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Review

Future challenges in environmental risk assessment of transgenic plants with abiotic stress tolerance

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Environmental risk assessment of transgenic plants is a prerequisite to their release into the target environment for commercial use. Risk assessment of the first generation transgenic plants with simple monogenic traits has been carried out with principles and guidelines enlisted in the Cartagena Protocol on Biosafety. For more complex traits such as abiotic stress tolerance, there is a growing need to examine for additional considerations in the risk assessment process based on the different nature of this trait. The salt tolerance-inducing *codA* gene is a representative of many abiotic stress tolerance genes that confer salt stress tolerance in transgenic plants. In comparison with simple monogenic Bt trait, the future challenge to environmental release of abiotic stress tolerance genes lies in the question whether these genes such as the salt tolerance-inducing *codA* will need additional considerations in the risk assessment process?. In the present work, we discussed the nature of abiotic stress tolerance trait, environmental risk assessment issues and comparison of the risk assessment elements on *Bt* and salt tolerance-inducing *codA* genes to examine needs for additional considerations in the risk assessment process. We concluded and recommended that the use of abiotic stress tolerance genes such as the salt tolerance-inducing *codA* gene in transgenic plants does not need additional considerations in risk assessment.

Key words: Transgenic plants, abiotic stress tolerance, environmental risk assessment, salt tolerance-inducing *codA*.

INTRODUCTION

Since commercialization in 1994, there has been substantial progress in the development and uses of transgenic plants. In 2009, the number of countries that adopted commercial cultivation of transgenic plants reached to 25 (James, 2009). During the period from 1996 to 2008, the total area under transgenic crops expanded from 1.7 million to 125 million ha (Qaim and Subramanian, 2010).

Environmental risk assessment has been a key issue of concern surrounding transgenic plants and their release into the environment (Nuclear Regulatory Commission, 2002; Andow and Zwahlen, 2006; Chandler and Dunwell, 2008). Since development of the first generation of transgenic plants with insect resistance and herbicide tolerance, issues related to environmental risk assessment have been dealt with according to the principles and guidelines laid down in the Cartagena Protocol on Biosafety to the Convention on Biological Diversity, and those formulated by the Organization for Economic Cooperation and Development (OECD) (Sumida, 1996; Baum and Madkour, 2006; Nickson, 2008). These guiding principles are being used to ensure that genetically modified plants with new traits do not pose adverse effects to the environment and to human and animal health.

During the last two decades, a large number of crop plants have been engineered with genes conferring abiotic stress tolerance traits such as salt, drought and extreme temperatures (Cherian et al., 2006; Bhatnagar et al., 2008). In the near future, these plants will be deployed for use in the abiotic stressed environment. Due to the complex nature of abiotic stress tolerance, the emerging challenges confronting deployment of these transgenic plants are mainly regulatory and environmental risk assessment (De Greef, 2004; Wolt et al., 2009). Transgenic plants with abiotic stress tolerance genes are confronted with issues, such as increased potential of persistence and invasiveness, and unpredictable non-target effects (Mallory and Zapiola, 2008; Warwick et al., 2009; Wolt et al., 2009). Recently, some abiotic stress tolerant transgenic crop plants such as maize, wheat, cotton and trees such as poplar and eucalyptus are under field trials (office of the gene technology regulator, 2008a, b, c, d; Kikuchi et al., 2006). Some other plants, engineered with genes encoding regulatory proteins, osmoprotectants and ion transporters are already in the pipeline to enter field trials. Therefore a scientific debate is timely to focus on the risk assessment issues on transgenic crops with genes conferring abiotic stress tolerance.

For environmental risk assessment of transgenic plants with abiotic stress tolerance genes, there has been a wide consensus that the current risk assessment paradigms are scientifically-sound and sufficiently robust. However, due to the specific nature of abiotic stress tolerance trait, there is a growing need to investigate whether risk assessment of these traits requires additional needs. To achieve this purpose, a comparative analysis of the basic risk assessment issues on insect resistance and abiotic stress tolerant transgenic plants was conducted to find: 1) whether additional risk assessment elements to be considered in the use of abiotic stress tolerance genes such as the salt tolerance-inducing *codA* gene; 2) whether different strategies or measurements are needed in the risk assessment methodologies to assess these additional risk considerations.

THE CARTAGENA PROTOCOL ON BIOSAFETY (CPB)

The cartagena protocol on biosafety (CPB) originated from the Convention on biological diversity (CBD) in 2000 and came into force in 2003 (Kinderlerer, 2008). It is a legal framework that deals with international movement of living modified organisms (LMOs). The main characteristics of the CPB are: 1) it distinguishes between import of LMOs for planting or for food; 2) it establishes risk assessment and risk management of LMOs; 3) it establishes biosafety clearing house (BCH); 3) it provides for capacity building, public awareness and participation; 4) socio-economic considerations (Baum and Madkour, 2006). In order to evaluate the potential risks of the LMOs in the target environment, the CPB provides certain regulations, listed in Annex 3 of the document. The main objective of this risk assessment process is to evaluate the potential adverse effects of LMOs on the conservation and sustainable use of biological diversity in the likely potential receiving environment, taking also into account risks to human health. The major principles listed in Annex 3, state that risk assessment should be carried out in a scientifically sound and transparent manner, risks associated with LMOs and/or their products should be considered in the context of the risks posed by the non-modified parental organism in the potential receiving environment, and risk assessment should be carried out on a case-by-case basis. Moreover, an important element of Annex III is the precautionary principle which states that “lack of scientific knowledge or scientific consensus should not necessarily be interpreted as indicating a particular level of risk, an absence of risk, or an acceptable risk.” Environmental risk assessment of transgenic plants uses the precautionary approach, because a lack of knowledge and unavailability of appropriate data regarding a potential ecological risk makes the assessment process difficult (Kinderlerer 2008). Based on the precautionary approach, a risk that has no scientific evidence or very low possibility to cause harm is considered for assessment.

PROCESS OF ENVIRONMENTAL RISK ASSESSMENT

Environmental risk assessment is a process based on scientific principles that aimed to evaluate the potential adverse effects of transgenic plants on the environmental entities of value. Risk is often defined as the probability of direct or indirect harm that a potential hazard may cause to the environment and human health and is the combination of the probability of environmental exposure to the hazard and the probability that the adverse effect will occur (Andow and Zwahlen 2006; Galatchi 2006). The conventional risk assessment process follows four major steps which are: hazard identification and assessment that examine the potential hazard; exposure assessment that includes levels and likelihood of exposure; effects assessment; and finally risk characterization that integrates hazard, magnitude of the potential consequences and the likelihood of occurrence (Nuclear Regulatory Commission, 1983; EPA ,1998).

