Genetically Modified Organisms in Food Production

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ABOUT THE AUTHOR

Dr Umaiyal Munusamy is a Plant Scientist and currently working as a post-doctoral at Institute of Plantation Studies UPM. She is also an affiliate member of the Young Scientist Network (YSN) Academy Science Malaysia (ASM). Her interest is mainly in science communication, plant biotechnology, transgenic research and plant microbe interactions. Currently she is also keen into looking at sustainable agriculture processes and technologies in accordance with the climate change. Besides research, her passion in teaching continues into educating refugee children voluntarily. Committed to science communication Dr Umaiyal Munusamy engaged in Kuala Lumpur Engineering Science Festival (KLESF) 2016, had delivered talk on Introduction of Agriculture Engineering to secondary school student, performed Science Show at FameLab 2017 competition, coaching for contestant participating in scientific communication competition. Last but not least, her passion in writing continues through this book.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

PREFACE

Plant fertilization begins when pollen hits the pistil, the stalk that protrudes from the center of the flower. A tube extends from the pollen grain down through the pistil to deliver sperm to the eggs stored. Animal reproduction happens when the sex cells of parent (male and female) organism joins together to produce a new organism. The offspring have DNA that differs from both parents, have a combination of physical characteristics from both parents but may not look exactly their parents. Without these two processes, earth will not exist and living organism will not present. Since human population are increasing coupling with climate change, natural reproduction of plant and animal unable to feed the entire population. However, we are so lucky to have emerging technologies and new system to improves the speed and accuracy of reproduction through artificial breeding. Breeders and researchers are working together to determine the genetic cues from both maternal and paternal genes for plants and animals to develop improved varieties. Genetic isolation, genetic and phenotypic manipulation, artificial crossing within and across species tamper with Mother Nature. Even though, controversies and public concern surrounding GMO and GMF focus on human and environmental safety, various approach such as proper labelling, intellectual property rights, ethics, food security, and safety are taking into consideration so that many gain maximum benefits from GMO and GMF. There will be 15 chapters covers in this book from history of GMO/GMF to why, what, and how about GMO/GMF. The topic is expanded to the importance of GMO/GMF and how breeding in plant and animal could produce sustainable GMO/GMF. How traditional and modern breeding contribute in production of GMO/GMF and a list of GMO/GMF database. Companies and countries that involve in GMO/GMF research, plantation and production. Finally, some examples of Plant/Animal Apps are also illustrated in this book.

ACKNOWLEDGMENT

This book would not have been possible without the help of several individuals who in one way or another contributed and extended their valuable assistance in the preparation and completion of this book. Mainly, the author would like to express her gratitude to all the photo contributors.

The author has given some opportunity for individuals who seem to inherit certain skills and talent that is required by the author. One of them is MC Nishanth who is one of her students from Tamil Forum Malaysia Community Learning Centre (TFM-CLC). The author who is voluntarily teaching science classes to a group of refugee's students noticed MC Nishanth is talented in taking photograph. To bring out the talent in him the author decided to collaborate with him to capture pictures for this book. This will help him to value his talent and taught him how he can expedite his talent for his better future. He can be contacted through his Instagram @nishphotography.

Secondly, thanks to Mythilee Munusamy who drawn the three images representing the prehistoric time. The author identified Mythilee Munusamy talent in drawing cartoon characters. She drawn cartoon characters such as smurfs during her early childhood. She can be contacted through mythilee.munusamy@gmail.com.

Special thanks to Predeeban Munusamy and S. P. Madathyattal Petchimuthu for their contribution of some pictures. The pictures were taken from Nokia 7 Plus and the rest which was taken by the author was captured by using Samsung Galaxy S5; Model Number SM-G900F; Android version.

CHAPTER 1

GENETICALLY MODIFIED ORGANISM IN FOOD PRODUCTION

CONTENTS

1.1. THE HISTORY

The genetically modified organism in food production started from the prehistoric time to the year 1900. Oxford Dictionary defined prehistoric time as time before history. In this time, there are already presence of food eaters. They are the living organism known as gatherers who search their own food. They gather, pluck and hunt foods that can be eaten safely as there is no genetic engineering or any other sophisticated tool or techniques that are available to produce genetically modified food.

While in 1900, European scientist begin to use Gregor Mendel's genetic theory (1800) to manipulate and improve plant species for better food variety.

From there, the work was further extended into publishing the three-dimensional double helix structure of DNA by James Watson and Francis Crick in 1962. This discovery was eventually lead to the competency of scientist to identify and splice DNA from one organism into the DNA of another organism. Upon identifying DNA, splicing was carried out and this lead to recombinant DNA organism which was successfully carried out by Herbert Boyer and Stanley Cohen in 1973.

IB404 - 2. Cloning and Sequencing - Jan 23

Cloning DNA fragments

Invented by Herbert Boyer (UCSF) and Stanley Cohen (Stanford) in the early 1970s. Boyer founded Genentech and Stanford eventually ran out of patent protection for the principles of cloning DNA.

Herbert Boyer

Stanley Cohen

The heat of the real genetically altered life forms happened in 1980 when Exxon oil company patented an oil-eating microorganism. In the next 2 years, US Food and Drug Administration approved the first genetically engineered drug known as Genentech's Humulin, a form of human insulin produced by bacteria. This is the first consumer product that was developed through modern bioengineering. Even though it is not a food, but it was consumed as a medicine.

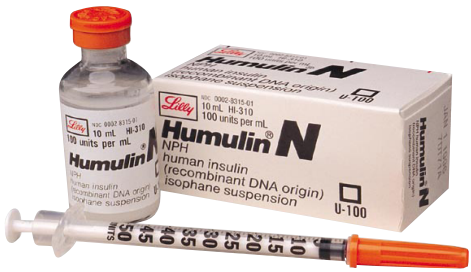


Photo credit: Umaiyal Munusamy

The first field tested of genetically engineered plants are tobacco and tomato. Tobacco was carried out in 1986 and followed by tomato in 1987.

The GM tomato crop was modified to be resistant towards usual rotting and decay better than the wild-type.

Genetically modified food become more popular and being produce widely when US Department of Agriculture approved genetically modified food for commercial production.

1.2. THE PRESENT

By using Google trends, *the author* was noticed that high percentage searched about GMO was detected globally and Palestine showed the highest interest in searching about GMO followed by Australia, Singapore, South Africa and Hong Kong. This proved that many people from all over the country are really concern to know and understand about GMO. In today's world, there are various food that are genetically modified such as wheat, potato, cotton, papaya, squash, canola, corn, alfalfa, apple, eggplant, carrots, strawberries, lettuce, cantaloupe and sugar beet. This is because of the necessity to feed all human population that is increasing day by day. All this genetically modified food is already being granted by USDA. Figure 1.1 showed genetically modified food that was already being deregulated by the USDA to plant and to distribute without restriction up to 2017. Furthermore, a total of 35 approvals had been granted to grow eight transgenic crops commercially.

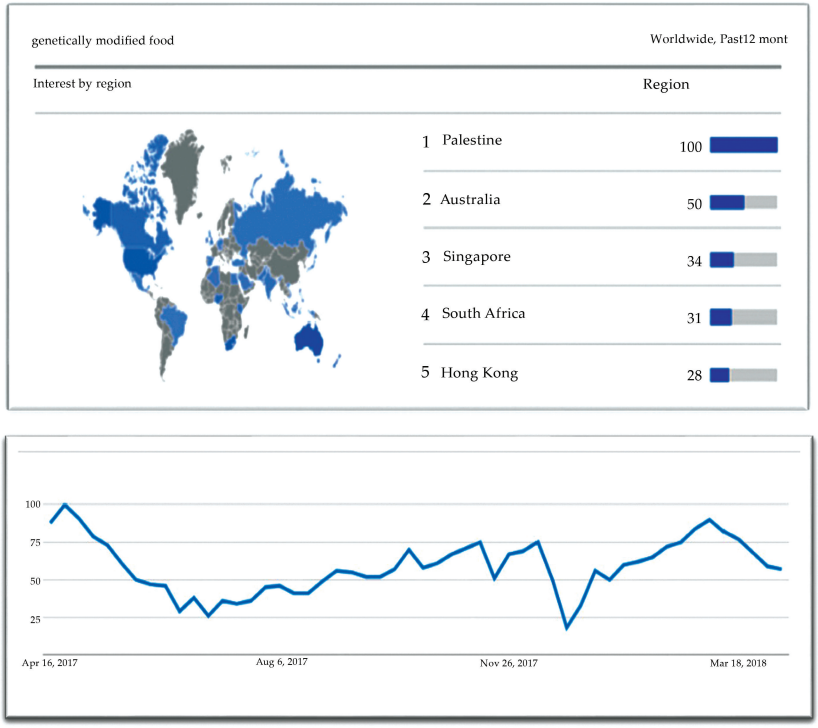
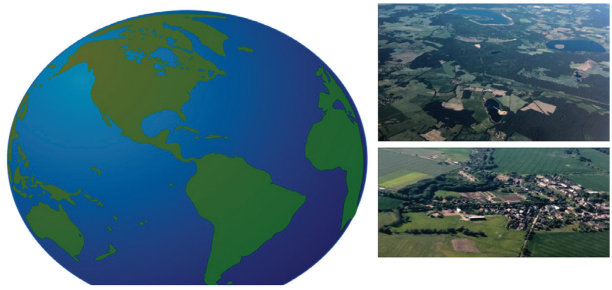


Figure 1.1: Percentage searched on GMO. (*Source from Google Trends*).

1.3. THE FUTURE

In the future, we believe more and more genetically modified food will be available on our market shelves. It is all due to the aim to cater all human population which is over 7.3 billion with sufficient food supply. According to the United Nations predictions, the population could have reached up to 9.7 billion people by 2050, and over 11 billion by 2100. Genetically modified foods are an intense topic to be debatable that has sparked a lot of controversy over the years as many fueled with lack of understanding and vast amount of misinformation. However, type of GM foods which are currently in the pipeline are include food ingredients, feeds, fibers and likely to include plants with improved resistance against plant disease or drought, variety of crops with increased nutrient levels and fish species with enhanced growth characteristics.



The NAKED TRUTH OF GMO

While, in these century, farmers have intentionally changed the genetic makeup of all the crops and their livestock that they have grown since domestic agriculture actually already began 10,000 years ago. Every fruit, vegetable, and grain including organic and heirloom seeds that is commercially available today has been altered by human hands.

CHAPTER 2

WHAT IS A GENETICALLY MODIFIED ORGANISM/FOOD

From the words itself, a layman will understand genetically modified food as a food that was genetically changed through artificial techniques. While, scientifically, genetically modified organisms (GMOs) is defined

as genetic material (DNA) of an organism such as plants, animals and microorganisms that has been altered differently in a predicted outcome away from natural process such as through mating or by natural recombination. Usually, genetically modified food was often being produced by using sophisticated tools and technology.

The technology that is used to produce GMO is known as modern biotechnology, gene technology, recombinant DNA technology and genetic engineering. The technology have moved towards GPS guided self-driving tractors to drones that monitors crop health. Today's modern farms use an array of innovative technologies to grow crops and to utilize resources more efficiently than ever before. It also involves with screening of desired selected individual genes to be transferred from one organism into another or sometimes between nonrelated species. Foods produced from or using GMO are often referred to as GM foods. All GMO product will only reach a consumer after a long lengthy process and it may be slightly costly. Each success story of an individual GMO usually will be after thorough different processes compared to the conventional processes.

These days, scientist is continuing to work on innovations in biotechnology to create GMOs that will benefit various range of people such as farmers, consumers, and stakeholders and in different environment such as drought, flood, dried and humid. GMOs also aimed to reduce food waste, agriculture pollution, maximizing crop yields, improving nutrition and using plant as a factory to produce medicine. Hence, current production of GMO is in hand with modern agriculture where most farmers will start to grow more food within usage of minimum resources.

Besides that, the main aim of developing GM plants-based organisms are to improve crop protection against diseases for example plant that resistance against plant diseases caused by insects or viruses or increased tolerance towards herbicides and environmental stress. Therefore, the GM crops which are currently in the market are mainly with an increased level of plant protection and plant productivity. Later, stakeholders and GMO producer realized that GM foods can also benefit themselves and consumer in terms of money, quality and quantity of the product.

Photo Credit: Umaiyal Munusamy

CHAPTER 3

WHY GENETICALLY MODIFIED ORGANISM/FOOD

CONTENTS

3.1. HUMAN POPULATION

Population growth is defined as an increase in the number of individuals in a population. Global human population growth are increasing. The global population have grown to 7.6 billion in 2018. The number is expecting to keep increasing and estimates reach 9.6 billion by 2050.

Technological advances in the current era have supported population growth in many ways. According to The World Bank, increases in human population will impact the natural resources. The increased population will give pressure on country's agriculture sustainability and biological diversity. A significant population growth will also negatively impact the availability of land for agriculture production and will aggravate demand for food and energy. Therefore, numerous solutions are initiated to boost food production by producing GMO that protect the biodiversity.

3.2. HUNGER

Figure 3.1 shows that the term hunger have been a popular search in New Zealand, Australia, and Ireland. It is because about 11% of childrens are living with food insecurity in New Zealand while, in Australia it is a hidden crisis with over 3.6 million people experiencing food insecurity at some point every year and 27% of them are childrens.

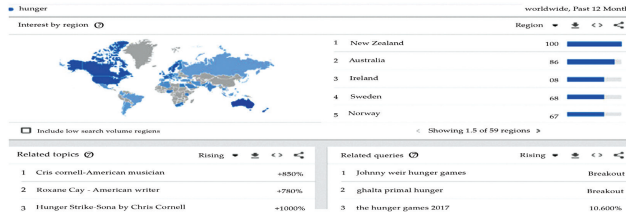


Figure 3.1: Data Shows countries that concern on hunger. (*Source from Google Trends*).

People facing hunger in certain countries are diverse and it affects all range of people such as shown in Figure 3.2.

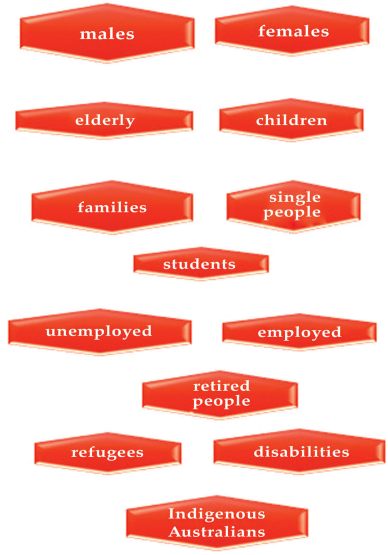


Figure 3.2: Example of the range of people that facing hunger. (*Source from Food and Agriculture Organization of the United Nations (FAO), International Fund for Agricultural Development (IFAD), the United Nations Children's Fund (UNICEF), World Food Programme (WFP)* and *World Health Organization (WHO)*).

According to food aid foundation, globally, 1 in 7 people are suffering from hunger. The majority of the world's hungry people live in developing countries were estimated about 12.9% of the population. It was reported that Asia is the continent with the hungriest people. The percentage in the southern Asia somehow have reduced while in western Asia the percentage showed slightly increased. In country that has high percentage of population such as

sub-Saharan Africa, usually one out of four people will be undernourished. While, about 23 million primary school-age children in Africa alone will often attend classes without any food. Frequently, the number of hungry people on the planet are always come from countries that affected with conflict such as politics, unstable climate conditions and under developed countries.

Besides that, as we know that hunger is not only meant when a 'person is without food' but it is also referred when a person having poor nutrition. Nearly 45% of deaths in children under age of five are due to poor nutrition in their diet. On the stunned issues in the growing children, data that was reported showed one in four children will be unconscious and the proportion will be raised in the developing countries. People living in the countries affected with significant portion of death risk, disease, and breakdown of their livelihoods will be more likely to be undernourished with 2.5 more than undernourished people from different location. World Food Programme estimated almost millions food need to be allocated to reduce the hunger among human population from various range age.

3.3. QUALITY OF THE ORGANISMS

Wild organisms such as wild plant and animal that are used as foods may have least quality. This type of organisms may have borne to various diseases and may easily deteriorated. In addition, they may also contain less nutrition. Genetically modified organisms (GMO) plant which developed through a precise process will be able to provide various type of nutrient that is needed by mankind. Using modern plant breeding methods, the generated so-called GMOs have shown that crop nutritional quality can be greatly improved. Many GMO varieties have been specifically developed to be resistant towards pests, tolerant to drought and contain beneficial nutrients. This leads to a reduction in insecticides usage. Therefore, GMO plant varieties are quality than the traditionally breed or wild type varieties.

3.4. ENVIRONMENTAL STRESS

Environmental stress is defined by three components. The first component will be the environmental stress itself, the second will be the interaction between biological systems and finally the responses. There are many types of environmental stress that was being identified:

a) Physical stresses such as volcanic eruptions and windstorm.

b) Wildfire due to agriculture practices.

c) Toxic pollution caused by agriculture practices.

d) Climate change such as heat, flood, and moisture.

e) Biological stresses such as competition, herbivory, predation, parasitism, and disease.

