することである。したがって、本プロジェクトの政策面での持続性は確保されると判断される。
	カウンターパート機関（Embrapaダイズ研究所）等では、本プロジェクトがどのように認識されているか。		Embrapaダイズ研究所では、本プロジェクトを大変重要なものであると考えている。なお、Embrapaダイズ研究所の所長からは、活動内容について、「アカデミックなものから、もっと応用的なものにして欲しい」との発言があった。これは、インパクトの項で述べたように、民間企業と連携して、乾燥耐性ダイズに除草耐性遺伝子も組み込んで、より市場が受け入れるダイズ品種を開発したいとの意向がある。この動き次第では、本プロジェクトに対するブラジル側の優先度が変化する可能性もある。

	カウンターパート機関に、本プロジェクトの成果（環境ストレス耐性ダイズの作出技術）を活用・発展させていくために必要な組織体制があるかどうか。（組織面）	プロジェクト終了後、Embrapa ダイズ研究所)は､環境ストレス耐性ダイズの作出技術を活用して､環境ストレスに適応したダイズの開発を円滑に実施できる組織体制を持っているかどうか。	Embrapa ダイズ研究所は、遺伝子組み換えダイズを開発するために必要な部署・組織体制、例えば、バイオテクノロジー、生態学、育種などの部署を有しており、高い資格を持つ研究者、テクニシャン、学生（ブラジル政府の奨学金をもらいつつ、Embrapa ダイズ研究所で研究活動に従事している）がいる。また、Embrapa ダイズ研究所は、民間企業と共同で遺伝子組み換えダイズ（除草剤耐性）の開発を成功させた実績を有する。したがって、環境耐性ダイズの開発を継続するに必要な組織面での持続性を有している。 以下、Embrapa ダイズ研究所の職員数についての情報。 1. 正規職員数： 310 人（研究者 70 人と研究補助者 240 人） 2. その他のスタッフ 2-1 技術移転担当： 7 人 2-2 学生： 167 人 2-3 パートナー及び外部委託労働者： 85 人 合計： 569 人
	カウンターパート機関には、本プロジェクトの成果を活用・発展させていくために必要な資金が確保されているかどうか、あるいは資金を獲得する能力を身につけているかどうか。（資金面）	プロジェクト終了後、Embrapa ダイズ研究所が独自資金で､環境ストレスに適応したダイズの開発を継続する資金的能力があるかどうか。	投入の項で述べたように、Embrapa は、機器類調達と新規のバイオテクノロジー・ビル建設などのためにかなり大きな予算を支出している。したがって環境耐性ダイズの開発を継続するに必要な資金的持続性は確保されていると言える。 なお、Embrapa ダイズ研究所の年間予算は、約 3,000 万ドル（約 23.4 億円）。Embrapa 全体の年間予算は、約 10 億ドル（約 780 億円）。
	カウンターパート機関の関係職員は、本プロジェクト終了後も、適切に、プロジェクトの成果を継続的に活用・実施できる能力を身につけているかどうか。また、プロジェクトに参加した職員の勤務の継続性があるかどうか。（技術面）	プロジェクトに参加した Embrapa ダイズ研究所の研究員の技術水準が十分高いかどうか。また､プロジェクトに参加した研究員の勤務の継続性があるかどうか。	Embrapa ダイズ研究所は、除草剤耐性の遺伝子組み換えダイズ開発実績を有する。また、Embrapa ダイズ研究所は、2003 年から JIRCAS と共同でストレス耐性ダイズの研究を行ってきている。したがって、Embrapa ダイズ研究所は、この分野で非常に高い専門性を有する。技術面で改善が必要な点は、アグロバクテリウム法による形質転換効率であり、現在、Embrapa ダイズ研究所が、そのプロトコールの最適化を進めている。いったん、プロトコールが最適化されれば、一定の望ましい効率で形質転換が継続可能となる。環境耐性ダイズの開発を効率的・効果的に継続するための技術的持続性は確保可能と考えられる。
	供与資機材の維持管理は適切に行われているか。また、協力終了後も適切に行われる見通しはあるか。		機器類及び各種施設は、適切に維持管理されており、協力終了後も適切に行われる見通しである。資金面でも適切な維持管理・補修を行う能力がある。
	持続性に影響を与える貢献・阻害要因は何か。		Embrapa ダイズ研究所は、その研究能力、資金力、組織体制において高いものを持っているので、持続性にマイナスの影響を与える要因は少ないと考える。

<table>
<tr><th></th><th colspan="2">評価設問</th><th rowspan="2">調査結果</th></tr>
<tr><th></th><th>大項目</th><th>小項目</th></tr>
<tr><td rowspan="5">実施プロセス</td><td>当初計画した成果を達成するためにどのような計画・実施体制の変更・軌道修正が行われたか</td><td>プロジェクト実施中に把握されていた課題は何か。その課題はどのように解決されたか</td><td>計画・実施体制については、特に、大きな変更や軌道修正は行われていない。
実施体制としては、ブラジル側プロジェクト・リーダーが、アメリカのラボに赴任し、不在であること、そして、リーダー代行を置いている状況であるが、プロジェクト・リーダーが、関係者と頻繁に連絡を取り合っていることで、これまでのところ、プロジェクトの進捗に大きな影響をもたらしていない。
JICA 雇用のテクニシャンの雇用期間が 2012 年 8 月末で終了したことに伴い、アグロバクテリウム法による形質転換作業にフルタイムで従事するテクニシャンが不在になった。これについては、英文の中間レビュー報告書で指摘し、Embrapa 側の善処を求めたところ、Embrapa ダイズ研究所の正職員の 2 名をパートタイムからフルタイムでのプロジェクト活動従事にするとの回答を得ている。しかしながら、アグロバクテリウム法のプロトコール最適化が進めば、実際に手を動かして作業するフルタイムのテクニシャンの配置が必要になる。
中間レビュー時のブラジル側のプロジェクト実施体制については、ミニッツの Annex 13 参照のこと。</td></tr>
<tr><td>共同研究や技術移転の方法に問題はなかったか。</td><td>問題がある場合、どの分野におけるどのような共同研究や技術移転方法に問題があったか。どのように解決されたか。</td><td>全般的には、共同研究活動や本邦研修における技術移転に大きな問題点は見られない。ただし、日本人研究者のブラジル派遣については、現地滞在期間が極めて少ない点（多くが 10 日以下）と、その際の技術交流が必ずしも十分に行われていない、といった意見がブラジル側にある。日本人研究者とブラジル人研究者との技術交流については、あらかじめその内容等について、十分調整し、より実りある技術交流にする必要がある。</td></tr>
<tr><td rowspan="2">相手国のオーナーシップ</td><td>①C/P 配置の適正さ
②予算手当ては適切か</td><td>研究者等の配置と予算手当については、概ね適切である。</td></tr>
<tr><td>Embrapa の本プロジェクトについての認識や参加度は高いか。</td><td>本プロジェクトに対する認識は高いと言える。参加度については、概ね良好であると考える。</td></tr>
<tr><td rowspan="2">プロジェクトのマネジメント体制に問題はなかったか。</td><td>JCC は、必要な時期に実施され、必要なテーマが話し合われていたか</td><td>
<table>
<tr><th>No</th><th>年月日</th><th>議論・テーマ</th><th>備考</th></tr>
<tr><td>1</td><td>2010 年 11 月 29 日</td><td>プロジェクト活動の進捗状況と 2011 年の作業計画
プロジェクトの円滑な実施に関わる事項</td><td></td></tr>
<tr><td>2</td><td>2012 年 3 月 9 日</td><td>プロジェクトの全体的進捗状況と 2011 年の成果
2012 年の活動計画（機材と研究員交換含む）
プロジェクトの円滑な実施に関わる事項</td><td></td></tr>
</table>
JCC は、最低年 1 回開催することになっている。1 回目と 2 日目の開催間隔が 1 年 3 ヶ月あり、若干開いているが、概ね良好と判断する。</td></tr>
<tr><td>その他の定例会議等を通じて、プロジェクト・チーム内（専門家、関係機関関係者及びカウンターパート）の意志決定メカニズムが十分機能しているか。</td><td>JCC 開催前には、技術的委員会が開催されている。これ以外については、ブラジル側のプロジェクト関係者の一部が、ミーティングを開催しているようであるが、ブラジル側の関係者全体（JICA 業務調整専門家と派遣研究員を含む）での定期的なミーティングは開催されていない。コミュニケーションの改善の余地がある。</td></tr>
</table>

		プロジェクトの進捗状況は、どのようにモニタリングされていたか。	JCC 等のミーティングを通じて、プロジェクトの進捗状況がモニタリングされている。
		日本人専門家・研究員とカウンターパート機関の研究員等とのコミュニケーションは、円滑に行われているか。	現地派遣日本人研究者とブラジル側研究者との間のコミュニケーションや情報共有が必ずしも十分に行われていない状況が見られた。
		JICA ブラジル事務所及び JICA 本部との連絡・協力が円滑に実施されたか。	概ね良好な連絡・協力が行われている。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