In case of transgenic plants, the initial step of risk assessment is termed as problem formulation (Figure 1). Problem formulation as the beginning of risk assessment, is an important step that leads the risk assessment process to successful risk characterization provided all individual components of this step are fully defined and integrated (Raybould, 2006; Wolt et al., 2009). Problem formulation begins with identifying assessment endpoints, developing a conceptual model and analysis plan (EPA, 1998). Nickson (2008) elaborated the problem formulation step with all its components in the context of environmental risk assessment of transgenic plants. The author emphasized the need for clear identification of assessment endpoints that must be some valued ecological entities to which the adverse effects are assessed. Developing a conceptual model is essential for generating risk hypothesis that leads to make assumptions

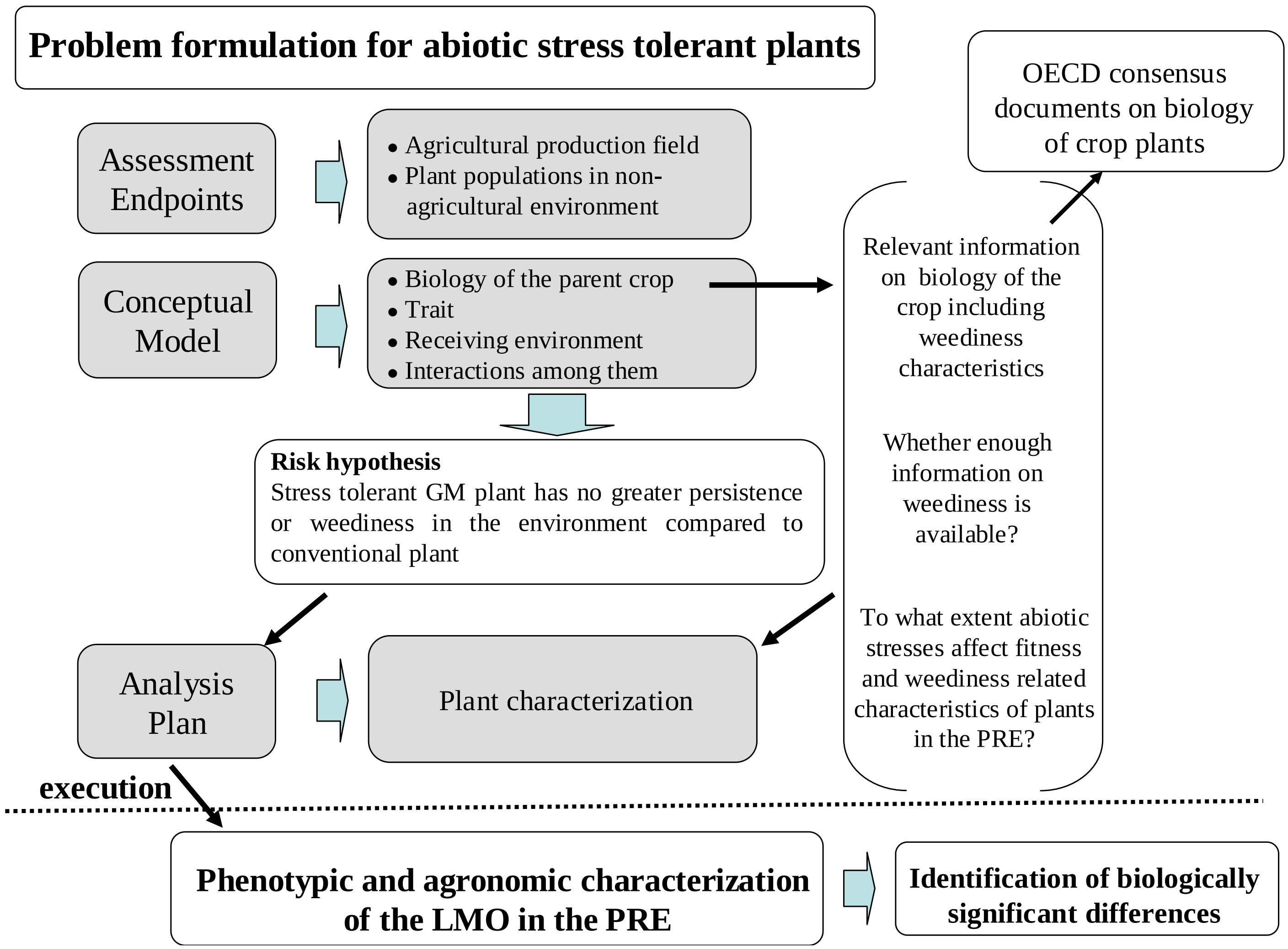

Figure 1. Schematic representation of the various steps of problem formulation in environmental risk assessment of transgenic plants. Problem formulation as the first step of risk assessment has three steps i.e. identifying assessment endpoints, developing a conceptual model and developing an analysis plan. Analysis plan is then executed for phenotypic and agronomic characterization of the LMO in the potential receiving environment and based on this, biologically significant differences are identified which are then subjected to the risk assessment process (modified from Nickson 2008).

regarding the potential effects of the stressor on the assessment endpoints. A conceptual model establishes links among the assessment endpoints, the stressor, exposure pathways and potential environmental effects. Analysis plan as the last step of problem formulation describes the nature of data needed and the kind of approach that is used for data collection.

Since commercialization of the first generation of transgenic plants with insect resistance and herbicide tolerance, risk assessment and its components have been successfully used to evaluate adverse environmental effects of these crops. Until now, no large environmental adverse effects of these crops have been documented and such risk assessment results provide limited basis for assessing risks of future transgenic plants modified with complex traits (Andow and Zwahlen, 2006; Wolt, 2009). The risk assessment process has never been complete and has continuously evolved over the years to address the emerging environmental constraints associated with transgenic plants. The new generation transgenic plants with complex traits may raise environmental concerns with unpredictable ecological consequences, which will require careful consideration of the existing risk assessment methodologies. Abiotic stress tolerance is one of such traits under investigation for potential environmental risks. Transgenic plants modified with abiotic stress tolerance genes are confronted with questions such as whether the risk assessment process will rely on the same elements with conservative methodologies for assessing ecological impacts; and whether additional issues to be included for consideration in order to assess their environmental effects.

Risk assessment of transgenic plants with genes conferring abiotic stress tolerance

The current strategy of developing transgenic plants with increased abiotic stress tolerance is mainly based on the use of regulatory and metabolic genes. Many of these genes may affect several aspects of plant development and fitness through their important roles in regulating gene expression, signal transduction, and influencing metabolic pathways. Due to this complex coordination among various elements of stress response mechanisms, the introduced genes with one stress tolerance may often influence responses to multiple abiotic stresses (Chinnusamy et al., 2004; Chan et al., 2009). These complex changes may also have the potential to induce unintended or secondary effects, which are considered to have unpredictable ecological consequences.