Therefore, GMOs are created to achieve a desired trait to cater food to all growing population. Few examples of the popular traits of GMO that are available shown in Figure 3.3.

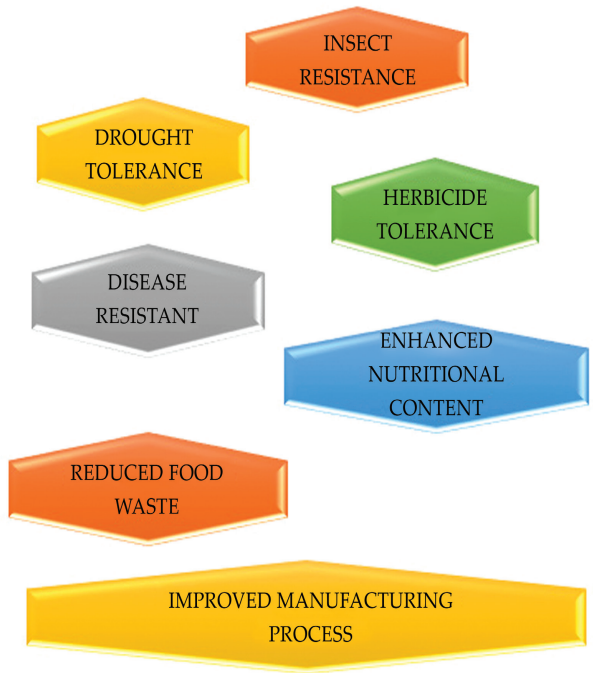
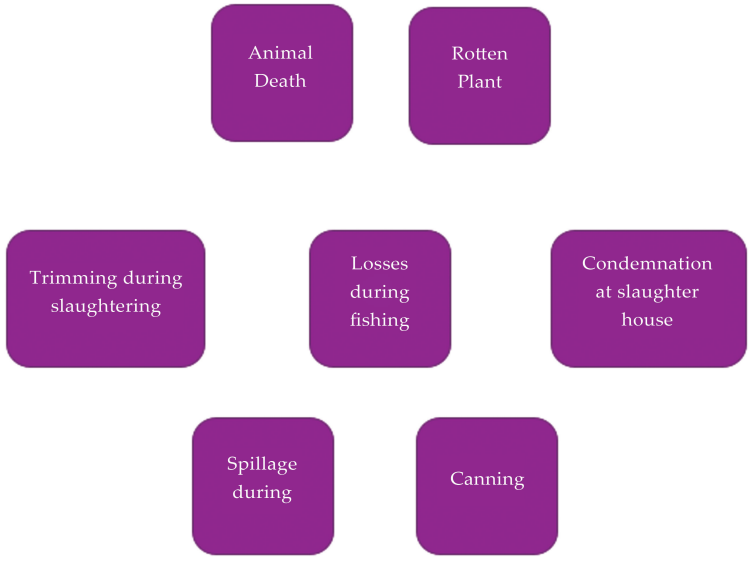


Figure 3.3: Example of popular GMO traits.

3.5. CONSUMER NEEDS

As population grows, the need towards food are increasing as well. However, lot of losses during agriculture production, postharvest handling, processing and during distribution have reduce the amount of the product that reaches the consumer. In agricultural production losses occur due to mechanical damage and/or spillage during harvest operation such as threshing or during fruit picking and sorting during post-harvest will reduce the total product

compared to the product that was harvested from the field. In addition, from the postharvest processes to storage and transportation between plantation field to the distribution location, losses due to spillage and rotten will also reduce the amount of food to be delivered to the consumer. In addition, the process of food processing such as during washing, peeling, slicing and boiling will also contribute some losses due to spillage and degradation. Moreover, even during food distribution, losses and waste in the market system can also occured. All these will affect the needs and expectation of consumers. Therefore, enhance production and processing are needed to minimize those losses. Below is the detected possibilities of losses in various sector.



Therefore, growing more food to cater the human population and to compensate the losses during post harvesting and production have become important. Increasing the volume of food production was considered the least important as this will only lead to maximum use of resources and increase in the production cost of the food. Therefore, genetically modified food which can be produce with less resources were considered. In addition, genetically modified food which derived from genetically modified organisms have possibilities of lesser tilling, capability in improving soil health and water retention, reducing runoff and greenhouse gas emissions. Additionally, genetically modified plants may have capabilities to use nitrogen more effectively, reduce the amount of fertilizer usage, and capable to increase

productivity. Furthermore, they also come with better taste, beautiful and enhanced adaptation.

Photo credit: Predeeban Munusamy

CHAPTER 4

TYPES OF GMO/GMF

CONTENTS

Genetically modified food is derived from a genetically modified organism. A standard pipeline that is present in any production of genetically modified food was elaborated in Figure 4.1.

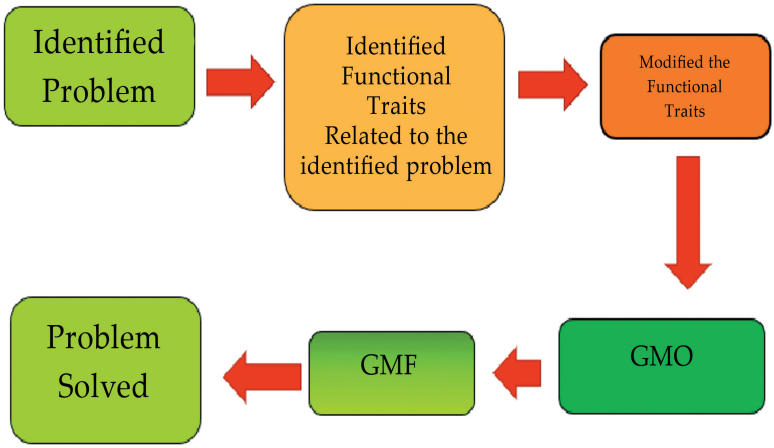


Figure 4.1: The standard pipeline that exists in the production of GMOs/GMF.

A genetically modified organism that available in the international market today have been designed using one of the basic traits as indicated in Figure 4.2. Genetically modified organism help maximize yields, provides farmers with season-long protection against target pests that reduces the need for pesticide applications, chemical usage, and fuel. Usage of this genetically modified organism will lead to lower input costs as well as reduce the postharvest cost. Furthermore, it can also protect our environment through preserving topsoil, preventing erosion, and reducing carbon emissions as farmers can till the soil much less often by using superior weed control of genetically modified crops or by applying targeted herbicides only when needed**.** By using GM crops that has better moisture retention and better endure drought conditions the need for additional irrigation can be reduced. A plant variety that lowered the production number due to infection can be minimized through the usage of disease resistant plant. Resistance against insects is achieved by incorporating the bacterium *Bacillus thuringiensis*

(Bt) gene for toxin production into plant. This toxin is used as a conventional insecticide in agriculture where plant incorporated with this gene will inhibit insect from destroying the plant. Fortunately, this gene is safe for human consumption and plants incorporated with this gene are no harmful for human being. GM crops that inherently produce this toxin have been shown to require lower quantities of insecticides in certain situations, e.g. where pest pressure is high. In addition, virus resistance plant is achieved through introduction of a gene from certain viruses that cause disease in plants. Virus resistance plants are plants that less susceptible to diseases caused by such viruses. This characteristic will enhance total crop yields. Furthermore, herbicide tolerance is achieved through the introduction of a gene from a bacterium conveying resistance to some herbicides. In the situations where weed pressure is high, the use of such crops have resulted in low herbicides application.

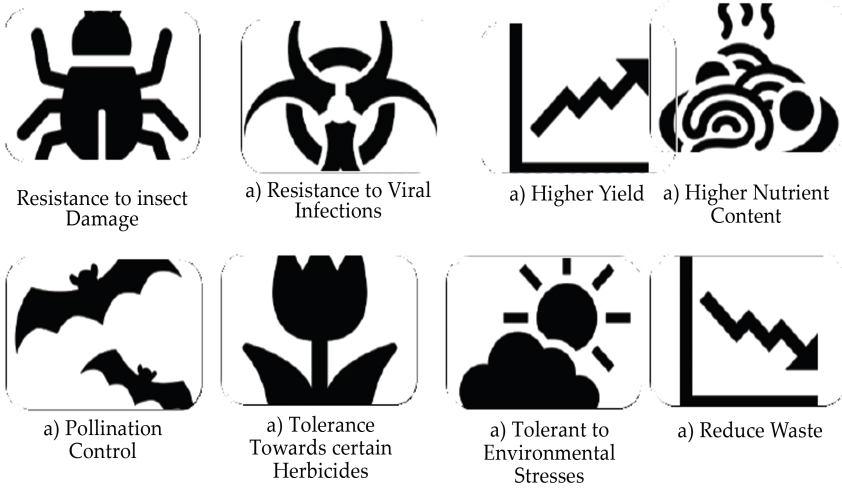
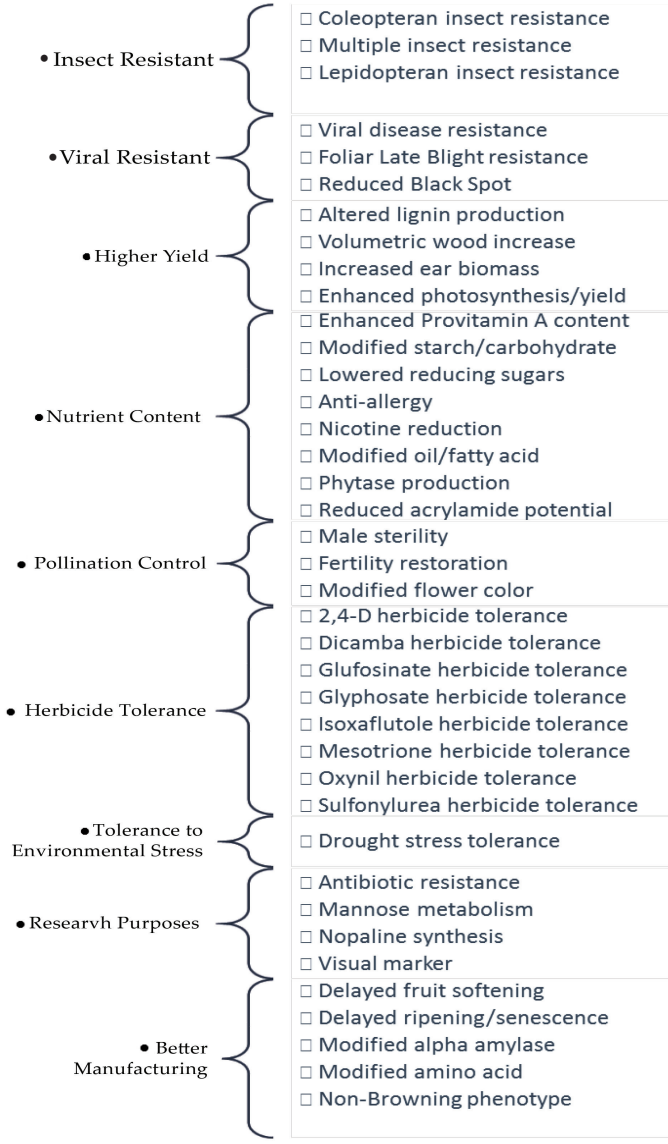


Figure 4.2: Basic traits in GMO.

Below is the breakdown of each trait of GMOs



4.1. WAY TO HIGHER YIELDS

Imagine a crop plants needs to be planted in low-phosphorus soil. Screening for root traits that can grow in such an environment is considered one of a tedious task. Therefore, sometimes focusing on the crop shoots rather than their roots are preferred. Let say, the root is the main concern, root depth

can be determined because phosphorus tends to stick to the upper layers of soil. There are two type of roots, basal roots that closer to the base of a plant that will grow at a shallow angle to produced 58% more above-ground biomass and steeper basal roots that showed lesser performance than the basal roots. Besides that, plants with long root hairs uptake phosphorus better and capable to improve the plant yield, relative to the plant variety with shorter root hairs. When root traits are knitted together, high yield are detected. For example, in Mozambique, beans with shallow roots and long root hairs gave higher yields per hectare in soils depleted with phosphorus, compared with local varieties. With this approach, farmers who cannot afford to purchase fertilizers still will able to produce better yield with this types of beans variety.

Table 4.1: Classification of Genetically Modified Food

Functional Gene	Gene Source	Product	Function
Insect Resistant			
cry1A	*Bacillus thuringiensis*	Delta-endotoxin of the Cry1A group	Resistance to lepidopteran insects
cry1A.105	*Bacillus thuringiensis* subsp. kumamotoensis	Cry1A.105 protein which comprises the Cry1Ab, Cry1F and Cry1Ac proteins	Resistance to lepidopteran insects
cry1Ab	*Bacillus thuringiensis* subsp. kurstaki	Cry1Ab delta-endotoxin	Resistance to lepidopteran insects
cry1Ab (truncated)	*Bacillus thuringiensis* subsp. kumamotoensis	Cry1Ab delta-endotoxin	Resistance to lepidopteran insects
cry1Ab-Ac	*Bacillus thuringiensis*	Cry1Ab-Ac delta-endotoxin (fusion protein)	Resistance to lepidopteran insects
cry1Ac	*Bacillus thuringiensis*-subsp. Kurstaki strain HD73	Cry1Ac delta-endotoxin	Resistance to lepidopteran insects
cry34Ab1	*Bacillus thuringiensis* strain PS149B1	Cry34Ab1 delta-endotoxin	Resistance to coleopteran insects particularly corn rootworm
cry35Ab1	*Bacillus thuringiensis* strain PS149B1	Cry35Ab1 delta-endotoxin	Resistance to coleopteran insects particularly corn rootworm
cry3A	*Bacillus thuringiensis* subsp. tenebrionis	cry3A delta-endotoxin	Resistance to coleopteran insects

cry3Bb1	*Bacillus thuringiensis* subsp. kumamotoensis	Cry3Bb1 delta-endotoxin	Resistance to coleopteran insects particularly corn rootworm
dvsnf7	Western Corn Rootworm (*Diabrotica virgifera virgifera*)	Double-stranded RNA transcript containing a 240 bp fragment of the WCR snf7 gene	Down-regulation of the targeted snf7 gene that lead to Western Corn Rootworm mortality
mcry3A	*Bacillus thuringiensis*-subsp. tenebrionis	Modified Cry3A delta-endotoxin	Resistance to coleopteran insects particularly corn rootworm pests
API	*Sagittaria sagittifolia* (arrowhead)	Arrowhead protease inhibitor protein A or B	Resistance to a wide range of insect pests
CpTI	*Vigna unguiculata*	Trypsin inhibitor	Resistance to a wide range of insect pests
ecry3.1Ab	*Bacillus thuringiensis*	Chimeric (Cry3A-Cry1Ab) delta-endotoxin protein	Resistance to coleopteran and lepidopteran insects
cry34Ab1	*Bacillus thuringiensis* strain PS149B1	Cry34Ab1 delta-endotoxin	Resistance to coleopteran insects particularly corn rootworm
Viral Resistant			
ppo5	*Solanum verrucosum*	Double-stranded RNA	Down regulate PPO5 transcripts which lowers black spot bruise development
Rpi-vnt1	*Solanum venturii*	Late blight resistance protein	Confers resistance to potato late blight
ac1 (sense and antisense)	Bean Golden Mosaic Virus (BGMV)	Sense and antisense RNA of viral replication protein (Rep); no functional viral replication protein is produced	Resistance to the Bean Golden Mosaic Virus (BGMV)
cmv_cp	Cucumber Mosaic Cucumovirus (CMV)	Coat protein of cucumber mosaic cucumovirus (CMV)	Resistance to cucumber mosaic cucumovirus (CMV)
plrv_orf1	Potato Leaf Roll Virus (PLRV)	Putative replicase domain of the potato leafroll virus (PLRV)	Resistance to potato leafroll virus (PLRV)
plrv_orf2	Potato Leaf Roll Virus (PLRV)	Putative helicase domain of the potato leafroll virus (PLRV)	Resistance to potato leafroll virus (PLRV)

Higher Yield			
athb17	*Arabidopsis thaliana*	A protein of the class II family of the homeodomain-leucine zipper (HD-Zip) transcription factors	Modulates plant growth and development and regulates gene expression
ccomt (inverted repeat)	*Medicago sativa* (alfalfa)	dsRNA that suppresses endogenous S-adenosyl-L-methionine: trans-caffeoyl CoA 3-O-methyltransferase (CCOMT gene) RNA transcript levels via the RNA interference (RNAi) pathway	Reduces content of guaiacyl (G) lignin
EgCAld5H	*Eucalyptus grandis*	CAld5H enzyme	Regulates syringyl monolignol pathway
bbx32	*Arabidopsis thaliana*	Protein that interacts with one or more endogenous transcription factors to regulate the plant's day/night physiological processes	Modulate plant's diurnal biology and enhance growth and reproductive development
Nutrient Content			
7crp	*Cryptomeria japonica*	Modified cry j 1 and cry j 2 pollen antigens containing seven major human T cell epitopes	Triggers mucosal immune tolerance to cedar pollen allergens
PhL	*Solanum tuberosum*	Double-stranded RNA	Downregulate PhL transcripts which lowers reducing sugars
R1	*Solanum tuberosum*	Double-stranded RNA	Downregulate R1 transcripts which lowers reducing sugars
Vlnv	*Solanum tuberosum*	Double-stranded RNA	Downregulate VInv transcripts which lowers reducing sugars

PhL	*Solanum tuberosum*	double-stranded RNA	Downregulate PhL transcripts which lowers reducing sugars
crt1	*Pantoea ananatis*	Phytoene desaturase enzyme CRTI	Catalyzes the conversion of 15-cis-phytoene to all-trans-lycopene
psy1	*Zea mays*	Phytoene synthase ZmPSY1	Converts geranyl-geranyl diphosphate into phytoene, and acts upstream of CrtI in the carotenoid biosynthesis pathway
crt1	*Pantoea ananatis*	Phytoene desaturase enzyme CrtI	Catalyzes the conversion of 15-cis-phytoene to all-trans-lycopene
gbss (antisense fragment)	*Solanum tuberosum*	No functional granule-bound starch synthase (GBSS) enzyme is produced; production of GBSS enzyme is suppressed by a gene silencing mechanism	Reduces the levels of amylose and increases the levels of amylopectin in starch granules
NtQPT1 (antisense)	*Nicotiana tabacum*	Antisense RNA of quinolinic acid phosphoribosyltransferase (QPTase) gene; no functional QPTase enzyme is produced	Suppresses the transcription of the QPTase gene, thereby reducing the production of nicotinic acid, a precursor for nicotine
cel1	*Arabidopsis thaliana*	CEL1 recombinant protein	Promotes a faster growth
phyA	*Aspergillus niger* var. van Tieghem	3-phytase enzyme	Increases the breakdown of plant phytates which bind phosphorus and makes the latter available to monogastric animals

phyA2	*Aspergillus niger* strain 963	Phytase enzyme	Degrades phytate phosphorus in seeds into inorganic phosphate to be available to animals when used as feed
phyA	*Aspergillus niger* var. van Tieghem	3-phytase enzyme	Increases the breakdown of plant phytates which bind phosphorus and makes the latter available to monogastric animals
Pollination Control			
barnase	*Bacillus amyloliquefaciens*	Barnase ribonuclease (RNAse) enzyme	Causes male sterility by interfering with RNA production in the tapetum cells of the anther
dam	*Escherichia coli*	DNA adenine methylase enzyme	Confers male sterility by interfering with the production of functional anthers and pollen
zm-aa1	*Zea mays*	Alpha amylase enzyme	Hydrolyzes starch and makes pollen sterile when expressed in immature pollen
barstar	*Bacillus amyloliquefaciens*	Barnase ribonuclease inhibitor	Restores fertility by repressing the inhibitory effect of barnase on tapetum cells of the anther
ms45	*Zea mays*	ms45 protein	Restores fertility by restoring the development of the microspore cell wall that gives rise to pollen
5AT	*Torenia sp.*	Anthocyanin 5-acyltransferase (5AT) enzyme	Alters the production of a type of anthocyanin called delphinidin

bp40 (f3'5'h)	*Viola wittrockiana*	Flavonoid 3,'5'-hydroxylase (F3'5'H) enzyme	Catalyzes the production of the blue-colored anthocyanin pigment delphinidin and its derivatives
cytb5	*Petunia (Petunia hybrida)*	Cytochrome b5	Cyt b5 protein acts as an electron donor to the Cyt P450 enzyme and is required for full activity of the Cyt P450 enzyme Flavinoid 3' 5' hydroxylase in vivo and the generation of purple/blue flower colors
dfr	*Petunia hybrida*	Dihydroflavonol-4-reductase (DFR) hydroxylase enzyme	Catalyzes the production of the blue-colored anthocyanin pigment delphinidin and its derivatives
dfr-diaca	*Carnation (Dianthus caryophyllus)*	Dihydroflavonol-4-reductase enzyme	Functions in the biosynthesis pathway of the pink/red-colored anthocyandin 3-O-(6-O-malylglucoside) pigment in carnations
hfl (f3'5'h)	*Petunia hybrida*	Flavonoid 3,'5'-hydroxylase (F3'5'H) enzyme	Catalyzes the production of the blue-colored anthocyanin pigment delphinidin and its derivatives
Herbicide Tolerance			
als	*Arabidopsis thaliana*	Herbicide tolerant enzyme acetolactate synthase (als)	Allows the synthesis of essential amino acids in the presence of sulfonylurea herbicides
csr1-2	*Arabidopsis thaliana*	Modified acetohydroxyacid synthase large subunit (AtAHASL)	Tolerance to imidazolinone herbicides
gm-hra	*Glycine max*	Modified acetolactate synthase (ALS) enzyme	Tolerance to applications of sulfonylurea – based herbicides

S4-HrA	*Nicotiana tabacum* cv. Xanthi	Herbicide tolerant acetolactate synthase (ALS) enzyme	Allows the plant to synthesize essential amino acids in the presence of sulfonylurea herbicides
surB	*Nicotiana tabacum*	Herbicide tolerant acetolactate synthase (ALS) enzyme	Tolerance to sulfonylurea herbicides and other acetolactate synthase (ALS) inhibiting herbicides
zm-hra	*Zea mays*	Herbicide tolerant acetolactase synthase (als) enzyme	Tolerance to acetolactate synthase-inhibiting herbicides such as sulfonylurea and imidazolinone
aad-1	*Sphingobium herbicidovorans*	Aryloxyalkanoate dioxygenase 1 (AAD-1) protein	Detoxifies 2,4-D herbicide by side-chain degradation and degrades the R-enantiomers of aryloxyphenoxypropionate herbicides
aad-12	*Delftia Acidovorans*	Aryloxyalkanoate di-oxygenase 12 (AAD-12) protein	Catalyzes the side chain degradation of 2,4-D herbicide
bxn	*Klebsiella pneumoniae* subsp. *Ozaenae*	Nitrilase enzyme	Eliminates herbicidal activity of oxynil herbicides (e.g., bromoxynil)
bar	*Streptomyces hygroscopicus*	Phosphinothricin N-acetyltransferase (PAT) enzyme	Eliminates herbicidal activity of glufosinate (phosphinothricin) herbicides by acetylation
pat	*Streptomyces viridochromogenes*	Phosphinothricin N-acetyltransferase (PAT) enzyme	Eliminates herbicidal activity of glufosinate (phosphinothricin) herbicides by acetylation
pat (syn)	*Streptomyces viridochromogenes* strain Tu 494	Phosphinothricin N-acetyltransferase (PAT) enzyme	Eliminates herbicidal activity of glufosinate (phosphinothricin) herbicides by acetylation

2mepsps	*Zea mays*	5-enolpyruvyl shikimate-3-phosphate synthase enzyme (double mutant version)	Decreases binding affinity for glyphosate, thereby increasing tolerance to glyphosate herbicide
cp4 epsps (aroA: CP4)	*Agrobacterium tumefaciens*strain CP4	Herbicide tolerant form of 5-enolpyruvulshikimate-3-phosphate synthase (EPSPS) enzyme	Decreases binding affinity for glyphosate, thereby conferring increased tolerance to glyphosate herbicide
epsps (Ag)	*Arthrobacter globiformis*	5-enolpyruvylshikimate-3-phosphate-synthase enzyme	Confers tolerance to glyphosate herbicides
epsps grg23ace5	*Arthrobacter globiformis*	Modified 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) protein or EPSPS ACE5 protein	Confers tolerance to glyphosate herbicides
gat4601	*Bacillus licheniformis*	Glyphosate N-acetyltransferase enzyme	Catalyzes the inactivation of glyphosate, conferring tolerance to glyphosate herbicides
gat4621	*Bacillus licheniformis*	Glyphosate N-acetyltransferase enzyme	Catalyzes the inactivation of glyphosate, conferring tolerance to glyphosate herbicides
goxv247	*Ochrobactrum anthropi* strain LBAA	Glyphosate oxidase	Confers tolerance to glyphosate herbicides by degrading glyphosate into aminomethylphosphonic acid (AMPA) and glyoxylate
mepsps	*Zea mays*	Modified 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme	Confers tolerance to glyphosate herbicides
hppdPF W336	*Pseudomonas fluorescens*strain A32	Modified p-hydroxyphenylpyruvate dioxygenase (hppd) enzyme	Confers tolerance to HPPD-inhibiting herbicides (such as isoxaflutole) by reducing the specificity for the herbicide's bioactive constituent

dmo	*Stenotrophomonas maltophilia* strain DI-6	Dicamba mono-oxygenase enzyme	Confers tolerance to the herbicide dicamba (2-methoxy-3,6-dichlorobenzoic acid) by using dicamba as substrate in an enzymatic reaction
avhppd-03	Oat (Avena sativa)	p-hydroxyphenylpyruvate dioxygenase	Tolerance to Mesotrione herbicide
bar	*Streptomyces hygroscopicus*	Phosphinothricin N-acetyltransferase (PAT) enzyme	Eliminates herbicidal activity of glufosinate (phosphinothricin) herbicides by acetylation
pat	*Streptomyces viridochromogenes*	Phosphinothricin N-acetyltransferase (PAT) enzyme	Eliminates herbicidal activity of glufosinate (phosphinothricin) herbicides by acetylation
Tolerance to Environmental Stress			
cspB	*Bacillus subtilis*	Cold shock protein B	Maintains normal cellular functions under water stress conditions by preserving RNA stability and translation
EcBetA	*Escherichia coli*	Choline dehydrogenase	Catalyzes the production of the osmoprotectant compound glycine betaine conferring tolerance to water stress
Hahb-4	*Helianthus annuus*	Isolated nucleic acid molecule encoding the transcription factor Hahb-4	The transcription factor Hahb-4 binds to a dehydration transcription regulating region of plant
RmBetA	*Rhizobium meliloti*	Choline dehydrogenase	Catalyzes the production of the osmoprotectant compound glycine betaine conferring tolerance to water stress

cspB	*Bacillus subtilis*	Cold shock protein B	Maintains normal cellular functions under water stress conditions by preserving RNA stability and translation
EcBetA	*Escherichia coli*	Choline dehydrogenase	Catalyzes the production of the osmoprotectant compound glycine betaine conferring tolerance to water stress
Hahb-4	*Helianthus annuus*	Isolated nucleic acid molecule encoding the transcription factor Hahb-4	The transcription factor Hahb-4 binds to a dehydration transcription regulating region of plant
Research Purposes			
aad	*Escherichia coli*	3”(9)-O-aminoglycoside adenylyltransferase enzyme	Allows selection for resistance to aminoglycoside antibiotics such as spectinomycin and streptomycin
aph4 (hpt)	*Escherichia coli*	hygromycin-B phosphotransferase (hph) enzyme	Allows selection for resistance to the antibiotic hygromycin B
bla	*Escherichia coli*	Beta lactamase enzyme	Detoxifies beta lactam antibiotics such as ampicillin
nptII	*Escherichia coli* Tn5 transposon	Neomycin phosphotransferase II enzyme	Allows transformed plants to metabolize neomycin and kanamycin antibiotics during selection
spc	*Escherichia coli*	Spectinomycin adenyl transferase enzyme (not expressed in plant tissues)	Confers resistance to spectinomycin/streptomycin antibiotics, which permits prokaryotic selection
aad	*Escherichia coli*	3”(9)-O-aminoglycoside adenylyltransferase enzyme	Allows selection for resistance to aminoglycoside antibiotics such as spectinomycin and streptomycin

nos	*Agrobacterium tumefaciens*strain CP4	Nopaline synthase enzyme	Catalyzes the synthesis of nopaline, which permits the identification of transformed plant embryos
dsRed2	*Discosoma* sp.	Red fluorescent protein	Produces red stain on transformed tissue, which allows visual selection
uidA	*Escherichia coli*	Beta-D-glucuronidase (GUS) enzyme	Produces blue stain on treated transformed tissue, which allows visual selection
dsRed2	*Discosoma* sp.	Red fluorescent protein	Produces red stain on transformed tissue, which allows visual selection
uidA	*Escherichia coli*	Beta-D-glucuronidase (GUS) enzyme	Produces blue stain on treated transformed tissue, which allows visual selection
pmi	*Escherichia coli*	Phosphomannose Isomerase (PMI) enzyme	Metabolizes mannose and allows positive selection for recovery of transformed plants
Better Manufacturing			
pg (sense or antisense)	*Lycopersicon esculentum*	No functional polygalacturonase enzyme is produced (transcription of the endogenous enzyme is suppressed by a gene silencing mechanism)	Inhibits the production of polygalacturonase enzyme responsible for the breakdown of pectin molecules in the cell wall, and thus causes delayed softening of the fruit
acc (truncated)	*Lycopersicon esculentum*	Modified transcript of 1-amino-cyclopropane-1-carboxylic acid (ACC) synthase gene	Suppresses the normal expression of the native ACC synthase gene, resulting in reduced ethylene production and delayed fruit ripening

acc (truncated)	*Dianthus caryophyllus*	Modified transcript of 1-amino-cyclopropane -1-carboxylic acid (ACC) synthase gene	Causes reduced synthesis of endogenous ethylene through a gene silencing mechanism and thus delayed senescence and longer vase life
accd	*Pseudomonas chlororaphis*	1-amino-cyclopropane-1-carboxylic acid deaminase enzyme	Metabolizes the precursor of the fruit ripening hormone ethylene, resulting in delayed fruit ripening
acc (truncated)	*Lycopersicon esculentum*	Modified transcript of 1-amino-cyclopropane-1-carboxylic acid (ACC) synthase gene	Suppresses the normal expression of the native ACC synthase gene, resulting in reduced ethylene production and delayed fruit ripening
acc (truncated)	*Dianthus caryophyllus*	Modified transcript of 1-amino-cyclopropane -1-carboxylic acid (ACC) synthase gene	Causes reduced synthesis of endogenous ethylene through a gene silencing mechanism and thus delayed senescence and longer vase life
accd	*Pseudomonas chlororaphis*	1-amino-cyclopropane-1-carboxylic acid deaminase enzyme	Metabolizes the precursor of the fruit ripening hormone ethylene, resulting in delayed fruit ripening
anti-efe	*Lycopersicon esculentum*	Antisense RNA of 1-amino-cyclopropane -1-carboxylate oxidase (ACO) gene (no functional ACO enzyme is produced)	Causes delayed ripening by suppressing the production of ethylene via silencing of the ACO gene that encodes an ethylene-forming enzyme
sam-k	*Escherichia coli* bacteriophage T3	S-adenosylmethionine hydrolase enzyme	Causes delayed ripening by reducing the S-adenosylmethionine (SAM), a substrate for ethylene production

anti-efe	*Lycopersicon esculentum*	Antisense RNA of 1-amino-cyclopropane -1-carboxylate oxidase (ACO) gene (no functional ACO enzyme is produced)	Causes delayed ripening by suppressing the production of ethylene via silencing of the ACO gene that encodes an ethylene-forming enzyme
sam-k	*Escherichia coli* bacteriophage T3	S-adenosylmethionine hydrolase enzyme	Causes delayed ripening by reducing the S-adenosylmethionine (SAM), a substrate for ethylene production
anti-efe	*Lycopersicon esculentum*	Antisense RNA of 1-amino-cyclopropane -1-carboxylate oxidase (ACO) gene (no functional ACO enzyme is produced)	Causes delayed ripening by suppressing the production of ethylene via silencing of the ACO gene that encodes an ethylene-forming enzyme
amy797E	*Thermococcales* spp.	Thermostable alpha-amylase enzyme	Enhances bioethanol production by increasing the thermostability of amylase used in degrading starch
cordapA	*Corynebacterium glutamicum*	dihydrodipicolinate synthase enzyme	Increases the production of amino acid lysine
fad2-1A (sense and antisense)	*Glycine max*	No functional enzyme is produced (production of delta-12 desaturase enzyme is suppressed by RNA interference)	Reduces desaturation of oleic acid to linoleic acid; increases the levels of monounsaturated oleic acid and decreases the levels of saturated linoleic acid in the seed

fatb1-A (sense and antisense segments)	*Glycine max*	No functional enzyme is produced (production of FATB enzymes or acyl-acyl carrier protein thioesterases is suppressed by RNA interference)	Decreases the transport of saturated fatty acids out of the plastid, thereby increasing their availability to desaturation to oleic acid; reduces the levels of saturated fatty acids and increases the levels of oleic acid
gm-fad2-1 (partial sequence)	*Glycine max*	No functional enzyme is produced (expression of the endogenous fad2-1 gene encoding omega-6 desaturase enzyme was suppressed by the partial gm-fad2-1 gene fragment)	Blocks the formation of linoleic acid from oleic acid (by silencing the fad2-1 gene) and allows accumulation of oleic acid in the seed
gm-fad2-1 (silencing locus)	*Glycine max*	No functional enzyme is produced (production of endogenous delta-12 desaturase enzyme was suppressed by an additional copy of the gm-fad2-1 gene via a gene silencing mechanism)	Blocks the conversion of oleic acid to linoleic acid (by silencing the endogenous fad2-1 gene) and allows accumulation of monounsaturated oleic acid in the seed
Lackl-delta12D	*Lachancea kluyveri*	Delta-12-desaturase	Converts oleic acid to linoleic acid
Micpu-delta-6D	*Micromonas pusilla*	Delta-6-desaturase	Convert a-linolenic acid to stearidonic acid

PGAS PPO suppression gene	*Malus domestica*	Double-stranded RNA (dsRNA)	Double-stranded RNA (dsRNA)from the suppression transcript is processed into small interfering RNAs (siRNAs) that direct the cleavage of the target mRNA through sequence complementarity and suppresses PPO resulting in apples with a non-browning phenotype
asn1	*Solanum tuberosum*	Double-stranded RNA	Designed to generate dsRNA to downregulate Asn1 transcripts which lowers asparagine formation

CHAPTER 5

IMPORTANT OF GENETICALLY MODIFIED FOOD

CONTENTS

5.1. FOOD ISSUES

Food consumption is an essential to human life. In these days food have become more specialized towards individual choice rather than as an economic commodity. Nowadays, as information becoming seamless, issues that arise regarding food are easily reachable to consumers. Consumer becomes more aware on the type and quality of food that they are eating. They are also demanding on the choices of specific taste, texture, and of a food color and information on origin and the processing method of a food that they consume. Basically, the usual food issues that arise either can be positive or negative. Producer, manufacturer together with researcher working together to provide better quality food through plant breeding and genetically modified organism. Through this approach, there will be many positive and negative impacts steaming from the creation of GMF. Previous, current and future GMF are likely derived from plants and animals product. Some of the positive food issues involve with plants that are improved resistance against plant disease or drought, crops with increased nutrient levels, fish and poultry species with enhanced growth characteristics. Other GMOs have been developed to extend the shelf life of fruits and vegetables and to reduce food waste. While some of the negative food issues are such as usage of antibiotic in meat production. However, reduction of antibiotic usage on poultry will have significant negative implications for animal health. Other than that, microbiological hazards, pesticide residues, misuse of food additive, chemical contaminations and adulteration are also some of the food issues that usually considered s negative food issues by consumers. Therefore, GMOs have been developed to improved crop and poultry with minimum impact on consumer and environment.

5.2. CLIMATE CHANGE

Agriculture is an important sector in many countries. The crops, livestock, and seafood production has contributed in the economy of each countries. Land cultivation, animal breeding, and fisheries are very close to the climate. A slight change in the climate gave a huge impact on agriculture sector. For example, increases in temperature and carbon dioxide (CO_2) can increase some crop yields in some places but can be detrimental at certain regions. Changes in the frequency and severity of droughts and floods has challenged farmers and ranchers subsequently threaten food safety. Meanwhile, warmer water temperatures are likely caused the habitat of many fish and shellfish species to shift. This parallelly had distrusted the whole ecosystem. Overall, climate change has made it more difficult to grow crops, raise animals, and catch fish through the same methods and at similar location. Therefore, the effects of climate change are crucial to be considered as it will change the whole farming practices and technology. This is where GMO will take its step, GMO have the capabilities to overcome most of the climate change crisis. With good GMO, quality GMF will be available on consumer plate.

5.3. COST

Some may have a thought why certain farmers are able to grow so much food per acre than other farmers, this is because some farmers have the access on certain nitrogen fertilizer that boost plant growth. However, usage of high volume of fertilizer sometimes can leads to runoff that subsequently pollute environment. Therefore, usage of crops that can completely utilize the nitrogen or plant that can fertilize themselves by pulling nitrogen from the air are preferable. Developing self-fertilizing plant will reduce the total cost of agriculture production as less fertilizer are being used. It would boost yields of poor farmers who can't afford purchasing additional fertilizer and at the same time it would also cut down on nitrogen runoff that creates water pollution. In addition, improved technology, as in the case of producing quality seeds or GM seeds or crops will improved farm yields. This was proven by Brookes and Barfoot through commercialization of GM crops which occurs at a rapid rate since the mid-1990s. It showed a very significant net economic benefits at the farm level. The value was detected to reached up to $15.4 billion in 2015. These returns also shown an increase among farmers which is about 49% from farmers in developed countries and 51% from farmers in developing countries.

5.4. QUANTITY

Quantity is the measurement stating how much is there or defined as an amount or number of the referred material. Quantity of crop, pastures, and food that is available are crucial to ensure zero hunger. In agriculture, the quantity of production is depending on the size of the agriculture land. Huge land will give high volume of agriculture production and smaller land will give lesser amount of production. In addition, an agriculture land can be used as an arable farming (land dedicated to grow crops), and pasture land (which includes meadows and pastures used for livestock rearing). In these days, as the population increase, most of the arable land are reducing. This can be clearly seen from the figure generated from the World Bank Data (Figure 5.1). In addition, FAO predicted that the finite amount of arable land available for food production per person will decrease from the current value which is about 0.242 ha to 0.18 ha by 2050. Therefore, normal breeding and plantation approach may not be able to cater food for everyone. This is because in normal agriculture processes it requires huge arable land area. In order to overcome this problem, agriculture processes with enhance technology should applied. For example usage of plant that can grow in any type of land such as high soil salinity as soil salinity has become a major problem in agriculture in certain country especially in the San Joaquin Valley. In addition, farmers can consider planting plant that requires less fertilizer, plant that resistant to insects and weeds as a way to preserve the surrounding environment by reducing the amount of pesticide and herbicide as it is a must to keep crops healthy and pest free. In addition, these herbicide and pesticide resistant plant variety will helps farmers from developing countries as farmers from this region may not have access on the information about safer herbicide and pesticides usage. By using quality GMO seeds farmer can also choose to use GMO seeds that is much smaller as it will give high yield/quantity with less herbicide and pesticide application to fight diseases. For example, maize that contains Bt toxinre quires zero external pesticides as it will act as a natural pesticide. This type of plant which is genetically modified can be developed through application of forefront technology that is currently available. With the usage of enhance plant material and processes, farmers can reduce cost saving by getting higher yield within limited resources and this will supersede the cost of either GMO plant or its seed investment.

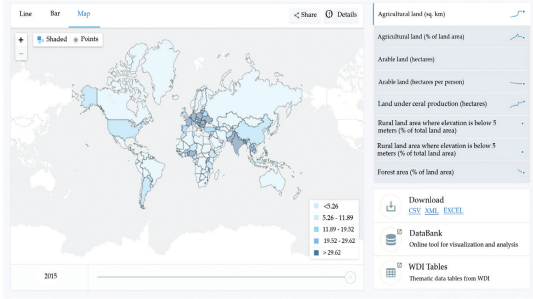


Figure 5.1: Arable land worldwide.

5.5. QUALITY

People are very concern about what they are eating as it has become everyone routine in this millennial age. People are looking forward to consume enhance nutritional food and avoids food that can cause allergy. As our food supply becomes increasingly globalized, the need to strengthen food nutrition and safety systems in and between all countries are becoming more and more necessary. This can easily achieve-able through GMO and our body digest food made from GMO crops the same way they digest non-GMO food. Today, we have GMO soybean seeds that produce healthier soybean oils, eliminating trans fats and containing increased levels of Omega 3. We do have banana with six times as much Vitamin A, Golden rice that produces and accumulate Vitamin A and beta-carotene. This type of nutritional food will help millions of children who suffer from Vitamin A deficiency globally every year. In addition, other biofortified crops with enhanced levels of iron and zinc will make it possible to produce better-processed food with enhanced nutrition. Numerous studies have shown that GMO crops are not linked to any negative health consequences including the incidence of cancer, infertility, allergies, ADHD, or any other health conditions.

CHAPTER 6

BREEDING

CONTENTS

6.1. PLANT BREEDING

Plant breeding is a process of producing progeny with enhanced characteristics. It can be done through 2 processes such as natural breeding or through artificial breeding. Plant breeding through a natural process occurs between two individuals from the same species and in certain plant species it is mediated by external factors such as insects, bees, moths, butterflies, birds, bats and sometimes even wind, water and rain can also act as a pollination agent.

While through artificial breeding, the process involve when humans use partially natural plant breeding methods with advance technology. This initiated by choosing desirable plant male and female either from similar species or between different species to selectively develop phenotypic traits (characteristics). In advanced artificial breeding, some process of natural breeding will be incorporated with technology to develop plant with desired characteristics. Both natural breeding and artificial breeding will cause character changes in the progeny however they are differed in terms of timing, processes, and quality of the product.

Therefore, before technology invention had taken place, producing better quality plant was very difficult. Scientist invested many years to produce one single variety of plant with desired characteristic by natural plant breeding. In plant breeding, farmers will either propagate plant with desired characteristics or some will actively collect the best seeds from the best plants for planting. Furthermore, in current situation initially plant breeders will work together with plant scientist to understand the structural and functional relationships of plant cells and tissues. By studying the morphology of plant cell and wall, organelles and other cellular compartments and narrow it to complex gene regulation and expression by using cell tissues analysis techniques and tools had become critical for crop improvement.

Case study: For example, a farmer who wanted to produce a plant that able to produce high yield with resistant to insect will first grow a plant under high insect attack and then the survival plant will be chosen. At the same time, another farmer will identified plant that can produce high yield. Both of this plant will be grown together. Pollen from the male flower will be transferred to female flower through hand pollination. The new seeds will now carry the desired characteristic such as resistant to insect and producing high yield. These seeds will be preserve for the next planting cycle. However, this process will be carried out many times until the desired characteristics are widely noticeable.

Till date, this practice is still being utilized globally by individuals such as gardeners, farmers, and professional plant breeders. Many international bodies believe that breeding new crops are important for ensuring food security as desired trait able to provide higher yielding, disease resistant, drought tolerant or regionally adapted to different environments and growing conditions. Even it is sound traditional, but plant breeders have developed sophisticated techniques to attain specific traits with better taste, high nutrition, and productivity. In Figure 6.1,examples of plant breeding that is categorized as traditional and modern breeding was described.

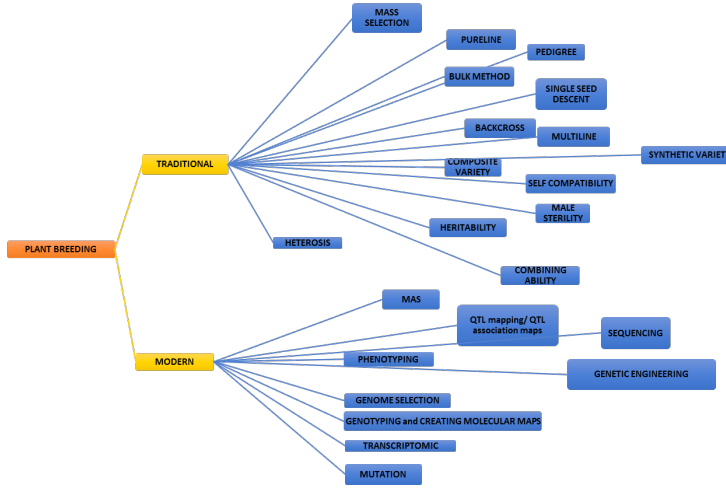


Figure 6.1: Plant Breeding Chart.

6.2. ANIMAL BREEDING

Like plant breeding, animal breeding is also aimed for maximizing number of progeny with desirable genetic traits. Animal breeding which meant for feeding human are categorized under commercial or personal purposes. Animals in terms of livestock will contribute to the global value

of agricultural output, contribute to the livelihoods and food security to almost a billion of people globally. Like plant breeding, animal breeding is also taken place through either natural breeding or through artificial breeding. Animal breeding through natural process occurs between two individuals from the same species which will be mated naturally. While through artificial breeding, it is a process by which humans choose animal with desirable characteristic and mated them through in vitro. This type of artificial breeding is done by using selectively develop phenotypic traits (characteristics) of animal males and females either among similar species or between different species. Both natural breeding and artificial breeding will cause character changes in the progeny however they are differed in terms of timing, processes and product quality. Therefore, before technology invention had taken place, producing better animal trait was very difficult. Scientist invested many years to produce one single variety of animal with desired characteristic by animal breeding. Advances in animal breeding such as genetics, genomics, and technologies had eased an efficient breeding.

Case study: For example, a farmer who wanted to produce a dairy cow need to select the best cow and a best bull. The best cow is the cow that can produce claves and have a good udder and teats. While the best bull is the type of bull that has the dairy blood. Finally, organize the mating between them. This type of mating is known as natural breeding.

Till date, this practice is still being utilized widely around the world by individuals such as farmers, and professional animal breeders. Many international bodies believe that breeding new livestock are important for ensuring food security as desired trait able to provide higher yield, disease resistant, or variety that regionally adapted to different environments and growing conditions. Even it is sound traditional, but plant breeders have developed sophisticated techniques to attain specific traits with better taste, high nutrition, and productivity. In the Figure 6.2 examples of animal breeding that is categorized as traditional and modern breeding was described.

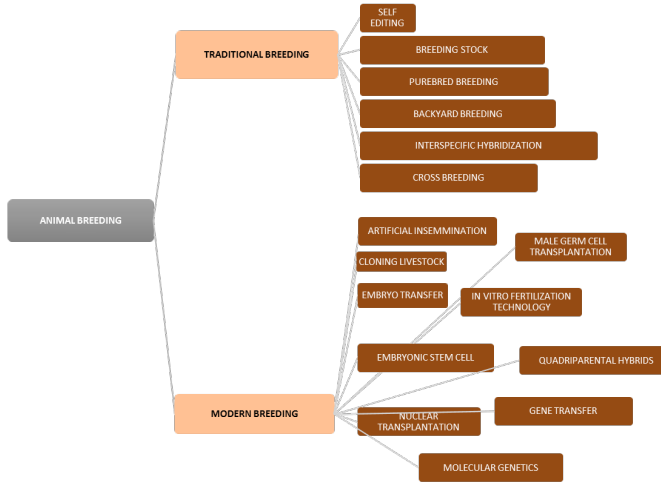


Figure 6.2: Animal Breeding Chart.

6.3. DIFFERENCES BETWEEN TRADITIONAL AND MODERN BREEDING IN PLANT AND ANIMAL BREEDING

TRADITIONAL BREEDING	MODERN BREEDING
Nature will select the favorable characteristics	Human will select the favorable characteristic
Selection pressure is bear by environment	Selection pressure is applied by human
Genetic diversity is high	Genetic diversity is less
More strength	Less strength
Lengthy time	Short time
No Technology needed	Involvement of Technology
Increases heterozygosity	Increases homozygosity
Occurs in wide scale of natural population	Occurs in between of selected plant and animal
Progeny will have better strength	Progeny will have less strength

CHAPTER 7

TRADITIONAL PLANT BREEDING

CONTENTS

Traditional plant breeding is sometimes also referred as a conventional plant breeding. This type of plant breeding is mostly accepted by mankind. This technique involves with manipulation of plant genome through natural mating process which randomly occurs in nature. Plant breeding methods will firstly involve with testing of many wild relatives of a domesticated crop for certain characteristics. It is followed by selecting the ones that exhibit the best characteristics and crossing those wild relatives with the domesticated crop. These processes are the most challenging task.

Sometimes, the crossings can remove desired qualities of the domesticated crop. Therefore, it often takes time for a crop to be enhanced completely. In nature, breeding usually will take place through pollination. There are many types of pollination agents that are available in the nature such as insects, birds, bats, water,wind, and sometimes even plants themselves can self-pollinated within a closed flower. There are two types of pollination besides self-pollination such as open-pollination, and cross-pollination.

Pollination is a process that involves pollen transfer from a male plant to a female plant. This followed with fertilization and seeds production. Pollination process can occur within a species however sometimes under certain circumstances it do occur between species. Pollination between species will produce hybrid offspring.

In an open pollination, from the phrase itself, the pollination is mediated by the external factors such as wind, insects, animal or any other natural based mechanism. Furthermore, in an open pollination, the resulting seeds will produce plants with almost identical to their parents. The seeds of

open-pollinated plants will produce new generations of the parent plants. However, since open pollination is sometimes uncontrollable, under certain circumstances the pollen (male parent) source is sometimes unknown and there are possibilities it may be derived from different species, therefore, it may result in plants that vary in unwanted genetic traits. Therefore, one of the challenges in regulating an open-pollination is by avoiding dispersion of pollen from another species/cultivar. Besides that, sometimes selected plant can be placed in the greenhouses. Some examples of open-pollinated plants are vegetables such as tomatoes, beans, and peas. In open pollination, the percentage of fruit production are much higher, they are also superior in physic-chemical characteristics in terms of volume of the fruit, specific gravity, acidity, size of the fruits which usually can be from medium to large in open-pollinated plants compared to closed pollination.

While in self-pollination, a plant will always self- pollinated itself. This type of pollination will only occurs within a plant that has male and female flower together. The pollination take place when the pollen from one flower pollinates the same flower or other flowers in the similar individual plant. In addition, self-pollination can also occur when a flower has both the male and female organs, which is known as stamens and pistil. Through this pollination, viable offspring which is called self-fertile progeny will be produce. Therefore, in traditional breeding, the aim is only to focus to bear more fruits unlike in modern breeding which is aimed to developed hyper-efficient plants. Through natural mating, various type of cultivars and plant lines can be produced. Example of crop that self-pollinated are apricots, figs, peaches, plum, apple, beans, peppers tomatoes, violets and sweet peas.

In cross-pollination, pollen will be transferred by external factor from different plant within same plant species to mediate the pollination. Besides that, the flower structure of the plant that carried out cross-pollination have taller stamen and shorter pistil. When cross-pollination taken place, the genetic material from the parent plant will combines and the resulting seeds from that pollination will have characteristics of both parent plant varieties and the progeny plant is consider as a new variety.

In the following section, the common methods of plant breeding will be discussed.

7.1. MASS SELECTION

In a mass selection, first plant that possesses distinct survivability will be observed from many populations. The seeds from a particular plant is

collected and further planted in a population for further selection. Therefore, in the next generation, there will be a mixture of different lines. The distinct survivability that are observable such as plant height, grain color, grain size, tilling needs, disease resistance is some of the obvious characteristic. Mass selection increase the frequencies of desirable characteristics in a large population. This type of selection can be carried out multiple times until strong characteristic is detected. Therefore, sometimes mass selected variety may contain considerable genetic variation. However, genetic variability is not created in mass selection, usually mass selection is utilize to improve the base population performance. Mass selection is used in both self-pollinated and cross-pollinated plant species. In case of cross-pollination, the mass selected varieties exhibit both heterozygous and homozygous. While, in self-pollinated species, these varieties exhibited as homozygous only. In addition, there are two types of mass selection, negative and positive mass. These types of mass selection are based on the observed phenotypes of a plant that are related to its genotype. The differences between these two types of mass selection is in positive mass selection, parent plants with desirable traits are selected from mixed population for producing new progeny while in negative mass selection, its involve with cutting out all negative characteristics to improve the productive progeny.

7.2. PURE LINE

The concept of pure line was adapted from Johansons Pure Line Theory (1903). Pure line cultivars are resulted through inbreeding of plants which have certain desired characteristics. In another words, survival plants will be self-fertilized among themselves to produce new progeny. In plant research, individual plant which exhibit desirable characteristics will be selected from a large group of plants. Therefore, it is also known as individual plant selection. These plants will be harvested individually, and their individual progenies are grown and further evaluated. Many layers of selection will be carried out to select the real pure line (Figure 7.1). Table 7.1 and 7.2 showed the advantage and disadvantage of pure line stock. Pure line and the variation within pure line is purely environment based and non-heritable. The genetic make-up in this type of breeding will be mostly homozygous as they

are genetically similar which also known as true breeding. Therefore, they have poor adaptability because they have narrow genetic base. However, they become genetically variable with time either through environmental stress or mutation. Hence, pure line become more susceptible to external factors such as environmental stress or diseases. Pure line can be used as a superior variety, development of new variety through hybridization and for plant mutation studies. The best progeny is released as a pure line variety. Cowpea, wheat, barley, rice, pulses, oilseeds, cotton, many vegetables, and oat are some examples of pure line stock.

Table 7.1: Advantages of the Pure Line

Advantages
Ability to achieve possible improvement over the original variety
Extremely uniform
Easier than hybridization
Requires less skill
Easily identifiable

Table 7.2: Disadvantages of Pure Line

Disadvantages
It is an expensive and laborious. The variety developed can't be easily maintained by the farmers. The varieties developed by pure line selection don't have wide adaptability and stability in production. The upper limit on the improvement is created by the genetic variation present in the original population.

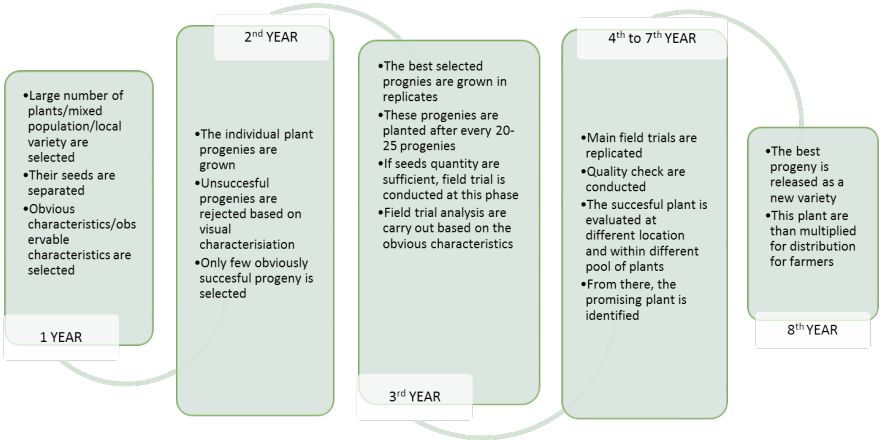


Figure 7.1: Process Producing a Pure Line.

7.3. PEDIGREE METHOD

In pedigree method, crossing between selected parent plant which is known as hybridization (the initial step) are carried out. Seeds obtained from this level is known as F1. These seeds are sowed properly according to a specific distance. Around 10 000 plants are grown from F1 seeds. Plant seeds from F1 plant is known as F2 seeds. In F2 generation, selection is carried out. Around 500 plants were selected and harvested separately. Seeds from 20 to 30 plants are harvested in bulk and further grown for F3 generation. About 30 progenies are grown from the selected F2 plant to produce the F3 generation. At this stage, 100–400 superior plants are selected. F3 seeds from the selected plant are further grown and the plant with best characteristic are again screened. Seeds from this plant which is F4 sire are harvested and further grown for F5 seeds and plant from F5 seeds are further grown for F6 generation. During planting of F4 to F6 generation, each plant progenies are planted spaciously and during each selection, the number of selected plants are narrowed down. Only in F7 generation, yield trials and quality control will be analyzed. In F8 to F12, multi-location are used for planting and quality control purposes for different characteristics. Finally, seeds from generation F10 or F13 are distributed (Tables 7.3 and 7.4).

Table 7.3: Advantages of the Pedigree Method

Advantages
Used to evaluate segregating generation from crosses of self-pollinated
Used to improve an established variety
To improve high heritability
Easy and quick selection
Record of the parent plant is available
Quality control involve with genotypic value
Breeding efficiency is increased

Table 7.4: Disadvantages of the Pedigree Method

Disadvantages
Costly Labor intensive Skill breeders is crucial Time-consuming

In pedigree method, origin of the selected individuals or lines are recorded and maintained as pedigree record. From the record, the progeny of the F2 plant can be determined. This record will also help in handling the segregation from crosses.

7.4. BULK METHOD

Bulk method is used to handle segregation generation from F2 to F6 generation. From the phrase itself, 'bulk' means that all seeds are collected in bulk. From minimum 20 seeds, it can goes up to 30 to 50 thousand seeds and selection of plant can reached 1000 to 5000 plants. Finally, from F7 to F8 generation, desirable plant is also selected in bulk. Characteristics evaluation and quality check is carried out from F9 to F13. Finally, in F14, seeds are collected for distribution.

Table 7.5: Advantages of the Bulk Method

Advantages
Simple and easy
Less cost
Less record management
Environment based selection
Homozygous selection

Table 7.6: Disadvantages of the Bulk Method

Disadvantages
Lengthy process No record of the parent plant Need to handle large number of progenies Suitable for small grain crop

7.5. BACKCROSS METHODS

Through backcross method, one can incorporate desired gene that may lacked in highly productive variety. The newly desired gene can be either dominant or recessive. From the phrase itself, backcross means one is going back or returning to the perfect cultivar with more desired characteristics by crossing it to the existing cultivar. The variety that receives the additional new gene is called as recipient while the plant that become the source of the gene is known as donor parent. This method can be apply anytime when there is a need to enhance new characteristics. Repeated backcross leads to homozygosity and it is important to ensure that 98% is consist of elite/ recurrent plant genes with newly desirable characteristics. Dominant and recessive gene can be both transferred through backcross method.

BACKCROSS BREEDING STORY

RECENTLY, A PLANT SCIENTIST NAMED DR. YAL DEVELOPED A TRANSGENIC PLANT WITH RESISTANT TO DISEASE. SHE THEN PLANNED TO MARKET THE DEVELOPED LINE. UNFORTUNATELY, THE DEVELOPED PLANT SHOWED LOWER YIELD. THEREFORE, SHE HAND OVER THE DEVELOPED PLANT TO MR THIYAGU WHO IS A PLANT BREEDER.

TO ENSURE THIS TRANSGENIC PLANT TO BE MORE MARKETABLE MR THIYAGU USED PLANT BREEDING METHOD. IT IS BECAUSE HE FOUND THAT THE TRANSGENIC LINE HAVE LOWER YIELDING THEN WHAT THE CURRENT ELITE LINE CAN PRODUCE. AS AN INITIAL STEP, HE PLANTED THE TRANSGENIC LINE AND ALLOWED THEM TO SELF POLLINATED.

THE SEEDS DERIVED FROM THE SELF POLLINATION WAS THEN SOWED PARALLEL WITH THE ELITE LINE. NOW, HE ALLOWED BOTH TRANSGENIC LINE AND THE ELITE LINE TO BE CROSS POLLINATED. THE SEEDS THAT WERE PRODUCED FROM THE ELITE LINE IS KNOWN AS F1. THESE F1 SEEDS WERE PLANTED TOGETHER WITH THE ELITE LINE AGAIN AND BACKCROSS-POLLINATION WAS MEDIATED. THE SEEDS PRODUCED IN THE ELITE LINE AT THIS STAGE WAS KNOWN AS BACKCROSS 1 (BC1).

AGAIN, HE PLANTED THE HARVESTED BC1 SEEDS TOGETHER WITH THE ELINE LINE. AT THIS STAGE, HE CARRIED OUT PROGENY TESTING TO ENSURE THAT THE TRANSGENIC GENE SUCCESFULLYINCORPORATED IN THE ELITE LINE. HE REPEATED THIS BACKCROSSING METHOD AROUND 5 TO 6 GENERATION.

AFTER 5 TO 6 TIMES OF BACKCROSSING. MR THIYAGU SELF-POLLINATED THE NEW ELITE LINE TO OBTAINED HOMOZYGOUS PLANT. THE HOMOZYGOUS IDENTIFICATON CAN BE DETECTED THROUGH PROGENY TESTING OR THROUGH IDENTIFICATION OF SELECTABLE MARKER. IN ADDITION, THIS WILL ALSO ENSURE THAT AROUND 98% OF THE ELITE GENE IS PRESENCE. FINALLY, PLANT WAS SCREENED AGAINST NEGATIVE SELECTION. NOW MR THIYAGU SUCCESSFULLY DEVELOPED ELITE LINE WITH THE TRANSGENIC GENE. THIS PLANT LINE CAN NOW BE MARKETABLE.THIS NEW ELITE LINE WILL POSSES RESISTANT TO DISEASE CHARACTERISTIC WITH HIGH YIELDING

7.6. SINGLE SEED DESCENT

Single seed descent is a modification of the bulk method. Instead of applying bulking approach during harvesting the seed, a single seed is selected randomly from each selected plant to makeup the whole bulk. In

this method, plant breeders usually minimize the record keeping as in the pedigree approach. Breeder works towards focusing on improvement of quantitative traits or characters such as yield rather than qualitative traits. But, it does not involve with artificial selection process, so chances of losing superior plants are comparatively more and does not eliminate weak plants as in other methods. The stages that involve in single seed descent method is hybridization, which involve with crossing of the selected plant and followed by seeds (F1) collection. F1 seeds were grown, pollinated and F2 seeds were harvested one from one plant randomly and mixed in bulk. This was repeated for up to five generation. Only at the sixth-generation superior plant selection was conducted and only 150 to 500 plants were chosen. Seeds are again collected, and plant progenies were further grown, and selected plants are harvested in bulk. In F8 generation, field trials and quality tests are conducted up to 13 generations. Finally, at 14th generation, seed multiplication and distribution is carry out (Tables 7.7 and 7.8).

Table 7.7: Advantages of the Single Seed Descent

Advantages
Requires little space
Saves time. Fast. Easy
Extensive field trials are not required
Applicable for low heritability
Little influence from natural selection

Table 7.8. Disadvantages of the Single Seed Descent

Disadvantages
No recombination Need to evaluate large numbers of inbred lines to find out superior ones Maximum productivity is only established after F2 generation

7.7. MULTILINE

From the phrase itself, multiline variety mean several lines. In this type of breeding, those several lines will have different genes to improve disease resistance. Therefore, when thinking of developing plant line with disease

resistant characteristic, they should be looking into multiline breeding program. Pure lines usually have similar phenotype such as plant height, seed color, flowering period, maturity time and other agronomic characteristics. While isogenic line that appears in multiline consist of different genes for the similar characteristic for disease resistance. The advantage is that, in multiline they do not reduce the yielding ability of each other when grown in a mixture (Tables 7.9 and 7.10).

Table 7.9: Advantages of Multiline

Advantages
Only one or few lines of the mixture will be affected during the outbreak
Less loss to the farmer
More adaptive to environmental changes than individual pure line

Table 7.10: Disadvantages of Multiline

Disadvantages
Changes need to carry out each time during outbreak No improvement in yield or other characters Lengthy time Not suitable for cross-pollinated plant All multiline variety that lack of the resistant gene for particular pathogen will be easily attacked

A STORY OF MULTILINE

A plant variety which was originally found to be resistant to brown rust was identified being susceptible to new races of pathogen. Therefore, several pure lines with different resistance genes were produced through backcross breeding using one donor and a recurrent parent. The donor parents were the one with different genes of the disease resistance. Every donor parent was used in separate backcross program so that each line receives different gene for disease resistance. Later, few lines with different alleles for disease resistance were then mixed to developed multiline variety. The mixed lines were determined by the pathogen races.

7.8. COMPOSITE VARIETY

Composite variety is a variety that is developed by mixing phenotypically outstanding seeds from various lines that possess similarities in characteristics like height, seed size, seed color and maturity. Composite variety is obtained by crossing between the selected variety through open pollination and cross-pollination approach. Basically, composite variety is heterogeneous. Composite variety seeds usually can be kept for up to 4 years. Figure below will explain how composite variety is carry out.

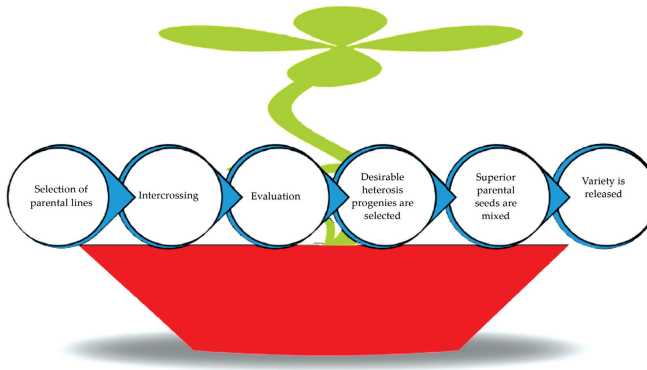


Figure 7.2: Process producing composite variety.

7.9. SYNTHETIC VARIETY

Synthetic variety is a variety that is developed by selecting several inbred lines with good general combining ability (GCA) and intercross them in various combinations and mixing. Besides that, they can be developed by using clones or inbreeds through cross-pollination but need to be further maintained through open pollination. The seeds of all the crosses are handled in equal quantity. Basically, all seeds derived from synthesis variety are heterogeneous. Synthetic variety possess additional advantages than composite variety such as hybrids as reconstitution is possible, more adaptive, less uniform and attractive, show some amount of heterosis and posses better disease resistance. It also proved to be better yield than the inbreeds but at the same time show lower yield than the single cross hybrids or double cross hybrids. Finally, less cost is required compared to hybrid varieties. Farmer can use the collected seed from the synthetic variety up to 4 years.

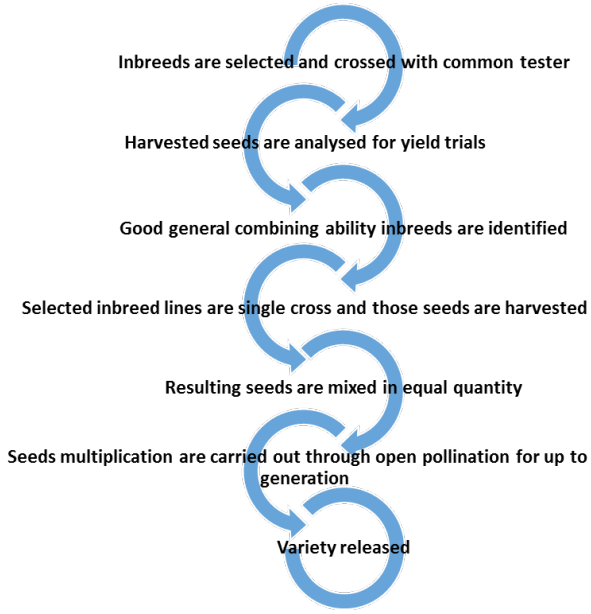


Figure 7.3: Development of synthetic variety from established inbred lines.

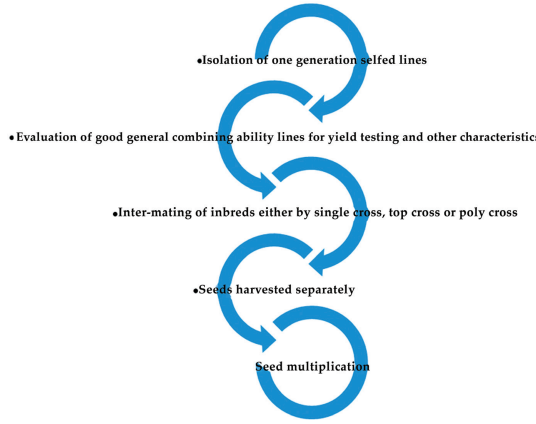


Figure 7.4: Production of synthetic variety from plants.

7.10. SELF-INCOMPATIBILITY

Self-incompatibility is a mechanism in flowering plants that prevents both inbreeding or selfing but mediates outcrossing. The outcrossing can be mediated at any stage between pollination and fertilization. The self-

incompatibility response is genetically controlled by one or more multi-allelic loci in a species population. Plant that possess this characteristic will have functional pollen that fails to set seed if it's are self-pollinated. It is incompatible between the male gamete pollen and the stigma which is the female reproductive organ. Therefore, it is unable to germinate in the plant where the pollen attached to the stigma. The benefits of self-incompatibility plants are, they have more chances to increase new genes combination through outcrossing. This new gene combination has more chances to exhibit new morphological, physiological, genetical or biochemical phenotypic characteristic and reduces homozygosity.

7.11. MALE STERILITY

Male sterility refers to either absence of pollen grain or plant with non-functional pollen grain, but with functionable female gametes. This phenomenal occurs through mutation and artificial. This type of plant prevents self-pollination but allowed cross-pollination. Therefore, the progeny will be heterozygous. There are many types of male sterility such as:

- Cytoplasmic male sterility (CMS) which governed by cytoplasmic genes.
- Genetic male sterility (GMS) which governed by nuclear genes.
- Cytoplasmic-genetic male sterility (CGMS) which governed by both nuclear and cytoplasmic genes.
- Transgenic male sterility which induced artificially through genetic engineering.
- Chemical-induced male sterility which induced artificially by using chemical.

7.12. HETEROSIS

Heterosis is a hybrid vigor, means it has enhanced characteristics and survivability. A progeny that is heterosis consist of genetic mixture of its parents. Desirable heterosis can be either positive or negative. Positive heterosis refer to yield, quality and disease resistance while negative heterosis refer to plant height and maturity duration. Heterosis is widely found in cross-pollinated plant. Heterosis generation are easily reproducible and directly proportional with heterozygosity. It can be exploited fully for

hybrids generation plant and partially for synthetic and composite varieties plant.

7.13. HERITABILITY

Heritability is the variation or variability which is transferred from parents to their offspring. It can be classified in two types such as broad and narrow sense depending on which component of variance is being used as a numerator. Heritability is used to decide which method should be utilized for selection, to construct selection index, to decide minimum population that is required to carry out selection effectively and to know responses of various traits.

7.14. COMBINING ABILITY

Combining ability is the capacity of an individual to transmit superior performance to its offspring. There are two types of combining ability which is known as General Combining Ability (GCA) and Specific Combining Ability (SCA). It is the phenomenon where inbred lines produce hybrid vigor after each cross. Progeny tests will be performed to predict combining ability of the characters governed by recessive genes. Various steps like parent selection for crosses, crosses performance, evaluation and interpretation will be done.

CHAPTER 8

MODERN PLANT BREEDING

CONTENTS

Technology has evolved over years, therefore any impossible processes or procedures are achievable and that includes producing genetically modified organisms and subsequently genetically modified food production. In this century, scientists can easily develop modified organism in a lesser time. Genetic engineering is a technique that has been used to change the genetic makeup of all the living things such as plants, animals or microbes. Genetic engineering represents the advanced procedure in the genetic modification of crops, through gene transfer. This procedure eliminates all taxonomic barriers. The differences in genetic modification through genetic engineering versus selective breeding depend on the number of genes that are being transferred into the crop plant. With genetic engineering, single genes of new desirable traits are preferable in a single crop. But in selective breeding, many genes are transferred into a particular crop. Therefore, genetic modification through genetic engineering is more precise than genetic modification through breeding. Irrespective of the differences in the techniques that lead to genetic modification, the goals are the same, the accumulation of desirable traits in a particular crop plants. In the next level of genetic engineering, development of markers that can able to connect genotype to phenotype have leads to advancement in genetically modified organisms. This is linked to the combination of alleles in different code of a particular genes in the plant which is knowns as genotype. Genotype usually controls the phenotype of a plant. This genotype and phenotype information can then be transferred through genetic engineering to produce

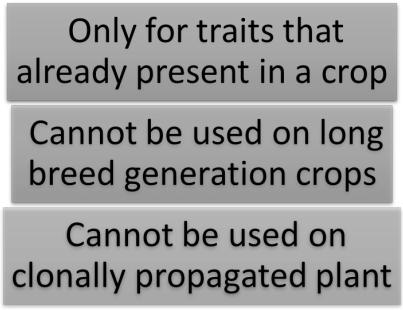
genetically modified organism. Therefore, traits or plant characteristics which are controlled by genes can be easily modulate through technology and genetic engineering. However, various precautions need to take into consideration to ensure that the genetically modified organism and food derived from GMO do not carry diseases or allergy compound. There are several mechanisms of DNA transfer that occurs in living organism naturally on a larger scale and there is also mechanisms that occurs beyond the natural processes which often carried out by the scientist in the laboratory. There are various techniques that involve in genetic engineering for crop improvement which known as plant molecular breeding. The development of genetically modified plants often requires complex design and assembly of DNA to achieve optimal effect. The tools that make it easier and faster for scientists to select plant traits will be illustrated under the section 'methods for trait introduction'.

8.1. METHODS FOR TRAIT INTRODUCTION

8.1.1. Marker Assisted Selection

Plant breeders using marker-assisted selection (MAS) to breed crops with desired traits. In MAS, molecular or genetic markers are used to identified the specific gene in a plant. The markers are a string or sequence of nucleic acid which makes up a segment of DNA. It is located near the DNA sequence of the desired gene and are transmitted by inheritance from one generation to the next generation. Since the markers and the desired genes are located close on the same chromosome, they tend to stay together at each generation of plants. This technique also known as gene selection. In maker assisted selection once genes or markers are identified, it is used for genotyping and selection decisions. Advancement of marker-assisted selection is known as Marker-assisted backcrossing (MABC), which involves with backcrossing of F1 with its parents. It is to transfer a limited number of loci such as transgene or disease resistance loci from one plant genetic background to another plant. Usually, the recipient of such genes are from a good adapted cultivar. Like plant breeding, about 4–6 generation of backcrossing are carried out to ensure that genetic background of the recipient genotypes is kept well. But the advantages through plant molecular breeding is that markers from the whole genome can be recovered quickly in 2–3 rounds of backcrossing faster than plant breeding solely. In addition, marker-assisted recurrent selection (MARS) include identification and selection of several

genomic regions (up to 20 or even more) for complex traits within a single population. However, molecular breeding somewhat limited compared to genetic engineering. The limitation is such as:



There are few examples of molecular markers such as:

1. Restriction Fragment Length Polymorphisms (RFLPs)
2. Random Amplification of Polymorphic DNAs (RAPDs)
3. Sequence Tagged Sites (STS)
4. Amplified Fragment Length Polymorphisms (AFLPs)
5. Simple Sequence Repeats (SSRs) or microsatellites
6. Single Nucleotide Polymorphism (SNPs)

These molecular markers have been widely used to monitor differences in DNA sequence within species and among species. Screening genetic markers that correlate with selectable phenotypes requires the right tools. Single nucleotide polymorphism (SNP), genotyping, gene expression profiling, and other genetic variation determination will help to associate the differences so that breeders can select the right plant seed line, move a transgenic plant to the greenhouse, or collect critical data. In addition, it can also allow a creation of new sources of genetic variation by introducing new and desirable traits from wild varieties into elite lines. While RFLP markers have been the basis for most genetic work in crop plants. Hence AFLPs and SSRs are used to ease in detection and automation. The adoption of the new marker system, such as SNPs, is highly preferred due to the increasing amount of sequence information, and fast determination of gene function because of overwhelming genomic research. SNPs are small variations in the DNA sequences that are associated with desired traits. They are used in conjunction with quantitative traits locus (QTL) mapping to develop, to improve and to enhance certain plant characteristics such as higher yield, better stress tolerance or enhanced pest resistance.

The main uses of these molecular markers are as follows:

- Assessment of genetic variability and characterization of germplasm
- Identification and fingerprinting of genotypes
- Estimation of genetic distances between population, inbreeds, and breeding materials
- Detection of monogenic identification of useful candidate gene sequences

8.1.2. Genotyping and Creating Molecular Maps

Once we are aware where is the markers being located on a chromosome and how they are close to a specific gene, genetic linkage map can be created. In genotyping and during creating molecular map, the commonly used markers are include simple sequence repeats (or microsatellites) and single nucleotide polymorphisms (SNP). The process of identification of plant genotypes are known as genotyping. Effective high throughput methods for genotyping analysis will ensure breeders to understand how to breed food crops for higher yields and better disease resistance. Rapid genotyping of plant species all over the world will allow breeders to determine which species will benefit the consumer. Development of SNPs has revolutionized the molecular breeding process as it helps to create dense markers. In addition, such a map would show the location of a markers and genes, and their distance from other known genes. By using detailed genetic maps and knowledge of the molecular structure of a plant's DNA, analysis through a small piece of plant tissue or from newly germinated seedling can be used to analyzed whether the seedling contains the appropriate gene or vice versa. If, the desired gene was not identified in a tissues or seedling, we can quickly move on to concentrate on analyzing another seedling or other organisms.

8.1.3. Phenotyping

Phenotyping tools are used to identify genes that associated with traits. It is important to measure the trait value in order to identify the phenotype. By using omics approach, the measurement of phenotypes is called phenomics. Here, the phenotype can be an indicative of the trait measurement either indirectly related or correlated. In addition, we can see a broader understanding of the structural and functional relationship of the plant cells and tissues. This information are also important in the process of deregulating new plant varieties.

8.1.4. QTL Mapping or Association Mapping

Quantitative trait loci (QTL) or quantitative trait genes are involved in controlling the trait of interest. This process is known as mapping. Mapping of such genes are done by using molecular markers. In QTL, the mapping can involve single large family, unrelated individuals or multiple families. However, the basic idea is to identify genes or markers associated with the phenotypic measurement.

8.1.5. Sequencing

Crops genome sequencing will provide insights on understanding the function and evolution of a crop genomes. It has become an ideal model for plant comparative genomics, evolutionary biology, and functional biology studies. Sequencing wild crop will provide valuable genomic resources for crop improvement. Furthermore, wild relative plant which has natural resistance against many pathogens and environmental stress can be identified. For example, 261Mb of compact genome sequence of *O. brachyantha* are caused by the silencing of long terminal repeats (LTR) retrotransposons and massive internal deletion was identifie by sequencing. Furthermore, this wild type genome is used to compare rice genome to identify the number of gene families that were expanded through tandem duplications and gene movements that mediated by double-strand break repair. These processes are responsible for the amplification of the gene families. Moreover, segmental and tandem duplication are further expanded by transposable element insertions that contribute to transition from euchromatin to heterochromatin. From this analysis, many important genomic mechanisms such has genome variation, gene movement and transition can be revealed. Genome sequencing can also disclose a map of structure and functions of the genes of the define crop. It also reveals clues on how a particular sequence can be useful for sustainable crop improvement and resilient food production. Moreover, it will also improve livelihood of small farmers and can be utilize to harness genetic diversity by broadening the genetic base of cultivated crop genes pool. Once genetic markers or low diversity genome regions are identified, this information can be used to developed superior varieties with enhance tolerance and disease resistant. Recently, sequencing plant genomes becoming easier and more cost effective. These dramatic improvements in sequencing technology are changing the way of plant breeders perspective on genomics and are paving the way for the next wave of remarkable plant discoveries.

8.1.5.1. Whole Genome Sequencing

Whole genome sequencing (WGS) is carried out to obtain sequence of the entire genome from of a plant. WGS enable to detect and identify known and novel mutations in experimental plant and characterized pathogens from infected plants. Furthermore, it can also help in identifying alleles or variations in the experimental genome.

8.1.5.2. Genome Sequencing

From the discovery of the Gregor Mendel's theory on DNA heredity towards exploration of decoding to enhance the life of living organisms, a very popular tools known as genome sequencing have impact the agriculture industry. Genome sequencing allows breeders and plant scientist to uncover genetic makeup of plants. The profile of plant genomic data will provide full set of information on plant disease, how transformative approach in crop could help feed the growing population and how genetic mutation and variation have determine the adaptation of certain species. The information obtained from this technique can be use to perform selective breeding through trait screening methods to enhance plant species.

8.1.6. Genomic Selection

Genomic selection is an approach that involve with selection that is made based on few markers. Rather than seeking to identify individual loci that significantly associated with a trait, genomics uses all marker data as predictors of performance and consequently delivers more accurate forecast. Genomic prediction combines marker data with phenotypic and pedigree data (when available) to increase the accuracy of the breeding prediction and genotypic values. Selection that based on genomic selection predictions, potentially will lead to more rapid and lower cost gains from normal breeding.

8.1.7. Mutations

A widely used method to identify mutations are by using the T7 Endonuclease I mutation detection assay. This assay detects heteroduplex DNA that results from the annealing of a DNA strand and including desired mutations with a wild-type DNA strand.

8.1.8. Transcriptomic

Agriculture sector utilize a variety of approaches to understand plant gene expression. In some cases, whole transcriptome profiling is preferred over targeted RNA sequencing. Transcriptomic is a complete set of transcripts in a cell or within a population of cells or quantity of transcripts at a specific developmental stage under specific conditions. Transcriptome sequencing can be utilized to analyze transcriptome profiles and translate it into information. Subsequently, transcriptome analysis can identify genetic function in cells and tissues. It will also provide understanding of the disease development and their impact on cell functions. Furthermore, transcriptome sequencing enables to detect rare and novel transcriptome, rare variants, quantify transcriptomes, analyze differential gene regulation, identify fusion gene, and alternative silencing. In addition, it can also help in detect single nucleotide polymorphisms (SNPs), insertions and deletions and single nucleotide variants. Transcriptome sequencing is much better than compared to traditional microarray technology, which can only be used for gene expression profiling in species with known transcriptome sequences. Next generation sequencing (NGS) technologies enable to perform these sequencing in any species with lower cost.

8.1.8.1. Metagenomics and Metatranscriptomics

Metagenomics is a study that involve of recovered DNA directly from complex environmental samples while metatranscriptomics is the analysis of the genes within those samples. The primary goals of these approaches are to characterize the organism and to identify the activities in the experimental sample. Metagenomics and metatranscriptomics are highly relevant in the agriculture research. It will enable to characterize the particular plant present in the certain environmental conditions. It is because certain phenotypic characteristic is changeable due to the environment that the plant being exposed. In addition, it will also allow scientist to investigate how certain activities change in response to the environment.

8.1.9. Genetic Engineering

Genetic engineering is one of the solutions to mitigate the impending food security crisis. Generating transgenic plants are a key to an introduction of a new crop traits in the field. The development of genetically modified plants often requires complex design of DNA elements to achieve optimum expression effects. DNA distinguish the differences in the plant's genetic

material between plants. DNA that is packed in chromosome strands of genetic material derived from each parent, male and female plant. Genes, which control a plant's characteristics, are located on specific segments of each chromosome. Together, all of the genes make up known as genome. Genetic engineering involves with the horizontal transfer of genes from one organism to another. This can be done by cloning, as plant cloning is a key step for genetic engineering, gene studies and other aspects of plant research both in discovery and applied research. In addition, in genetic engineering, an organism that is receiving genes from different organism can be done precisely by isolating individual gene from the wild relatives of a domesticated crop or other species that code for increased production of certain nutrients. Furthermore, stacking of few genes that code for few proteins can be also done through genetic engineering. This provides limitless opportunity in breeding plant. Traits like flower color may be controlled by only one gene, crop yield or starch content that influenced by many genes can be easily manipulated through genetic engineering.

8.1.9.1. Way to Genetic Engineering

The jellyfish *Aequora victoria* has discrete glowing point of this bioluminescence around the edge of its umbrella-like structure. Bundles of about 6000 to 7000 special photogenic cells produce this ethereal light. These cells packed with aequorin (a light producing cell) and a color shifting secondary protein that accepts the blue light emitted from aequorin, re-emitting it as green light, hence there comes the name green fluorescent protein (GFP). GFP gene is originally found in jellyfish. It produces a greenish glow to scares off the predators. The greenish glow is produced by the green fluorescent protein without any external factors. Many cnidarians utilize green fluorescent protein (GFPs) as an energy transfer acceptor. *Aequora victoria* uses this light to lure prey, to frighten off attackers or to attract mate. Green fluorescent is mainly found in marine environment where it can be categorized as a rich source of both biological and chemical diversity that has intrigued humans for centuries. The whole sequences of GFP gene is consist of 5170 nucleotides. This sequence contains 1723 triplet codon but only 238 amino acid is involved in expressing the fluorescence. This shows that the sequence contains non-coding region known as introns. For the molecule to function well, it needs two polypeptides chain recognized as chain A and chain B. The protein is in the shape of a cylinder, comprises of 11 strands of beta sheet with an alpha helix inside and short helical segments on the ends of the cylinder. The motif with beta structure on the outside and

2 alpha helix inside of both chain, represent a new protein fold which can be modified easily. The GFP molecule has been shown to emit green fluorescent (maximum emission at 509 nm) when irradiated with light of 395 nm and 470 nm wavelength. Therefore, the green fluorescent protein that lights up the jellyfish has proven to be useful for biologist to mark a genes that they are studying and to further track the gene expressions as it will glow green when exposed to ultraviolet or blue light. Since scientist can't look at the DNA or its products in a living cell, they can insert the DNA fragment of the green fluorescent protein next to the gene of interest. In any time, when the gene is turned on, the cells will glow green. Green fluorescent protein is now being used in mammals as markers to identify the gene of interest. By understanding the three-dimensional structure and properties of the green fluorescent protein, scientist could tailor the GFP protein to produce many versions with different colors, such as red or orange. Having different colored gene tags would allow researchers to look at several different genes rather than just one in a living organism at similar time. By knowing the three-dimensional structure, it opens new avenues for scientist to engineer gene markers with different properties and to engineer green fluorescent protein for other purposes as well. It is a very powerful and popular tool in the genetic engineering field.

8.1.9.1.1. The Uses of GFP Gene

The GFP gene have several non-optimal properties including low brightness, a significant delay between protein synthesis and fluorescence development and complex photo-isomerization. Fortunately, it can also re-engineered by mutagenesis to ameliorate this deficiency and shift the excitation and emission wavelengths to create different colors. It can be used as reporters of gene expression, tracers of cell lineage and as fusion tag to monitor protein localization within living cells due to its ability to be attach to the gene of interest. This puts the GFP gene under the control of a gene regulation and to study gene expression in living cells in vivo, in-situ and in real time. It can also be used as recombinant DNA to transfect cells to synthesize a chimeric protein that contains GFP gene fused to the protein of interest. This fusion protein where GFP is attached to the gene of interest will not affect the novel function of the gene of interest. In addition, it will not participate in the normal activities of the cell. The advantage of GFP-fused protein is that it can be easily viewed under UV-Light. Exposure of the protein to the UV will result in the emission of fluorescent light. It can provide a powerful tool for the detection of the exact location of the protein.

8.1.9.1.2. Fluorescent and GMO

This section will describe how GFP gene was incorporated into a bacteria cell known as *Lactococcus lactis*. In future, this engineered bacteria cell can be used for different research purposes. Since there is engineered commercial *E. coli* with GFP is available, the GFP gene can be easily isolated from a genetically engineered versions of green fluorescent protein (GFP) known as pGFP-N1 in a *E. coli*. It is actually encodes the green fluorescent protein from the jellyfish *Aequorea victoria*. The GFP gene is extracted from a TOP10 *Escherichia coli* (TOP10 *E. coli*) cells by using mini-prep plasmid isolation techniques. The GFP coding plasmid region was amplified by using polymerase chain reaction (PCR) method by using specific primers, which is constructed based on the GFP sequence data available in the database (Gene Bank Accession AU19279). In these days, primers can be easily design by using primer design software that freely available in the bioinformatic platforms. By including restriction enzymes *PST*1 on forward primer and *Hind*III on reverse primers, the pGFP-N1 are further amplified. This is to increase the concentration of the pGFP-N1 fragment. The amplified DNA is digested and separated through agarose gel electrophoresis to further isolate the correct fragment sizes (720 bp). The lactococcal vector, pMG36e (3611 base pair) which is used to clone the GFP gene, is extracted by using miniprep plasmid isolation techniques methods. This is because mostly all plasmid will be marketed in a transformed bacterial cell. Both the digested vector and the DNA is ligated to transform the freshly prepared *Lactococcus lactis* MG1363 competent cells by using electro-transformation techniques. Transformants containing the recombinant product is first selected based on erythromycin resistance. Finally, the presence of the GFP gene is confirmed by extracting the recombinant plasmid followed with restriction enzyme analysis. This approach is used to develop a new vector system that can function perfectly in *Lactococcus lactis*.

8.1.9.1.3. Recombinant DNA

Recombinant DNA is also use to engineer bacterial genes with plant DNA fragment so that it will work properly in plants DNA. Through this method, several bacterial and plant proteins could be produced simultaneously into a functional enzyme.

8.1.9.1.3.1. PCR Amplification of GFP Gene

GFP gene, which is isolated from jellyfish, is first need to be amplified. This is done by using the polymerase chain reaction (PCR) methods. PCR is a rapid and effective method for replicating specific fragments of DNA. The principal consideration in PCR is the choice of primers. The primer sequence determined the fragment of DNA that will be amplified from the entire genome. To amplify a gene or a piece of a gene, we need to know enough information about the gene sequence to synthesize suitable primers that will form base pairs with both ends of the target region. In the case of PCR, primers are made of single-stranded DNA, instead of RNA which act as natural primers. Therefore, the primers do not need to be replaced with DNA in the living cells instead the primers in the PCR cycle will become part of the newly synthesized DNA strand. The PCR cycles are automated in a machine called thermal cycler that raises and lowers the temperature of the samples according to a pre-set program. The PCR product is then analyzed by using an agarose gel electrophoresis to clarify the accuracy of the amplification. Gel electrophoresis permits researchers to separate DNA fragment from one another to visualize the differences in the DNA as different length represents different genetic variation. It is also use to identify the molecular weights of DNA fragment. DNA that has been mixed with a blue dye is added to the wells, DNA fragments migrates through the gel towards the positive electrode and away from the negative electrode. From the result, we can detect that the length of the GFP gene that is amplified is between 500 bases to 1.0-kilo base pair. This is the DNA that will be inserted into a vector.

The marker that is commonly used in research is 1 kb ladder. The 1 kb DNA ladder is suitable for sizing linear double-stranded DNA length from 500 bp to 12 kb. It is prepared from a plasmid containing repeats of a 1018 bp DNA fragment. The ladder consists of 12 fragments ranging from 1018 bp to 12 216 bp. In addition to these 12 bands, the ladder contains vector DNA fragments that range from 75 to 1636 bp. The double-stranded ladder can be visualized on 0.5 to 1.0% agarose gels upon ethidium bromide staining. This ladder can be labelled using T4 polynucleotide kinase, T4 DNA polymerase, DNA polymerase 1 or the large fragment of DNA polymerase 1 which is known as Klenow fragment.

The remaining of the PCR products from the first step is then purified to isolate clean GFP-PCR products. This cleaned GFP PCR products is then

utilized in the restriction enzyme digestion. It can also stored in –20°C for future usage.

In this step, the vector, which is PRSET A is excised with restriction enzyme (BamH1 and EcoR1) and the cleaned GFPPCR product is also excised with the same restriction enzyme. This step is carried out in different tube and the excised fragment is run on gel electrophoresis to detect whether the restriction enzyme has cut both end accurately.

The band in the agarose gel is isolated while viewing it under the UV light. Those bands are then purified separately to obtain a purified vector and insert. In later part of this, both vector and insert is mixed so that both strands can become closer and could ligated in the followingt step. In this step, the elution solution for ligation from the previous step is, ligated with T4 ligase for overnight. T4 ligase is functioned to glue the both ends of the vector and insert.

Bacterial cells can take up DNA from their surroundings through transformation, a technique that involve with the transfer of DNA into a bacterial cell from the cells environment. In nature, transformation can take place when DNA from a ruptured bacterial cell is taken up by another bacterial cell. If the introduced DNA is homologous with a portion of the chromosomal DNA in the recipient cell, the introduced DNA can recombine with chromosomal DNA. Transformation is a rare event both in nature and in the laboratory. Some species of bacteria do not take up DNA simply from the environment. In some species, the rate of transformation can be increased by treatments such as exposure to high concentration of calcium ions, which increases the permeability of the bacterial cell to take up the DNA. Cells that has been treated to increase their ability to take up DNA is called competent cells. Plasmid are closed circular molecules of DNA that must be introduce as a complete and intact molecule to be completely function in the cell. They enter the cells as double-stranded DNA and do not invade the chromosomal DNA, because plasmid contains its own origin of replication, it can replicate independently as a separate entity from the chromosomal DNA. Some plasmids replicate so profusely that a single cell may contain up to hundreds of copies of the plasmids. However, some plasmids do not replicate as readily and may be present in only one or two copies per cell as plasmids DNA do not invade the chromosomal DNA, when there is no homology between the plasmids and chromosomal DNA. Typically, cells that are capable of plasmid transformation must be treated to make them competent. *E. coli* is an example of a bacterial species that is

capable of plasmid transformation when its cells are competent. The ligation mixture which has been incubate for overnight is then mixed with *E. coli* (XL-BLUE). Then after few steps, this mixture is then plated on agar plate and incubated for overnight.

Satellite colonies will tend to form around a single colony if the culture plate is incubated for long. Satellite colonies are colonies without plasmid. However, it can grow due to the digestion of antibiotic by the main colony that consists of the recombinant plasmid which carry antibiotic resistant gene. The main single colonies are picked up by using tips and punch into the PCR tube and on duplicate agar plate. Duplicate plate is prepared for future analysis.The PCR tube, which contains the colonies were used for PCR amplification. The PCR product is analyzed to confirm the presence of recombinant DNA through gel electrophoresis. The colonies that contain the desired recombinant DNA was traced back in the duplicate plate and is further grown on agar plate. To obtain the high yielding colony, more than one single colony is grown separately. The grown colonies on agar plate is further multiplies in broth culture. The overnight broth is used to isolate the recombinant plasmid. The isolated recombinant plasmid is digested with respective enzymes to ensure the accurate length of the insert. The colony that produce thick band is considered as the high yielding colony.

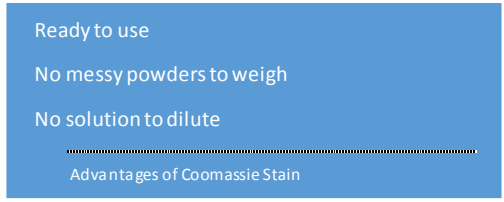
8.1.9.1.4. Recombinant Protein Expression Analysis

Plants are increasingly being examined as an alternative recombinant protein expression system. In this section, the author shared how the standard recombinant protein expression analysis is done. Recombinant pRset construct is transformed into BL 21 (DE 3) PlysS and is selected for resistant transformant amplification. It is a common *E. coli* that usually used for protein expression analysis, while DE 3 is an enzyme that express T7 polymerase. After transformation, *E. coli* need to be grown on a agar plate prior to liquid culture so that many copies of transformant can be produced. Next, IPTG is added prior harvesting the grown bacterial cells. The harvested bacterial cells will then be used for protein expression by using SDS PAGE, Electro-Blotting, and Western-Blot.

8.1.9.1.5. Protein Separation through Denaturing SDS PAGE

SDS PAGE in the presence of reducing agent (2-mercaptoethanol) is an anionic detergent which denature proteins by wrapping around the

polypeptide backbone. SDS binds to protein specifically in a mass ratio of 1:4:1. It confers a negative charge to the polypeptide which turn it into rods of negative charge cloud with equal charge or charge densities per unit length. It is usually necessary to reduce disulfide bridge in proteins before they adopt the random-coil configuration which is necessary for size separation. In denaturing SDS PAGE separations, fragment migration is determined not by intrinsic electrical charge of the polypeptide but by molecular weight. There are two types of buffer systems in electrophoresis, continuous and discontinuous buffer systems. A continuous system has only a single separating gel and uses the same buffer in the tanks and the gel. In a discontinuous system, a non-destructive large pore gel called stacking gel is layered on top of resolving gel. Each gel is made with a different buffer, and the tank buffer is different from the gel buffers. The resolution obtained in a discontinuous system is much greater than that obtained with a continuous system.



8.1.9.1.6. Coomassie Stain

Once SDS-PAGE electrophoresis is completed, the gel was stained with Coomassie Blue R-250 to visualize the polypeptide bands. This staining is used for visualizing electrophoresed protein because it is sensitive and reproducible even though it is time-consuming. In Coomassie staining , both proteins and gel matrices are stained and removal of the stain from the gel matrix requires several hours before the desired proteins are exposed. The molecular weight of the polypeptide is inversely proportional to its mobility. The molecular weight of the polypeptide subunit can be estimated directly from a semi-log graph of the molecular weight of the protein standard versus their mobility or from a plot of log of molecular weight versus mobility. Separation of proteins by SDS-PAGE is an excellent technique for producing purified proteins.

8.1.9.1.7. Electro-Blotting

Electro-blotting is a technique that is used for electrophoretic transfer of DNA, RNA or protein onto a suitable membrane. This technique is used to produce pure sample for amino acid analysis and for N-terminal sequencing. For example protein is usually transferred onto a nitrocellulose membrane while for protein sequencing and amino acid analysis, the proteins are transferred onto a chemically stable membrane known as polyvinylidene difluoride (PVDF).

Nitrocellulose is a good general-purpose membrane for smaller peptides as a smaller pore sizes membrane is recommended for protein separation. Binding to nitrocellulose membrane occurs primarily through hydrophobic bonds. While, PVDF membrane is more hydrophobic than nitrocellulose membrane and will bind more proteins more tightly and tolerate more SDS in blotting system. Consequently, PVDF generally requires more stringent blocking conditions than nitrocellulose. Therefore, it is suitable, for protein sequencing, nylon membrane binding through both hydrophobic and electro statistic interactions and furthermore it is recommended primarily for northern and southern blotting.

In electro- blotting, by using the wet transfer technique, proteins are first separated by SDS-PAGE, the gel is then removed from the electrophoresis cassette and equilibrated in transfer buffer without methanol. However, PVDF membrane is activated by dipping it in methanol, it is then placed in transfer buffer (pH8.3) where most proteins which are negatively charged will migrate to the anode (positive electrode). In case a particular protein are suspected to have pH greater than 8.3, a PVDF membrane can be placed at the cathode-side of the gel. Alternatively, the pH of the transfer buffer can be adjusted to a higher pH. After transfer, the membrane is stained with Coomassie Blue R-250 and further destined to locate the protein bands. Section containing the proteins bands is excised for amino acid analysis and N-terminal protein sequencing.

In Western blotting, it is a specific test that is used for detecting protein instead of DNA or RNA. It allows one to visualize antibodies directed against each viral protein after being electrophoresed on the gel. As the proteins migrate through the gel they are separated based upon the protein size and charge. Characteristically, smaller proteins migrate through the gel faster than larger proteins. Sufficiently, separated proteins in a SDS-PAGE can be transferred to a solid membrane such as nylon or nitrocellulose filter. In this procedure, an electric current is applied to the gel so that

the separated proteins being transfer through the gel onto the membrane. Careful consideration is required during optimizing the electro-blotting of proteins. The goals are to transfer all the protein (large or small) out of the gel efficiently. However, some protein always will be remained on the gel. This can be detected by simply staining the gel after it has been transferred. The protein that has been transferred out of the gel should be invisible on the side of the membrane that contacts the gel. If in doubt a second back up membrane during the blotting, which can be stained to check for any signs of leftover proteins. In case some of the protein is left on the gel due to a poor membrane binding certain parameters should be optimized. For example,both SDS and alcohol are used in most blotting protocol however they have opposing effects on the success of protein transfer therefore it is need to be optimized according to the proteins that is being transferred. Furthermore, SDS required for protein mobility and it is particularly useful to facilitate the transfer of large proteins out of the gel. Since membrane binding requires hydrophobic interactions, too much SDS can also prevent binding, especially when nitrocellulose membrane is used. If too much SDS stripped from the protein, the protein may not completely transfer out of the gel. As a rule, if membrane binding appears to be efficient but some proteins still remained on the gel, addition of 0.01% SDS in the transfer buffer may promote complete transfer. Alcohol, on the other hand, is used to enhance hydrophobic binding of protein to the membrane by stripping some of the SDS out of the protein. Typically, addition of 20% methanol in the transfer buffer will improve binding of the nitrocellulose membrane. The lower the acrylamide percentage the more easy proteins transfer will happens. Therefore, always choose the lowest possible gel percentage that will resolve the protein completedly.

Gradient gel is ideal for blotting a range of protein sizes since the porosity of the gel matrix is well matched with different protein sizes. A protein will transfer more easily out of a thinner gel, therefore 1.0 mm gel over a 1.5 mm thickness is better. If the voltage is too low and the transfer time is too short, some of the protein will be left on the gel. If the voltage is too high, smaller proteins may pass through the membrane without binding. If proteins are left on the gel for some time after blotting, under certain conditions, increasing of the voltage by no more than 5 volts may be helpful in further protein transfer. However, please note that once SDS has been stripped from protein, lengthy transfer time or higher voltage will not help. Once bound, most proteins will remain on the membrane even with extended transfers.

8.1.9.1.8. Bacterial Lysis for Probond Purification

In this step GFP pRSET plasmid in *E. coli* Nova Blue (DE3) is used. This plasmid is need to be lysis by using sonicator. This sonicator is used to break the cell wall of the bacteria instead of using SDS to rupture a bacterial cell. This technique is used to analyzed prepared transform bacteria that is needed for development of genetically modified organisms.

8.1.9.1.9. Purification

Probond is a nickel-charged agarose resin that can be used to purify recombinant protein containing a polyhistidine (6X His) sequence. Proteins bound to the resin is eluted with either low pH buffer or by competition with imidazole or histidine. The probond resin is pre-charged and capable to bind 1–5 mg of recombinant protein per ml resin. It is provided as a 50% slurry in a 20% ethanol. The resin will appear blue in color when charged with Ni^{2+} and can be easily stored in 4°C. Probond resin is guaranteed stable for 6 months when properly stored.

8.1.9.2. Plant Metabolic Engineering

Metabolites are the end products of any cellular functions. Their levels depend on the response of plants towards biological and environmental stimuli. The term engineering implies that there is some precise understanding of the system that can be modified. Metabolic engineering in plants involves in the modification of endogenous pathways to increase flux and desirable molecules, to enhance the production of a natural product and to synthesize a novel compound. Plants have been the choice for metabolic engineering as they are the major source of carbohydrates, lipids, proteins, minerals, and vitamins for human and animals.

8.1.9.2.1. Strategies for Metabolic Engineering

Strategies in metabolic engineering can be done either by increasing or reducing a targeted metabolic compound or by producing a new metabolite in a crop. This involves with upregulating several or specific enzymes in a pathway, while suppressing those in another competing pathway or by using regulatory genes to establish multipoint control over more pathways.

To increase the production of a metabolite compound, a single gene that corresponds to an enzyme will be over-expressed. The gene can either from homogenous or heterologous sources. However, sometimes these strategies

will lead to some limitations to the subsequent steps that involved in the targeted pathway that will result in the decrease of the metabolite compound instead of increase. To overcome this, several genes that correspond to several enzymes that are involved in the pathway are crucial to be up-regulated at similar time.

To reduce the levels of certain metabolite compound, gene disruption and gene silencing can be used. In gene disruption, the target gene is mutated, while in gene silencing, either the gene transcript is destructed, or inhibited. Technologies such as transposon mutagenesis, *Agrobacterium*-mediated, and chemical mutagenesis approach are also favorable for gene disruption and gene silencing. Metabolic engineering in higher plant for metabolite increase and reduction can be carried out by using RNA interference (RNAi) or co-suppression.

8.1.9.2.1.1. RNAi

RNAi technology is currently being used in the development of innovative agriculture to feed the hungry. RNAi is a biological process that occurs in all organism. Here, RNA molecules inhibit gene expression, by causing the destruction of specific mRNA molecules. This technology is being used to create molecular approach to cure disease. Usually, the DNA contains the information to make RNA will form proteins. Sometimes, these proteins formed abnormal structure/compound which can be silenced or its formation can lead to various diseases. RNAi can be used to precisely target and control specific insect by protecting crops and beneficial insects. It can also provide broad resistance spectrum against pathogens with higher degree of genetic variability. RNAi related processes also play an important role in plant stress adaption. RNAi is also consider as a degradation mechanism in animal. Long doubled stranded RNA derived from the introduction of a gene will trigger the RNAi pathway. This results in the formation of an enzyme known as Dicer. Dicer will cleave the dsRNA into short strands that known as small interfering RNA (siRNA). An RNA-induced silencing complex (RISC) will recognize the two siRNA strands as either sense or antisense. The sense strand will be degraded, while the antisense will be incorporated with RISC. When RISC complex meets the complementary sequence, mRNA degradation is initiated, and protein synthesis is inhibited.

There are two types of small RNA molecules that function in RNAi. The first type comprises synthetic short interfering RNA (siRNA) molecules that target mRNA cleavage effectively by knocking down expression of a gene

of interest. It is a traditional type of RNAi that used for gene knockdown by usage of synthetic RNA duplexes that consist of two unmodified 21-mer oligonucleotides annealed together to form short/small interfering RNAs (siRNA). For better RNAi results it is improved with some chemical modifications. The second type is microRNA (miRNA) molecules that regulate gene expression by binding to 3' untranslated regions (UTRs) of target mRNAs to inhibit their function. MicroRNAs (miRNAs) are short, highly conserved small noncoding RNA molecules that naturally occurring in the genomes of plants. miRNAs are 17–27 nucleotides long and regulate post transcriptional mRNA expression, typically by binding to 3' untranslated regions (3' UTR) of the complementary mRNA sequence for translational repression and gene silencing. There are several ways to induce RNAi a) synthesis molecules b) RNAi vectors and c) in vitro dicing.

8.1.9.2.1.2. Co-Suppression

Co-suppression in plant is a RNA degradation mechanism. The mechanism of co-suppression mainly involves in the inactivation of RNA transcript of both transgene and endogenous gene through post-transcriptional gene silencing (PTGS) mechanism. This happens when some regions of the DNA sequence of the transgene and endogenous genes are homologous and both sequences can be actively transcribed into functional mRNA. When high accumulation of mRNA/aberrant RNA occurs in the cytoplasm, it triggers the PTGS mechanism to degrade it. As a result, both transgene and endogenous mRNA is degraded causing the silencing of the gene. The benefit of co-suppression strategy is that the effect of co-suppression will trigger a switching on alternative pathways that will result in positive feedback.

8.1.9.3. Plant Transformation

The genetic makeup of plants can be altered in the laboratory by a process called transformation. Transformation can be a laborious and expensive process that leads to low success rates. Several methods to accomplish plant transformation have been revised but nowadays, transient transformation and floral dip transformation are becoming popular as they are easier to handle and requires less lengthy steps for plant regeneration. Researchers that lack of plant tissue culture background can still carry out plant transformation through this technique.

Agrobacterium tumefaciens (a soil-borne microorganisms) is a mediator to provide mechanism of T-DNA integration for natural gene transfer in

the production of a transgenic plant. It works well on a broad range of dicotyledonous and monocotyledonous plant species. It is the easiest, simplest and most widely used transformation technique.

8.1.9.3.1. Transient Transformation

There are many types of transient transformation and the type that is being discussed here is transient transformation through agro-infiltration. Agroinfiltration is a fast and convenient method compared to traditional plant transformation. Agroinfiltration is a method that is used to induce transient expression of foreign genes in a particular plant to produce a desired protein.

Here, a suspension of transformed *Agrobacterium* will be applied into a plant leaf through the air spaces inside the leaf stomata, or sometimes through a tiny incision made to the underside of the leaf, where it transfers the desired gene to the plant cells. On the other hand, vacuum infiltration is used to penetrate *Agrobacterium* deep into plant tissue. Air is forced out from the stomata and filled with vacuum, when the vacuum is applied and released the differences in the pressure force *Agrobacterium* solution into the mesophyll. The *Agrobacterium* will remain in the intercellular space and transfer the gene of interest in high copy numbers into the plant cells. The gene is then transiently expressed.

Many studies on RNA silencing were carried out through agroinfiltration. Plant species that were studied using this method include green *Amaranthus* sp. *Nicotiana benthamiana, Triticum aestivum*, *Capsicum chinense* Jacq., *Vitis vinifera* and *Arabidopsis thaliana*.

8.1.9.3.2. Floral Inoculating Methods

Germline transformation is a type of transformation that target the plant ovule by dipping the plant floral into *Agrobacterium* suspension. The floral dip technique reported by Clough and Bent (1998) is the first method of a stable plant transformation. This technique is simple and relatively inexpensive to produce transgenic plants.

The mechanism starts when the pollen tube penetrates the stigmatic surface and continues to grow towards the ovule, occasionally carrying *Agrobacterium* from the stigma down to the style towards matured ovule through similar route. Once the *Agrobacterium* reaches ovary, it will transform the ovules or zygote. Sometimes, some researchers prefer to use vacuum infiltration technique to increase the chances of *Agrobacterium* to penetrate the floral material. However, it is only suitable if the inflorescence

plants that are to be inoculated are short, as it is not suitable for plants that has long inflorescence. Successful transformation through floral dip techniques will be achieved if the inoculated flowers are matured and slightly open at the top prior to anthesis so that they are completely exposed to *Agrobacterium*. Besides vacuum filtration, some researchers used floral spray, but this technique produce high level of contamination.

8.1.9.3.3. Agent of Plant Transformation

Promoter is the DNA sequence that regulates the expression of a gene. All genes are accompanied by promoters. A promoter is located upstream of a gene. It has the information on when and where a gene need to be expressed. RNA polymerase II binds at specific regulatory region on the promoter for transcription initiation.

There are many types of promoters, promoters that are active all the time, promoters that are active only at certain tissues and promoters that are activated in response to external signals. The promoters that drive the gene expression will have information on the timing, location and the level of expression of a gene for an efficient selection of elite events.

8.1.9.3.3.1. Constitutive Promoters

Promoter that is active at all time is known as constitutive promoter. Constitutive promoters are strong promoters that are often use as selectable marker genes to ensure high expression of successful selection of transformed tissues. These promoters can be apply across species. Examples of constitutive promoters are Maize ubiquitin promoter, Ubiquitin extension protein (uep1) promoter, Glycine max polyubiquitin (Gmubi) promoter, Cauliflower mosaic virus (CaMV), 35S, rice actin 1 (Act-1), Octopine synthase promoters, Nos promoter and maize alcohol dehydrogenase 1 (Adh-1).

Ubiquitin and actin genes are expressed in almost all plant species and tissues. Therefore, their promoters (Maize Ubi and Rice Act-1 promoters) can drive gene expression in a broad range of plant species and tissues. CaMV35S promoter is the most commonly used constitutive promoter to drive high levels of gene expression in dicot and monocot plants. This promoter is usually active in cells of younger tissues that has a high metabolic activity and vascular tissues of leaves and roots.

Typically, monocotyledon plants have highest expression level with monocot derived promoters, while dicots have high levels of expression with

monocot or dicot promoters. Earlier reports showed that gene expressions between monocot and dicot differ with respect to transcription factors and the recognition of promoter sequences therefore, monocot promoter activity is inhibited in dicot. However, current studies have revealed that certain monocot or dicot promoters do work in both dicot and monocot plants, however, the level of expression between plants is different.

8.1.9.3.3.2. Tissue-Specific Promoters

Promoters that are active only in certain tissues, organ, and development stage are known as tissue-specific promoters. These types of promoters will influence gene expression during flowering, fruiting, seedling, rooting stages, or during the vegetative, or seed-setting stage. They drive gene expression only in particular tissues, organ or development stage that is actively functioned. Expression of a gene controlled by a tissue-specific promoter will be confined to a specific tissue with less detrimental effects on the normal growth and development of the whole plant in comparison to a constitutive promoter. Successful gene expressions in targeted plant parts or development stages are often lead by promoters from closely related species. Some examples of a tissue-specific promoters are fruit-specific (E8) promoter in tomato, mesocarp-specific promoter in oil palm, light harvesting chlorophyll A/B binding protein promoter or also known as leaf- specific promoter, chromoplast-specific promoter in Solanum and seed storage protein (GT 1) promoter.

8.1.9.4. Modifying Photosynthesis Gene

Photosynthesis is the basis for almost all plant life. It has the potential to use the energy of the sun so efficiently. There is a great opportunity to maximize this system within the plant as well as in terms of the surrounding environment of the plant. Crop productivity can be increased by 20% if the amount of light used in the photosynthesis are raised. Three genes were targeted that is functioned to protect plant cell from damaging when plant is overexposed to light. By increasing the expression of those gene, it is believed that 14–20% in the productivity is achieved. In photosynthesis, plants use the energy from the sunlight to take up carbon dioxide from atmosphere and convert into biomass which we utilize as food. When there is too much sunlight, the photosynthesis machinery in chloroplast can be damaged, so plants do need photoprotection. Inside chloroplast, plants have a system called non-photochemical quenching (NPQ). When there is too much sunlight the

pressure in the cell increase and this turn on the NPQ. The NPQ will release the energy slowly. Leaves located in the shade provides lower pressure and turn off the NPQ slowly as well. These circumstances will lead to reduce efficiency in the photosynthesis and thereafter reduce plant production. By overexpressing the 3 genes exist in the NPQ, the system can turned off much faster and efficient photosynthesis can be carried out efficiently. This hypothesis was studied on tobacco plant as it is the best model crop plant. Besides NPQ that present in the plant itself, certain bacterial species are identified to have faster enzyme such as Rubisco, which can carry out photosynthesis much faster than the RUBISCO that found in the plant. Therefore, the gene function of Rubisco enzyme from bacterial cell can be incorporated in the plant DNA. Successful incorporation will increase the photosynthesis level in the plant. Further incorporation of micro compartment called the carboxysome will further increase the level of photosynthesis too. Another way to increase photosynthesis can be done through making a plant to carry out C4 photosynthesis instead of C3 photosynthesis. It is because, in C3 photosynthesis, there is a tendency where plant captured oxygen rather than carbon dioxide. When this happen, the energy obtained from the light is being wasted as the selected oxygen is released without any product being produced. While in C4 photosynthesis, carbon dioxide is captured by another protein which can distinguished between O_2 and CO_2. The captured CO_2 is moved to another part of the plant where it is released to Rubisco to make useful product. To ensure this approach available in the plant species, genes that are responsible in C4 plant are identified. Study on the structure of the leaves and the amount of protein that exist in different part of the plant are crucial to develop C3 plant into C4 plant. Finally, steps into looking at turbo-charging photosynthesis approach in crops that has reached a plateau are recently being considered.

8.1.9.5. Mediating Flowering

A plant can reproduce successfully only if it flowers. During flowering, energy complex network of photoreceptors and other protein will evolve parallelly with the changes in the environmental condition such as light and temperature. Induction of photoreceptors and other protein leads to expression of FLOWERING LOCUS (FT) gene. The produce FT protein will migrate from the leaf to the tip of the shoot for flower formation. Besides overexpressing FT protein, one can also regulate energy process not in the form of light but in the form of sugar. The sugar molecule trehalose-6-phosphate (T6P) is detected to influence the flowering. Flowering is stop due

to T6P suppression besides that this sugar is also crucial for the expression of the FT protein. In another words it means suppression of T6P will greatly delayed the accumulation of FT protein in the leaf. Therefore, energy in the plant can be in the form of sugars that derived from photosynthesis. This proved that plant convert sun's energy into usable chemical form to perform all metabolic task.

8.1.10. Bioinformatic Analysis

Using bioinformatic analysis to reconstruct the path of evolution with considerable accuracy.

8.1.11. Clustering Analysis

Clustering analysis of the predicted genes can be used to identify the generated clustered specified for the plant. From here, one can identify the specific trait that belong to a particular crops.

8.1.12. Comparative Analysis

A detailed comparative analysis of crop genomes will facilitate the understanding of the genomic architecture, which can contribute to cultivation, and further utilization of the information.

8.1.13. Imaging System

Digital photography and automating imaging system can provide detailed image and information about the plant parts. The resulting images are uploaded to a server and analyzes with a software based on many different parameters. The result of the analysis will provide information that is needed for a gene search that exhibit best characteristics.

CHAPTER 9

TRADITIONAL ANIMAL BREEDING

CONTENTS

9.1. SELF-EDITING

Cephalapods have evolutionarily conserved RNA re-coding sites which are known as coleoid. RNA editing in current cephalopods are much higher than the primitive one, it is because the coleoid nervous system is enriched with proteins that act as the key players in neural excitability and neuronal morphology. In current cephalopods, many editing events are highly conserved unlike in mammals. To ensure that editing occurs in the RNA level, natural selection mutation are being suppressed.

Octopus, squid, and cuttlefish are known for exhibiting complex behavior. They have a tendency of editing the coding regions of their own RNA, specifically in the nervous cell system by using an enzyme known as ADAR. ADAR enzyme has the capability to change the adenosine nucleotide into inosine nucleotide and allow to generate different versions of proteins from similar gene. Therefore, it has the effect of diversifying the proteins that it cells can produce. For example, more than 60% of RNA transcripts in the squid brain are re-coded for editing itself to adapt the surrounding environment.

9.2. BREEDING STOCK

A breed is identified as an organism based on its body shape or style. Planned breeding is referred to a group of animals that are used for a breeding. This happens when individuals are looking forward to breed animals with valuable traits. Example of valuable traits are such as fast-growing, muscular, lean, and high reproductive. Identifying the best breed is done through visual appearance of a phenotypic which is appear based on the response between genetics and environment. Phenotypic characteristic such as body conformation, muscling and based on recorded data can be used for making breeding selection. In addition, if the breeding is for a commercial purpose, traits such as good reproduction rate, more meat than fat, milk and disease resistant are usually considered as good traits.

9.3. PUREBRED BREEDING

Mating animals of the same breed is referred to as purebred breeding. It is done to maintained a same breed that has a perfect trait. This involves culling where superior qualities are gathered together than the non- superior one. However, pure-breeding creates a limitation in the gene pool therefore

purebred animal is often susceptible to a wide range of congenital health problems. