

Transgenic plants and their applications



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Aim of study

Rice is one of the main staple foods in this world. It is particularly interesting that studies be done on enhancing the rice production. There are biotic and abiotic factors that can reduce the production of rice but the aim for this study is to examine further into genes that can enhance rice tolerance towards drought as drought one of the main factors that decreases the annual production of rice in the world. In addition, genes conferring tolerance to transgenic rice will also lead to other positive phenotype effect such as salt tolerance (Hu et al., 2006), disease tolerance (Chen and Guo, 2008) and many more.

The research of transgenic plants is still in its infant stage as technology progress to further extend. As this transgenic research is still new, there is a lot of problem surrounding this new research and it is particularly important for one to know every aspect in transgenic rice. Aspects such as benefits and setbacks should be provided so that the aspects can be reviewed and to find a balance point. Balance point is where the benefit will outweigh the setbacks.

Furthermore, review would be more wholesome and informative and the information provided will lead to invoking thought of scientists that are interested to study further in the transgenic field.

Rice Production

According to Food and Agriculture Association (FAO) of United Nations, rice is the second largest produced cereal in the world. Around 350 million tons of rice was produced in the early 1990s and by the end of the century it had

reached 410 million tons. Asia countries are the major rice producer in the world (90%) with China and India producing one-third of global population supply (ref?). Currently, rice is grown and harvested on every continent except Antarctica, where conditions are not suitable for rice growth. Other major rice producer includes India, Japan, Indonesia, Thailand,

Philippine, and Bangladesh. Currently, more than 550 million tons of rice is produced annually around the globe. Few thousands of new strains of rice are produced till today. That includes those grown in the wild and those which are cultivated as a crop. Globally, more than 3 billion people from Asia and other countries depend on rice (*Oryza sativa*) as their staple food, and by 2025 at least 60% more rice must be produced to meet the demands of the growing human population (Yarasi et al., 2008). Currently, rice yield around the world are just barely enough to support the people that depend on rice as food (Karaba et al., 2007). This problem has become worrying because food shortage related to rice may escalate out of control and sends billions into starvation.

Factors affecting rice production

This shortage may be caused by biotic and abiotic factor (Capell et al., 2004). Plants had to face periodic or unpredictable biotic and abiotic stresses, such as salt, drought, cold, pests, and disease. The most significant abiotic stresses faced by plant are high salt environment and drought. These stresses inhibit crop growth and development and usually result in plant death. As crops fail, the production of food will decrease accordingly.

Biotic Interference

Production of rice is negatively impacted by numerous biotic factors includes insect infestation and microorganism invasion. An approximate 52% of the global production of rice is lost annually owing to the damage caused by biotic factors, of which around 21% is attributed to the attack of insect pests (Brookes and Barfoot, 2003).

Insects belonging to plant hopper (Delphacidae) and leaf hopper (Cicadellidae) are hard to control and monitor. So, a lot of rice yield are lost due to insect infestation. Insects not only cause direct losses to the agricultural produce but also act as vectors for various plant pathogens that causes disease (Dahal et al., 1997; Foissac et al., 2000)The most known pests of rice are viz, brown planthopper (*Nilaparvata lugens*, BPH), green leafhopper (*Nephotettix virescens*, GLH) and whitebacked planthopper (*Sogatella furcifera*, WBPH) are known to cause severe damage. They have a hugh appetite for plant sap, and in large numbers, they can suck the sap of plants to the point where plants will lose important nutrient in sap and die. They also act as vectors for major viral diseases (Yarasi et al., 2008).