A number of crops and traits are under confined field trials in Canada, Australia and the United States (Warwick et al., 2009; Beckie et al., 2010). In addition, transgenic plants with drought, salt and other abiotic stress tolerance genes have entered field trials for risk assessment studies throughout the world (Table 1). Risk assessment studies of some transgenic plants have been completed, while others are still under investigation for their effects on the environment and biodiversity. One such example is the risk assessment of transgenic wheat and barley with drought tolerance, under field trials in Australia (OGTR, 2008a, b). Environmental risk assessment of transgenic wheat with salt tolerance was also conducted (OGTR, 2005). Similarly, transgenic eucalyptus, cotton and sugarcane with abiotic stress tolerance genes are under field trials for risk assessment studies (BCH, 2005; 2007; OGTR 2008c, d; OGTR 2007; OGTR 2009). Moreover, the Monsanto led drought tolerant maize is under development that is expected to be launched in the USA in 2012 and in Sub Saharan Africa by 2017 (James, 2008). Studies on other drought tolerant crops such as soybean and cotton are in the pipeline (Monsanto, 1995).

Assessment of adverse environmental effects of these plants is based on the same basic risk assessment paradigm that has been used for Bt-transgenic crops. However, there is a growing need to search for additional needs on a case-by-case basis that may be required in the risk assessment of transgenic plants with abiotic stress tolerance genes. In the following section we will discuss the specific nature of abiotic stress tolerance trait and the environmental concerns that may arise. This will highlight the needs for any further considerations to be taken in the risk assessment process.

Nature of abiotic stress tolerance trait

The case of transgenic crops with genes conferring abiotic stress tolerance is different and more complicated than that of insect resistant or herbicide tolerant plants. Abiotic stress tolerance is a quantitative trait controlled by many genes working in several stress response pathways (Vinocur and Altman, 2005). Transformation of crop plants with genes encoding regulatory, metabolic and membrane proteins confer stress tolerance by influencing gene regulation, signal transduction and intersecting metabolic pathways (Shinozaki and Yamaguchi, 2007; Bhatnagar et al., 2008; Khan et al., 2009). For example, regulatory genes encoding transcription factors function as “master switches” that induce expression of a large number of genes involved in stress tolerance. In addition, these transcription factors also mediate expression of other genes working in plant physiological and developmental processes. Therefore, the term “abiotic stress tolerance” is too limiting to define and encircle the magnitude of molecular, metabolic and physiological changes that occur at the whole plant level. The process involves changing the whole architecture of the plant to confer abiotic stress tolerance. In such situation, the risk assessment process will focus on the whole plant and the receiving environment, where the plant is introduced (Chaves et al., 2003). Abiotic stress tolerance is a fitness enhancing trait that confers selective advantage under stress conditions and may increase competitive ability of transgenic plants compared to their conventional counterparts. Therefore, risk assessment of such crops will logically focus on questions of increased volunteer and weediness potential in agricultural environments and invasiveness in natural environments. Other than weediness and invasiveness potential, evaluation of other ecological and non-target effects will require full understanding of the stress-associated physiological and metabolic changes that occur during abiotic stress tolerance. So far, the technology has met with limited success to fully explore the knowledge and understanding of the required metabolic changes that occur during abiotic stress tolerance (Vinocur and Altman, 2005). Due to these knowledge gaps in understanding of stress-associated metabolic profiling, the problem formulation step in the risk assessment would require an appropriate comparative approach and analysis plan to consider the consequences of metabolic changes with respect to weediness, invasiveness and other non-target effects (Wolt et al., 2010).

ABIOTIC STRESS TOLERANCE, FITNESS, WEEDINESS AND INVASIVENESS POTENTIAL

Abiotic stress tolerance is considered to be a fitness enhancing trait that increases the reproductive and vegetative growth and competitive ability of plants subjected to selection pressure. Compared to the first generation insect resistant and herbicide tolerant crop plants which have not been more invasive in natural habitats (Beckie et al., 2006; Beckie and Owen, 2007), the second generation transgenic plants with abiotic stress

Table 1. Examples of abiotic stress tolerant transgenic crop plants under field trials for risk assessment studies.

Abiotic stress category	Transgene	Crop	Implementing organization	Reference
Drought tolerance	*TaDREB2/TaDREB3*	Wheat/Barley	The University of Adelaide	OGTR (2008a)
Drought tolerance	CCI	Wheat	Victorian department of primary industries	OGTR (2008b)
Drought tolerance	Pyrroline-5-carboxylate reductase (P5CR)	Soybean	The agricultural research council (ARC), South Africa	De Ronde (2005)
Drought tolerance	*OsDREB1A, ZmDof1*	Sugarcane	BSES Limited, Australia	OGTR (2009)
Salt tolerance	Ornithine aminotransferase (*oat*)	Wheat	Grain Biotech Australia, Pty Ltd	OGTR (2005)
Salt tolerance	Choline oxidase (*codA*)	*Eucalyptus camaldulensis*,	University of Tsukuba, Japan	Japan Biosafety Clearing-House (2005) and Kikuchi et al. (2006)
Salt tolerance	*codA*	*Eucalyptus globulus*	University of Tsukuba, Japan	Japan Biosafety Clearing-House (2007)
Water use efficiency	Transcription factors	Cotton	Monsanto Australia Limited	OGTR (2008c)
Water logging	*Adh*/*Pdc2*	Cotton	CSIRO Australia	OGTR (2008d)
Water use efficiency/Nitrogen use efficiency	*OSMAX3*, *OSMAX4-1*, *SOTB1*, *EcTPSP*, *AtMYB2*, *ZmDOF1*	Sugarcane	BSES Limited, Australia	OGTR (2006)
Freeze tolerance	*CBF* transgene	Eucalyptus	ArborGen	USDA (2009)

(-) Information is not known; CCI, confidential commercial information; OGTR, Office of the Gene Technology Regulator; CSIRO, Commonwealth Scientific and Industrial Research Organisation

tolerance genes have more potential to confer increased fitness under stress conditions. Increased fitness advantage of transgenic plants, in turn, may confer persistence or volunteer potential in agricultural environments and invasiveness in natural environments (Ellstrand and Hoffman, 1990; Ellstrand, 2001; Lu, 2008). Grant (1981) indicated that abiotic stress tolerance trait may extend the range of transgenic plants beyond the area where they were previously cultivated, to areas closer to their wild relatives. This may increase the likelihood of transgenic plants to sexually hybridize with their wild relatives, to which they had never previously hybridized due to geographic isolation. In similar fashion, drought or salt tolerance trait transfer to wild and weedy relatives could confer them selective advantage under abiotic stress conditions. Compared to adjacent plant populations, the wild relatives with increased reproductive and vegetative growth may cause damage or replace the former. For example, salt tolerance in transgenic crops and their weedy hybrids may enable them to colonize, reproduce and spread to saline areas where other plant species can not easily grow (Warwick et al. 2009). This situation is quite different from that of insect resistant Bt crops, where the ecological impact assessment of transgene escape to weedy relatives is difficult to predict due to limited knowledge of the role of Bt-susceptible herbivores in regulating the density and range of crop weedy relatives. It is speculated that the abiotic stress tolerance transgenes in wild relatives will have the same fitness advantage under stress condition with unpredictable ecological consequences. The ecological consequences may appear in the form of abundance of weeds, replacing or damaging populations of other species in natural environments. In agricultural fields, increased fitness may increase the volunteer and weediness potential of transgenic plants resulting increased burden on weed management practices.

Fitness may be defined as the ability of an individual of a certain genotype to reproduce or fitness is the number of alleles that an individual contributes to the next generation (Orr, 2009). An allele that increases survival or seed production has higher fitness and it will increase and multiply in the population. Transgenes may confer significant ecological advantage, if they increase the recipient plants competitiveness and invasive ability and decline in herbivores or plant pathogens that limit their growth and reproduction (Weis, 2005). However, fitness enhancing transgenes may not necessarily increase weediness and invasiveness potential due to the many plant characteristics associated with weediness. Backer (1991) listed 13 plant characteristics that may contribute to weediness. Some of these are; 1) rapid seedling growth, discontinuous germination and prolonged seed production; 2) high seed output under favourable conditions; 3) self compatible; 4) germination and seed production under a wide range of environmental conditions; 5) special adaptations for dispersal; 6) highly competitive through allelochemicals and; 7) vigorous vegetative reproduction in case the plant is perennial. These characteristics have played an important role in the evolution of weedy species. Crop plants that are domesticated to agricultural conditions have lost many of these characteristics and have acquired domestication traits which are opposite to weediness traits.

Abiotic stresses are one of the factors that limit the ability of crop plants to develop self sustaining populations under cultivated or natural environments. Tolerance to abiotic stress may increase biomass, survivorship and fecundity in crop volunteers and wild and weedy relatives under stress conditions. However, increase in these parameters such as fecundity may not predict population expansion or invasiveness (Cummings and Alexander, 2002). Weed species may be good models to predict the ecological impact of a single or multiple stress tolerance traits in the host/target environments (Warwick et al., 2009). The consensus point is that there are limited data available which could evaluate the potential for increased weediness or invasiveness in a transgenic plant with fitness-enhancing abiotic stress tolerance trait. This poses a serious challenge for evaluation of weediness potential during phenotypic and agronomic characterization of the transgenic plant in the risk assessment process.

Although these concerns regarding abiotic stress tolerant transgenic plants have been raised in several research articles, evidences rather establish that there is almost no or negligible risk of transgenic abiotic stress tolerant plants to become weeds or attain increased persistence or volunteer potential in agricultural environment and invasiveness in natural environment. It is well established that the present day modern abiotic stress tolerant crop varieties, developed through conventional approaches also involved the same alteration of stress-related physiological and metabolic profiles. However, none of these conventionally bred crop varieties was found with an increased potential of persistence in agricultural habitats and invasiveness in natural habitats. In addition, pollen mediated gene flow is a natural process that occurs between crops and their weedy and wild relatives. From conventionally developed drought and salt tolerant plants, no reports have been documented so far that could claim transfer of these fitness enhancing traits to their wild relatives resulting adverse ecological consequences. Moreover, biological regulation of pollen-mediated gene flow through chloroplast transformation and production of apomictic seeds could be attractive options for mitigating environmental constraints that could arise from these plants. However, these technologies are not fully developed to be implemented for practical application (Watanabe et al., 2005).

The escape of transgenic plants to non-agricultural environment and establish there as weeds is not logical in the sense that crop plants have adapted to agricultural environments through a long process of domestication. During this process, these crop plants have lost most of the typical weed like characteristics such as seed shedding, bare seeds production, and rapid vegetative growth. The OECD consensus documents on the biology of crop plants reveal that these crops have almost no weediness characteristics, can not compete with grasses, trees and shrubs and are unable to establish in non-agricultural environments[1].

Moreover the existing management practices can be easily used to control any volunteers of these abiotic stress tolerant transgenic plants in agricultural environment. Therefore, these plants do not pose any weediness or invasiveness concern for the environment. As the plants lack weediness characteristics, therefore modification with fitness enhancing abiotic stress tolerance genes can not make them potential weeds or to cause them invasive in non-cultivated areas. In natural environments, the spread and reproduction of weedy plants are regulated by many factors including many biotic and abiotic stresses, and soil nutrient conditions. Therefore, enhancing fitness of these plants by abiotic stress tolerance genes may not increase their spread and reproduction, as these are controlled by many factors.

Selective advantage under abiotic stress conditions

The signals of abiotic stress stimuli are mostly overlapping, there is a possibility that the inserted genes conferring one type of abiotic stress tolerance might also affect molecular response mechanisms to other abiotic stresses (Taylor and McAinsh, 2004; Yoshioka and Shinozaki, 2009). If a transgenic plant with genes conferring drought or salt tolerance, also acquires increased survival under cold stress might persist in agricultural habitats for longer durations compared to non transgenic plants. Under cold stress, seeds of transgenic plants may attain cold hardiness and remain dormant in soil compared to that of non-transgenic plants (SBC, 2007). In next generations, the germinated seeds in the form of volunteer plants may potentially compete with the crop under cultivation for space, CO_2, light, moisture and

[1]http://www.oecd.org/document/51/0,3343,en_2649_34387_1889395_1_1_1_1,00.html

soil nutrients (White et al., 2004). In case, the transgenic plant escapes to natural habitats may become invasive resulting damage to plant community structure. If the transgene escapes through pollen to non-transgenic plants of the same crop or weedy relatives may also produce the same consequences. In other words, this may expand the geographic range of the species beyond the cultivation area, inflicting damage on surrounding natural plant community structure.

On the other hand, changes in the ABA metabolism may also alter plant responses to cold stress. Many of the genes involved in abiotic stress tolerance work in an ABA-mediated signaling pathway (Tuteja, 2007). Other than its function in stress signaling, ABA also regulates key processes in seeds such as dormancy and storage of lipids (Kermode, 2005). Any genetic modification that targets ABA metabolism may alter seed characteristics so that the seeds survive under cold winter for longer times resulting increased potential for persistence in agricultural environments and invasiveness in natural environments (SBC, 2007).

Despite these concerns, the potential of salt and drought tolerant transgenic plants to become persistent, weedy or invasive is extremely low. The growth and spread of plants in agricultural and natural environment is regulated by many environmental factors including biotic and abiotic factors such as pests and diseases, salt, drought, low and high temperatures, UV irradiation, anoxia, soil nutrient conditions and other environmental factors. In addition, the cross-protection involves physiological and metabolic burdens, due to which transgenic plants may not have enhanced fitness and potential to become weeds.

Selective (dis) advantage of transgenic plants under biotic stress

Transgenic plants with salt and drought tolerance genes may also show a slight selective advantage or disadvantage to biotic stress conditions. There exists a cross-talk between molecular response mechanisms to abiotic and biotic stresses. Plant hormones such as abscisic acid (ABA), jasmonic acid (JA), ethylene (ET) and salicylic acid (SA) regulate plant responses to environmental stresses at the molecular level involving signal recognition, signal transduction, signal response and a multidimensional network of gene expression and regulation (Vinocur and Altman, 2005; Fujita et al., 2006). The ABA-dependent signaling pathway regulates stress-inducible gene expression through several positive and negative regulators (Shinozaki et al., 2003), while ET, JA and SA regulate biotic stress signaling upon pathogen infection. The two different hormonal pathways are not totally independent of each other as there exist some level of synergistic and antagonistic actions during response generation to biotic and abiotic stress stimuli. For example, one study found that ABA and JA antagonistically regulated the expression of salt stress-inducible transcripts in rice (Moons et al., 1997). Apart from that, several studies have also demonstrated that the ABA and ethylene signaling pathways also interact antagonistically to modulate plant development (Beaudoin et al., 2000; Ghassemian et al., 2000). The antagonistic action of ABA and JA-ET signaling pathways to modulate responses to biotic and abiotic stresses has been demonstrated in ABA and ethylene signaling mutants of Arabidopsis (Anderson et al., 2004). Authors of this study found that exogenous ABA application suppressed both basal and JA-ethylene-activated transcription of defense genes. By contrast, the mutation of ABA synthesizing genes resulted in up-regulation of transcription of JA-ethylene responsive defense genes. In addition to the above, they also demonstrated that by disruption of *AtMYC2* encoding a basic helix-loop-helix transcription factor and a positive regulator of ABA signaling resulted in transcription activation of JA-ethylene responsive defense genes. These results showed that this antagonism in ABA and JA-ethylene signaling pathways may be a strategy adopted by plants to avoid simultaneous expression of biotic and abiotic stress related genes.

Despite the phenomenon of cross-talk between biotic and abiotic stress responses, it is highly unlikely that the abiotic stress tolerant transgenic plants may show a changed response to populations of herbivores, predators, parasitoids and pathogens.

Other unintended effects

Genetic engineering of plants with genes conferring various traits may result into unintended effects. These unintended effects may include; 1) insertional effect, changed expression of a gene at the site of insertion; 2) pleiotrophic effect, altered expression of an unrelated gene at an other loci through changing its chromatin structure, methylation pattern and regulation of signal transduction or transcription; 3) generation of new products through interaction of the introduced protein with endogenous molecules; 4) high level transgene expression and the resultant metabolic burden and; 5) secondary effects due to changed substrate or product levels (OGTR, 2008b). In addition to other adverse outcomes, unintended effects may also result into weediness potential. However, these unintended effects are not restricted only to plants developed through transgenic technology. Other non-transgenic approaches of plant development may also have the potential to generate unintended effects. For example a potato variety developed through conventional biotechnology accumulated high levels of toxic glycoalkaloids (Haslberger, 2003).

Interactions with target and non-target organisms

There are no reasons to assume that genes conferring salt and drought stress tolerance (for example, *codA*, DREBs and antiporters) will have effects on target organisms (herbivores, parasites and pathogens) or non-target organisms such as pest predators, beneficial insects, pollinators and populations of other organisms. In fact for abiotic stress tolerance trait, no target species to which adverse effects are evaluated may be defined. Neither the abiotic stress tolerance gene such as the *codA*, DREBs and antiporters, their encoded proteins, and their endproducts have no known effects on these mentioned organisms, nor is the engineered abiotic stress tolerance trait in transgenic plants aimed to confer resistance to biotic stresses. On the other hand, there has been a slight cross-talk between biotic and abiotic stress response pathways, due to which transgenic plants with salt and drought tolerance genes may show slightly changed responses to biotic factors. However, the likelihood of this to happen is extremely low.

COMPARISON OF RISK ASSESSMENT ON INSECT RESISTANCE BT AND SALT TOLERANCE TRAIT IN TRANSGENIC PLANTS (SPECIFIC CASE OF SALT TOLERANCE-INDUCING-*CODA* GENE)

One of the fundamental principles listed in Annex 3 of the Cartagena Protocol on Biosafety is to conduct risk assessment on a case-by-case basis. Based on this principle, the risk assessment process deals transgenes on individual basis, taking also into account nature of the host plant, the trait, the potential receiving environment and the likely interactions among them. So far, the current risk assessment procedures have been used to evaluate and identify the adverse environmental effects of first generation transgenic plants engineered with simple monogenic traits such as insect resistance and herbicide tolerance. And there has been a wide consensus in the scientific community that the current risk assessment procedures are equally applicable to new generation transgenic plants engineered with genes conferring abiotic stress tolerance. However, risk assessment may consider additional measures based on the nature of the transgene and the trait in question.

For the purpose of searching for additional considerations for the salt tolerance-inducing *codA* gene, a comparison of the risk assessment elements on Bt and *codA* is summarized in Table 2. The *codA* gene is isolated from the soil bacterium, *Arthrobactor globiformis*. The *codA* gene encodes an enzyme called choline oxidase (COD) that works in the biosynthetic pathway of glycine betaine, an osmporotectant. Glycine betaine, in turn, protects vital cellular organelles, enzymes and membranes from the damaging effects of abiotic stresses including salt stress (Gorham, 1995). During the current decade, a large number of transgenic plants have been developed that harbored the bacterial *codA* gene. These transgenic plants exhibited multiple abiotic stress tolerance. However, in many plants, the *codA* gene conferred enhanced salt tolerance and transgenic plants showed improved vegetative and reproductive growth (Chen and Murata, 2008; Khan et al., 2009). For commercial use of these transgenic plants under the target saline soils, risk assessment studies are important to evaluate the adverse environmental effects.

The assessment of potential environmental effects of salt tolerance-inducing *codA* gene is based on the current risk assessment procedures. However, depending on the nature of salt tolerance-inducing *codA* gene, there is a need to look for additional considerations in the risk assessment. In case of insect resistance Bt transgenic plants, the main issues which are considered for risk assessment include 1) competitiveness, weediness and volunteer potential; 2) gene flow to wild and weedy relatives and increased weedines and invasiveness potential in agricultural and natural environments; 3) adverse effects on non-target organisms such as predators, parasitoids, beneficial insects, and endangered and charismatic insects, and soil microbial activities; 4) production of harmful compounds that may affect biodiversity; 5) and assessment of resistance evolution in the target insects against the Bt proteins. The risk assessment practice on insect resistance transgenic plants is not new and has been carried out for the last several years with environmental release for commercial use. On the other hand, the risk assessment of abiotic stress tolerance genes such as salt tolerance-inducing *codA* is still under development. Based on the nature of salt tolerance-inducing *codA* transgene in transgenic plants, the current risk assessment is considered to evaluate 1) competitiveness, persistence and volunteer potential; 2) gene flow to wild and weedy relatives and weedines and invasiveness potential in agronomic and natural environments; 3) and production of harmful substances (allelopathic influence) that could affect biodiversity including plant communities, interacting insects, and other organisms; 4) and soil microbe analysis. These issues are already under consideration for risk assessment of insect resistance Bt transgenic plants. For salt tolerance-inducing *codA* transgenic plants, these risks will be evaluated in the same way as for insect resistance Bt plants. In the following section, these issues are discussed in a more detailed way.

Both insect resistance and salt tolerance are fitness enhancing traits that may increase the competitive ability of transgenic plants compared to the surrounding plant communities. Transgene flow to wild and weedy relatives may confer selective advantage, which may increase their weedines and invasiveness potential in both agricultural and natural environments. In case of insect resistance Bt transgenic plants, the ecological consequences resulting from *Bt* gene escape to weedy relatives in natural environment are unpredictable and less defined. The knowledge is still limited regarding whether the same *Lepidopteran* insects feed on wild relatives and to what extent these insect pests regulate the survival and spread

Table 2. Comparison of risk assessment elements on insect resistant-Bt and salt tolerance-inducing *codA* gene.

Environmental concerns/Hazards	Insect resistance *Bt* (cry) genes	Salt tolerance-inducing *codA*
Competitiveness and weediness potential	Bt derived proteins may confer selective advantage under *Lepidopteran* attack, which may increase competitiveness and weediness potential	The *codA* gene confers selective advantage under salt stress that may increase fitness of plant The fitness advantage may affect competitiveness and weediness potential in agricultural habitat Generally crop plants are non-competitive and have very low or negligible weedy characteristics
Gene flow to wild and weedy relatives	Bt-derived proteins may confer increased weediness and invasiveness potential. Invasiveness in natural habitat depends on the degree at which the spread of weedy relatives are regulated by *Lepidopteran* insects	The *codA* gene escape may increase invasiveness potential of wild relatives in natural environment However, the selective advantage is limited and unable to affect the spread and survival of wild relatives Other environmental factors may still regulate and limit the spread of such wild plants
Gene flow frequency	No significant effect on pollen viability and dispersal characteristics	There is unlikely that the *codA* gene may affect pollen metabolism, viability and dispersal characteristics
Production of harmful compounds	Risk assessment considers evaluation of harmful compounds that may negatively affect biological diversity	Although *codA* is not likely to produce harmful compounds, allelopathic assessment is considered in risk assessment
Toxicity to non-target and beneficial insects	The Bt-derived proteins are specifically toxic to *Lepidopteran* insects. Risk assessment considers evaluation of adverse effects on non-target insects and other organisms	The *codA* gene, choline oxidase, GB and the salt tolerance trait have no adverse effects on insect populations and other organisms This concern is not considered in risk assessment
Effects on soil micro-organisms	Assessment of soil micro-organisms and their activities is an essential element in the environmental risk assessment of Bt trait	The *codA*, choline oxidase and GB have no known effects on soil microbes and their activities However, salt tolerance through changed water and nutrient uptake may affect microbial diversity and their enzymatic activities
Development of insect resistance to Bt toxins	The risk of resistance development in insect pests to Bt toxins is well established	No such risks

of these weedy relatives. As discussed in the previous section, abiotic stress tolerance is the product of gene action working in multiple stress response pathways.

These responses are mostly overlapping and there exists a cross-talk between them. Due to this phenomenon, transgenic plants with stress tolerance genes often exhibit tolerance to multiple abiotic stresses. In similar fashion, the *codA* gene manipulation in several transgenic plant species conferred salt tolerance and also drought and extreme temperature tolerance in some cases (Chen and Murata, 2008). The selective advantage of salt tolerant transgenic plants under cold stress, for example may increase the persistence or volunteer potential in the following cropping seasons. Transgene escape to wild and weedy relatives may confer selective advantage under salt stress compared to the surrounding plant populations. The consequences could be predicted in the form of improved vegetative and reproductive growth of wild relatives under salt stress resulting damage and loss to other plant communities.

The third environmental concern that is considered during risk assessment for both insect resistance and salt tolerance-inducing *codA* transgenic plants is the risk of production of harmful compounds due to transgene presence, position or pleiotrophic effects. For this purpose, various allelopathic tests are conducted. The purpose of allelopathic assessment is to determine whether the presence of transgene, its encoded protein and the end product in dried parts of transgenic plants or in the root exudates in the soil have allelopathic influence on the growth and germination of the surrounding plant communities and soil microbe activities. In case of salt tolerance-inducing *codA*, there is no reason to assume that the encoded protein and the final product, glycine betaine will produce harmful compounds. However, based on the precautionary principle, risk assessment on salt tolerance-inducing *codA* considers evaluation of allelopathic effects on the surrounding plant communities and valued soil microbe activities.

Moreover, evaluation of adverse effects on non-target organisms is an essential element of the risk assessment of insect resistant Bt transgenic plants. The non-target effects of insect resistance Bt trait are well studied. Knowledge of the mechanism of Bt toxicity to insects is well established that provides a basis for evaluation of adverse effects on non-target organisms. In some instances, adverse effects have been documented on some non-target and beneficial insects such as green lacewing and the monarch butterfly (Hilbeck et al., 1998; Losey et al., 1999). On the other hand, there are no reasons to assume that the salt tolerance-inducing *codA* gene will have any direct, indirect, immediate or delayed effects on non-target organisms. There have been no reports documented to date that the *codA* gene, its product or the salt tolerance trait have posed any adverse effects on non-target organisms including valued soil microbe activities. In contrast, there exists a slight cross-talk between abiotic and biotic stress responses and due to this phenomenon, transgenic plants with abiotic stress tolerance genes may show slightly altered responses to biotic factors. However, the chances of this phenomenon to happen are extremely rare.

Based on the above discussion, it is clear that the environmental risk assessment issues confronted with salt tolerance-inducing *codA* gene are already under consideration for the current generation transgenic traits such as insect resistance Bt trait. Moreover, the current risk assessment procedures will be used in the same way as used for insect resistance Bt trait. In comparison with insect resistance Bt trait, the salt tolerance-inducing *codA* does not need additional considerations or extra measurements in the risk assessment methodology. This is also evident from the environmental risk assessment of transgenic Eucalyptus plants transformed with the salt tolerance-inducing *codA* gene. Environmental risk assessment of transgenic Eucalyptus provides the only practical example of evaluation of environmental risks of the salt tolerance-inducing *codA* gene.

BIOSAFETY ASSESSMENT OF TRANSGENIC EUCALYPTUS IN JAPAN: A CASE STUDY OF BIOSAFETY ASSESSMENT OF SALT TOLERANCE-INDUCING *CODA* GENE

In Japan, two transgenic eucalyptus tree species, *Eucalyptus camaldulensis* and *Eucalyptus globulus* were transformed with the salt tolerance-inducing *codA* gene. Transgenic plants of both species exhibited salt tolerance under semi-confined conditions (Kikuchi et al., 2006; Yu et al., 2009). These transgenic eucalyptus plants were evaluated for environmental biosafety in a net-house (type II use) under the Japanese law on environmental biosafety for future field application (type 1 use).

In Japan, the biosafety of transgenic plants considers four items for assessment (Taniguchi et al., 2008). These include assessment of 1) competitiveness and weediness potential; 2) production of harmful compounds, allelopathic effects on biodiversity; 3) cross-ability, gene flow to wild relatives and increased invasiveness potential; 4) and other properties. Under net-house conditions (Type II use), environmental biosafety of three *Eucalyptus camaldulensis* genotypes was evaluated for the above mentioned risk assessment items. In environmental risk assessment, allelopathic studies on transgenic and their non-transgenic genotypes are important to investigate production of any harmful compounds. Some plants have allelopathic influence in nature and the transgene presence may enhance their negative effects on the surrounding plant communities and non-target organisms interacting with them. During environmental risk assessment, the direct negative effects of these transgenic plants on the biodiversity are evaluated and compared with the non-transgenic control plants.

In case of *codA* transgenic plants, the allelopathic effects are unlikely. The *codA* product, choline oxidase catalyzes the glycine betaine biosynthetic pathway. Glycine betaine is naturally produced in several plant species where its increasing content is directly correlated with increased stress tolerance. Also in transgenic plants, the increased glycine betaine accumulation conferred increased stress tolerance without any negative effects on plant growth. Therefore, the *codA* transgene, its encoded choline oxidase enzyme and the end product glycine betaine should not produce harmful substances or allelopathic effects. This is evident from the allelopathic studies conducted as part of environmental risk assessment of salt tolerant *codA* transgenic *Eucalyptus camaldulensis* and *Eucalyptus globulus* plants.

Transgenic *E. camaldulensis* plants harbored the *codA* gene under the constitutive *CaMV35S* promoter. These transgenic plants were found salt tolerant compared to their non-transgenic counterparts under controlled conditions. For environmental safety of these transgenic plants, allelopathic tests were conducted to evaluate the negative effects that could arise due to the *codA* transgene, its encoded protein and the glycine betaine product (Kikuchi et al., 2009). The authors of this study used four different tests for allelopathic assessment. They used sandwitch method to determine differences in root and hypocotyl growth of lettuce seeds grown on media that contained dried leaves from the three transgenic and non-transgenic genotypes. Soil germination test was conducted to evaluate differences in germination of lettuce seeds grown on soil taken from transgenic and non-transgenic genotypes. Volatile and phenolic compounds in transgenic and non-transgenic genotypes were analyzed through Gas chromatography and HPLC respectively. The authors reported no significant differences between the three *codA*-transgenic *E. camaldulensis* genotypes and their non-transgenic counterparts for the studied parameters. In addition, the salt tolerant transgenic *E. camaldulensis* plants harboring the *codA* transgene were evaluated for adverse effects on soil microbe activities and compared with those of non-transgenic control plants (Japan Biosafety Clearing-House 2005). No differences were found between microbial activities in soil samples taken from both transgenic and non-transgenic Eucalyptus plants grown in a special-netted house. Similarly, for environmental biosafety assessment, impact on allelopathic effect and soil microbes was investigated on salt tolerant *codA*-transgenic *E. globulus* and the non-transgenic plants grown under special-netted house conditions (Yu et al. 2008; Lelmen et al. 2009). No significant differences were found between transgenic and non-transgenic plants for both allelopathic effects and soil microbe communities.

These tests provided useful informations regarding the environmental safety of *codA* gene. From the environmental biosafety studies on the salt tolerance-inducing *codA* transgenic Eucalyptus plants, it was also pointed out that; 1) the risk assessment approaches and procedures used for environmental biosafety of *codA* transgenic eucalyptus were the same as currently under use for the first generation transgenic plants; 2) and risk assessment of *codA* did not need additional considerations under the Japanese government. In conclusion, the use of salt tolerance-inducing *codA* in transgenic plants are using the same risk assessment procedures as used for other transgenic crops modified with for example, insect resistance trait. Therefore, the *codA* gene does not need additional considerations in environmental risk assessment.

Examples of other field trials and risk assessment studies conducted on salt and drought tolerant transgenic plants by various regulatory authorities

Many transgenic plants with abiotic stress tolerance genes are under field trials for environmental risk assessment studies in Australia, Canada, USA, New Zealand, Philippines and the European countries. These are listed in Table 1.

The Monsanto developed drought tolerant maize will be ready for commercial use in the United States by 2012, and it has already applied for commercial release of transformation event MON 87460 in USA, Canada, Australia/New Zealand, Philippines and the European Union.. Much of the information regarding field trials and risk assessment, however has been undisclosed. Some of the details on risk assessment of MON 87460 are now available from the summary notification information format (SNIF), submitted by Monsanto. MON 87460 expresses a cold shock protein B (CspB) isolated from *Bacillus subtilis*. The transgenic maize is developed to perform better and show reduced yield loss under limited water conditions compared to the conventional counterpart. The information revealed that the MON 87460 is equivalent to conventional maize under well watered conditions. However under limited water, transgenic maize performs better in terms of reduced grain loss compared to the conventional maize. Under severe water limitation, transgenic maize like the conventional maize will be unable to grow. During comparative assessment, MON 87460 revealed no significant differences with the conventional maize except the intended trait that conferred a selective advantage only under limited water level that negatively affect plant yield. However, this selective advantage under limited water conditions is highly insufficient to alter the transgenic maize as a volunteer plant or its escape to non-agricultural fields. There may be some other important environmental factors that would limit the survival of maize in the potential receiving environment. The SNIF did not mention about cross-talk between different abiotic stress responses, or whether transgenic maize with the CspB protein also shows tolerance to other abiotic stresses other than the intended drought tolerance.

Transgenic sugarcane lines expressing the *OsDREB1A* and *ZmDof1* genes were developed that showed drought tolerance and improved nitrogen use efficiency (NUE). These transgenic lines were assessed for environmental risks. Regarding risk assessment, OGTR concluded that the limited and controlled release of these transgenic sugarcane lines pose negligible risks to the environment (OGTR, 2009). Regarding weediness potential of these transgenic sugarcane lines, it was mentioned that it is highly unlikely that the introduction of these genes could change all of the characteristics that regulate or limit the persistence of sugarcane. Moreover, the occurrence of any unintended pleiotrophic effects could have been detected during the pre-trial stage. However, further uncertainties, if still existed could be judged through containment measures and monitoring.

Transgenic wheat lines transformed with the Ornithine aminotransferase gene showed salt tolerance under greenhouse and field conditions (OGTR, 2005). The OGTR risk assessment and risk management plan concluded that modification of wheat with the Ornithine aminotransferase gene and the resultant proline accumulation confers selective advantage in some environmental conditions compared to conventional counterpart. However, this modification is unlikely to alter other characteristics associated with weediness potential. Transgenic wheat and barley (DIR077) contained drought responsive transcription factors from wheat, *TaDREB2* and *TaDREB3* (OGTR 2008a). About risk assessment and risk management, the OGTR concluded that the use of these genes pose negligible risks to human health and the environment and these negligible risks do not require specific risk treatment measures.

Moreover, several genes have been transferred into cotton to increase its water use efficiency (OGTR, 2006; 2008). The OGTR in the RARMP concluded that these genes are not likely to alter all of the characteristics that limit the spread and persistence of cotton such as dormancy, seed survival in soil for long time, length of life cycle, large amount of seed dispersal. Moreover, transgenic cotton showing multiple tolerance with these genes is not likely to show enhanced fitness; rather the multiple stress tolerance will make the plant less fit due to metabolic and physiological burdens.

In these field trials of transgenic plants with various abiotic stress tolerance genes, it is mentioned in their RARMP that 1) these genes may confer selective advantage to transgenic plants only under stress condition that enables the plant to perform better than the conventional counterpart. Under non-stress condition, the transgenic and non-transgenic plants are equivalent; 2) Due to cross-talk in stress response mechanisms, transgenic plants with these genes may show stress tolerance other than the intended one. However, this selective advantage under other stress conditions may not alter all the characteristics associated with weediness or invasiveness traits, or in other words may not make transgenic plants weedy or persistent in agricultural or natural environments: 3) these genes are mostly taken from plants, and their introduction or over expression in the host plants modify an already existing stress response mechanism. Therefore, it is concluded that these genes, their encoded proteins and the end products will pose no harm to the environment and living organisms including humans. For future large scale release, the RARMP concluded that additional information will be required regarding characteristics indicative of weediness including measurement of altered reproductive capacity, tolerance to environmental stresses, and disease susceptibility.

CONCLUSION AND RECOMMENDATIONS

Transgenic plants with abiotic stress tolerance genes are under development. Future environmental release of these transgenic plants requires assessment of their adverse effects. The abiotic stress tolerance genes are using the same risk assessment procedures as used for the current first generation transgenic plants with insect resistance Bt and herbicide tolerance genes. However, depending on the different nature of abiotic stress tolerance trait, needs for additional considerations should be examined in the risk assessment process. In comparison with insect resistance Bt genes, the abiotic stress tolerance genes are mostly taken from plants and their encoded proteins or at least their end products are not new to plants. The encoded proteins of these genes modify the already existing stress response mechanism and do not introduce novel pathways or functions in transgenic plants, which may pose adverse effects to the environment and human health. The use of these genes such as the salt tolerance-inducing *codA* gene in transgenic plants causes no risks to biodiversity including plant communities and non-target organisms. Therefore, the use of abiotic stress tolerance genes (for example, salt tolerance-inducing *codA* gene) does not need additional considerations in the already established and rigorous risk assessment procedures. In addition, as indicated in the field trials and risk assessment studies on several transgenic plants, the use of abiotic stress tolerance genes confer tolerance only under stress condition that enable transgenic plants to reduce yield losses compared to the conventional plants. Moreover, the salt and drought tolerance genes do not confer weediness, persistence and invasiveness potential to transgenic plants compared to the conventional non-transgenic plants. In agricultural and natural environments, salt and drought stresses are not the only factors that limit the growth and spread of plants. Regarding invasiveness, it is generally observed that the salt and drought tolerance of transgenic plants is considerably lower than the levels required sustaining and thriving in extreme saline and drought affected natural environments. Therefore, there is no risk of these plants to invade natural environments. Despite these observations, the abiotic stress tolerance trait in transgenic plants involves metabolic and physiological changes and the potential of selective advantages under other abiotic stresses. The uncertainties due to these specific aspects and their potential effects on weediness, persistence and invasiveness must be addressed through further information on weediness of the host plant, implementing an appropriate risk management strategy to control potential weeds and volunteers and continuous monitoring of the LMO in the potential receiving environment as mentioned in the protocol. In addition, the following points should be focused in order to overcome any uncertainties.

1) A well organised problem formulation: Increased emphasis is needed on elements of problem formulation (Figure 1). Defining assessment end points and developing a comprehensive conceptual model and analysis plan are essential to address the uncertainties that may arise in the risk assessment of salt and drought tolerant transgenic plants. Familiarity with biology of the crop, characteristics of the trait and the potential receiving environment is important for developing conceptual model and generating risk hypothesis. For generation of conceptual model of an abiotic stress tolerant LMO, informations on the biology of the crop will particularly include information on weediness characteristics of the conventional plant. In addition, questions will be raised such as whether enough information on weediness is available? And to what extent abiotic stresses affect fitness and weediness related characteristics of plants in the potential receiving environment? The OECD consensus documents provide a valuable source of information on the biology of the crop plants including information on weediness, volunteer and invasiveness potential. These all information will be used to develop an analysis plan for plant characterization in the potential receiving environment. Identification of meaningful differences between the LMO and the conventional plant will be subjected for further assessment.
2) Weediness characteristics: For weediness and invasiveness potential, the relevant information on weediness should be collected during the problem formulation step. However, there is limited data available that could evaluate the potential of increased weediness or invasiveness of a transgenic crop plant with fitness-enhancing abiotic stress tolerance trait and its comparison with the conventional counterpart. This poses a challenge in risk assessment. To meet this challenge, emphasis should be placed on; 1) phenotypic and agronomic traits associated with fitness and weediness characteristics in conventional crop plants and; 2) the effects of salt and drought stress tolerance on these traits. Baseline informations are needed on the characteristics of weeds in general and on the factors that limit the spread and persistence of conventional crop plants in particular (OGTR 2008). Further efforts are needed to understand; 1) the factors that control the population size and range of both the crop volunteers and their wild relatives and; 2) the degree by which abiotic stresses such as salt and drought regulate the survival and reproduction of crop plants in the field
3) Comprehensive phenotypic and agronomic characterization in the potential receiving environment: As stress tolerance genes may involve physiological and metabolic changes that confer selective advantage under abiotic stresses due to cross talk among various stress response pathways, careful phenotypic and agronomic characterization is needed to identify meaningful differences between the LMO and the conventional counterpart. The unintended changes may be considered for further evaluation in the risk assessment process. In relation to phenotypic characterization of the LMO in the potential receiving environment, the following points should be focused on:

1) Consideration of other environmental conditions prevalent in the potential receiving environment, while planning comparative analysis. Potential receiving environment for salt and drought stress tolerance transgenic plants will be the same as that for non-transgenic conventional plants. However, in some cases genes with one stress tolerance may also confer tolerance to other stresses. Therefore, these stresses should also be considered in that environment.
2) Choice of the conventional comparator (may be a commercial salt, drought tolerant variety) for comparative phenotypic and agronomic assessment in the potential receiving environment
3) Knowledge of and data availability on the response of conventional plant to the stress condition and to the potential receiving environment. If the conventional comparator has not been under cultivation in that area, comparative analysis will be a challenge.

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