Abiotic Interference

Plants are non motile organism and needs to find suitable environment to continue on growing. Sometimes the environmental conditions are not suitable for them. So the challenge is the plants had to adapt to its environment by some biological mechanisms that are able to help plants to flourish in stressful environment. They manage to do so by series of event. First sensing a stress, and then signaling the stress through a series of components, leading to activation of a large number of stress-related genes

and synthesis of a variety of functional proteins. This includes transcription factors, enzymes, molecular chaperones, ion channels, and transporters (Zhou et al., 2009). Functional proteins helps the plant can change in their physiological and metabolic reaction according to the stress endured. This ensure the plant continue to triumph in stressful abiotic environment (Bray, 1997).

Counter Measures

There are numerous physical ways to counter these problems. For example, pesticides can be use for the prevention of insect infestation. However, this counter measures are not without any negative consequences. Chemical control of insect pests is an effective method but not efficient. Pesticides cause a lot more today and the usage depends mainly on the weather conditions. Uncontrolled usage of chemical pesticides will not only build up resistance in insect pests but also affect other beneficial organisms such as pollinators, nutrient cyclers and natural pest-controlling predators (Yarasi et al., 2008). They can pollute the environment and as well as depleting other natural minerals of soil. As to solve drought problems on plants' growth, government had tried counter measure such as cloud seeding.

Cloud seeding

Cloud seeding works by the use of airplanes to spray expensive chemicals such as silver salt into the sky in hope for rain. Cloud seeding is not preferred method to reduce the effect of drought on food production because the silver salt used are very expensive and success percentage of seeding clouds are very low. Now scientists are looking forward in finding the best solution to

increase the production of rice. The most interesting and the most widely studied is the transgenic method.

Transgenic Approach

Transgenic comes from the word “trans-gene”, which means introduction of foreign gene that is considered beneficial to the wild type species. Any foreign genes that are considered a help to confer stability of rice to environmental stress are being studied. Genetic enhancement of rice through conventional methods is often constrained by narrow gene pools. So, transgenic technology can be used as a better alternative approach for hybridization of wild species genes with foreign gene to produce a better rice species in terms of survivability in nature (Yarasi et al., 2008). With current advancement in biotechnology, we can look forward to produce transgenic rice that can have higher survival chance from biotic and abiotic interference.

Beneficial Transgenic Effect In Rice

Through transgenic method, rice will be more resistant to diseases and insects when foreign genes are introduced into the wild type rice gene to express certain proteins that repel insects (Yarasi et al., 2008). For example, the introduction of Tobacco OPBP1 gene into rice may improve the disease resistance of rice (Chen and Guo, 2008) and the introduction of *Allium sativum* leaf lectin gene into rice to repel sap-sucking insect (Yarasi et al., 2008). Besides that, rice will become more likely to survive abiotic catastrophe such as drought and high salinity condition as introduction of new genes confers the ability of rice to mediate metabolic ways to react to these stresses. For example, insertion of *Arabidopsis* HARDY (HRD) gene in rice improves water use efficiency, the ratio of biomass produced to the

water used, by improving photosynthetic assimilation and reducing transpiration (Karaba et al., 2007). There is also the insertion of *Triticum aestivum* salt tolerance-related gene (TaSTRG) derived from salt-tolerant wheat mutant RH8706-49 enhances salt and drought tolerance of rice (Zhou et al., 2009). Other than that, transgenic process can be used to insert a bacterial chlorocatechol dioxygenase gene into rice so that rice plants are able to degrade pesticides (chlorinated compounds) to less harmful form. Several herbicides and pesticides containing chlorinated compounds have been used and have spread in the environment. They will destroy the delicate balance of nature by many ways. If left unattended, the environment will have some non reversible consequences. Therefore, bio-remediation of these chemical compounds will be a powerful technique to degrade chlorinated pollutants in soil. Transgenic rice plants that express foreign genes encoding enzymes to degrade chlorinated chemical compounds would enable farmers to remove these harmful chemical compounds from soil and water surrounding the fields (Alexander, 1981). By applying transgenic research, rice plant can be further enhanced to be able to survive stresses and as well to help remediate the environment. Indirectly, the rice production will also increase so that food crisis will not happen.

The Super Green Rice Project

Currently, scientific community are on the verge to produce transgenic rice called Super Green Rice which possesses numerous beneficial properties such as resistances to multiple insects and diseases, high nutrient efficiency, and drought resistance (Zhang, 2009). So this new kind of hybrid rice are hoped to reduce the consumption of pesticides, chemical fertilizers, and

water. Super Green Rice also needs to have high yield quality regardless of multiple stress environments (Zhang, 2009). For this production of Super Green rice to be realized, they are undergoing enormous efforts to focus on identifying all the hereditary material in a single species and discovering genes for resistance to diseases and insects, nitrogen and potassium use efficiency, drought resistance, grain quality, and yield. The steps adopted include screening of germplasm collections and mutant libraries, gene discovery and identification, microarray analysis of differentially regulated genes under stressed conditions, and functional test of candidate genes by transgenic test (Zhang, 2009). Genes that are considered beneficial to the production of rice are now being isolated and are gradually incorporated into wild type rice gene. It is anticipated that such strategies and efforts would eventually lead to the development of Green Super Rice (Zhang, 2007).

Problem of transgenic plant in food

Transgenic research seems to have a bright and brilliant prospect ahead to help solve the world food crisis problems, but, the introduction of foreign genes into wild type rice does not come without any major public concern (Yarasi et al., 2008). In all new scientific projects worldwide, there will be problems because the technologies and knowledge is yet to be fully explored. There are some worries that the lack of transgenic effect on food crop may have negative consequences. For example, effect of the transferred foreign toxic gene such as the *Bacillus thuringiensis* (Bt) insect-resistance gene to other species other than rice (Yarasi et al., 2008). This induces the rice to produce endotoxin to repel insect from eating the crops. This Bt gene inserted into food crop can help to reduce insect destruction

and increase the food yield, but, there are concerns that the inserted gene into food crops will have adverse effect on the nutrient quality of crops produced (Yarasi et al., 2008). The gene may induce the plant to undergo different metabolic process and produces toxic substances as by-product. Although with the insertion of Bt gene into rice will increase its yield, but the rice produced are not suitable for human consumption. This may cause the crops produced not fit to be consumed.

Transgenic Plant and Environment Concerns

Through the introduction of transgenic plant in our environment, scientist began to think about the consequences that might occur. There is concern about the consequences of transgene escape to wild type relatives.

Transgenic plants have genes that are different from the wild type gene but both transgenic and non-transgenic plants are still able to communicate and transfer genes (Lu and Yang, 2009). Communicate in this term means the ability to interbreed with each other. So, the transgene can flow to the wild type species. There are three pathways for gene flow to occur which is pollen-mediated, seed-mediated and vegetative propagule-mediated gene flow (Lu and Yang, 2009). Transgenes can escape from a genetically modified (GM) crop to its wild relative species via pollen-mediated gene flow. There are many factors that can affect the pollen-mediated gene flow such as wind, animal, water current and other factors. Moreover, types of vectors for pollination and environmental conditions, such as the strength, and direction of wind, temperature, light intensity, and air humidity, will also influence pollen-mediated greatly (Lu and Yang, 2009). It is natural to have pollen-mediated gene flow because it helps in plant evolution. However, the

movement of transgenes from genetically modified plant to wild type species may have adverse effect. This is because wild or weedy plants that acquire transgenes will continue to evolve, subject to natural and artificial selection in the agricultural ecosystem and beyond, posing potential ecological consequences (Lu and Yang, 2009). Once transgenes have moved into populations of wild or weedy species, it is nearly impossible to remove them from the environments if the transgenes can persist and spread in the populations. Different consequences will arise if the wild type relatives acquire transgene from genetically modified plant relative. If the transgene is able to confer favorable traits such as pest resistance, drought tolerance, and enhanced growth ability, the transgene followed by gene flow would persist to and quickly spread in the populations of wild relatives through introgression. Then individual plants which contain the transgene are out surviving the individuals without the transgene in natural selection. This will indirectly increase the invasiveness of transgenic plant and sooner or later, the habitat will be filled with transgenic species rather than the wild type species (Lu and Yang, 2009). On the other side, if the transgene reduces the survivability of wild relatives, the frequencies of individuals that contain the disadvantageous transgene will decrease gradually. This process will cause the extinction of local populations by the so-called swarm effect (Ellstrand and Elam, 1993). Therefore, transgenes escape via pollen-mediated gene flow from a GM crop to populations of wild relatives and its ecological impacts have been a major concern. Another concern over transgene escape from GM rice to its wild relatives is for the consequences of genetic diversity. The presence of transgenes in the germplasm of wild rice relatives may represent a form of pollution. It is theoretically possible that strong selection

for fitness enhancing transgenes could generate selective sweeps, in which portions of the crop genome that are linked to these transgenes displace corresponding portions of wild genomes (Lu and Yang, 2009). This can be particularly dangerous as the transgenic plant displaces its wild type relatives, the gene pool will be less diverse. So, if there is a new disease caused by new type of bacteria or viruses, and coincidentally the transgenic plants lack of gene to fight off the disease, the whole population of the plant species are endangered of being wipe out and becomes extinct. Besides that, with the reduction of gene variety, there will be surely affect the plants natural evolution. As gene pool decreases, the evolution of transgenic plant in the future will not be as vary and diverse as the plants of wild type gene. The newly introduced transgenic rice may alter the soil composition and its microbes community due to its different physiological need compared to its native species. These might render the soil useless and unsuitable for other plants to grow in.

Transgenic plant and health concerns

Majority of people are starting to get worried about the consequences of the transgenic genomes transferred into the native species and other major complication caused. Those complications include health concerns where foreign genes are transferred into the rice gene may cause health complication. Some scientists have argued that protein products are not the only potential source of toxicity in transgenic plants (Connor and Jacobs, 1999). An experiment had been conducted when researchers fed rats either wild-type, wild-type containing lectin or transgenic potatoes expressing the lectin protein. Lectins are of commercial interest because of their pesticidal

properties. The result shows that only the transgenic potato-fed group experienced physiological changes such as intestinal damage and they concluded that the genetic transformation process itself caused the observed complications. By getting the result from the rats, we can subsequently predict the analogous effect of unsuitable transgene on human health. Humans' health is in great danger if they consume such transgenic crop.

Other than that, people around the world are more alerted to the food safety of GM food that introduces allergens into the food supply. The allergenicity of the GM food is determined by the allergenicity of the substance. This means that gene products that are not allergenic normally will not become allergenic when expressed in a transgenic plant. For example, plant ferritin has no allergy cases reported, so, transgenic iron-enriched rice that contains the plant ferritin gene (Goto et al., 1999) poses no allergenicity risk. On the other side, if the gene product is a known allergen, then it will also be an allergen in a transgenic plant. As an example, when a Brazil nut albumin was expressed in soybean to boost methionine content, it resulted in nut-allergic individual reacted to the transgenic soybean (Nordlee et al., 1996).

Therefore, people with an allergy to Brazil nuts would now also be allergic to those GM soybeans, even though they were not allergic to native soybean before. However, allergenicity of food is much more complicated when the allergenicity of a transgenic protein is unknown. For example, if a substance is not tested for allergenicity before, then the question may be asked if people started to consume food with the unknown substance, will they develop food allergy. There are more than 200 food allergens have been

identified and sequenced (Gendel, 1998), but there is no definite sequence of protein are determined to be the source of allergenicity. Most known food allergens are stable to digestion (Ashwood et al., 1996). Therefore, protein's digestion stability test in the digestive process environment is one way to identify potential allergens. It is unlikely to reach immune cells to cause a hypersensitivity response if a protein is degraded in the stomach and small intestines. Stable proteins should be examined further. These experiments can be coupled with a comparison of sequence similarity to known allergens. Novel proteins with a significant sequence similarity can be tested for reactivity with serum from subjects who are allergic to the homologous allergen. Although these tests may not be comprehensive in identifying potential allergenicity, the limited variety of source foods suggests that the vast majority of transgene proteins will be safe for consumption (Lehrer et al., 1996). More than 90% of the people who have food allergies are allergic to one or more of either cow's milk, wheat, nuts, legumes, eggs or seafood.

Transgenic plant and effect on non target species

Transgenic crops that express insecticidal transgenes to control agricultural pests may also affect non-target organisms (Hilbeck et al., 1998; Losey et al., 1999; Saxena et al., 1999). Studies using corn transformed with a Bt-insecticidal transgene have non-target effects. For example, Lacewings (*Chrysoperla carnea*), an insect predator, suffered from higher death rates by feeding on corn destructive insect, European corn borer (*Ostrinia nubilalis*) reared on Bt corn.

The result shows lower and more significant less death rate using corn borers raised on non-Bt-transformed plants (Hilbeck et al., 1998). However,

this was a laboratory study. It would seem a low probability for lacewings to be exposed to European corn borers that have ingested Bt toxin in the field. In another study, Monarch butterfly larvae (*Danaus plexippus*) that consumed milkweed (*Asclepias curassavica*) leaves dusted with Bt-containing corn pollen had decreased feeding, growth and survival rates, compared to larvae that consumed leaves with non-transgenic corn pollen (Losey et al., 1999). This result may indicate that the usefulness of some genes to deter some destructive insect may have some adverse and unwanted consequences of other species within the food chain. This indirectly will cause some species to be greatly in danger by this newly introduced transgenic plant. If the problem persisted, some species affect maybe extinct and then it will generate cascade of negative effect on the fragile natural food chain.

Transgenic plant and resistance issue

There is also the issue of insect developing resistance to the transgenic plant and thus render the transgenic plants useless. For example, the diamondback moth, an important pest to Brassica crops worldwide, was the first documented pest to develop resistance to Bt toxins applied as microbial formulations in open-field populations (Tabashnik, 1994). Until now, there is no dominantly inherited Bt resistance genes have been documented. Using this information, various resistance management strategies have been proposed to delay the resistance building by insect, with plantation of a high expressing transgenic plant coupled with a non-transgenic plant (Shelton et al., 2000). The non-transgenic plant allows Bt-susceptible pests to survive on the field population and mate with Bt-resistant individuals. The goal of this

strategy is to keep the recessive Bt resistance genes at low levels in the target populations and thus limit the rate at which the entire population will acquire Bt resistance. The effectiveness of this strategy depends on the population size (Shelton et al., 2000).

Transgenic plant and ethical issue

Besides that, there are also some ethical issues where transgenic rice contains foreign genes that cannot be consumed. For example, the vegetarian will think twice before consuming the transgenic rice containing animal genes inside the transgenic rice. There is a strong sense of consumerism where consumers want to know what is in their food.

Transgenic plant and its economic issue

Economically, there are also a few setbacks regarding the production cost and the research cost for the transgenic rice. It is true that the transgenic rice will give us more yields but there are some worries that the money needed for the production and research of transgenic rice may overcome the benefit. As commercial crops are the main applicator for transgenic research, it may be hindered to progress in poor nation. So indirectly, the transgenic rice project might be not economically feasible. However, there is still no exact amount that can be taken into account but there is a bright prospect that the benefit of higher yield will overcome the cost needed.

Transgenic rice is something to look forward to in the future because it may put a stop to the world food crisis. By mastering transgenic technology, there is a hope where the 'perfect' rice plants can be produced greatly without much interference abiotically or biotically. It also have good prospect for

better future study of alteration of rice nutritional value to suit the ever more demanding human population.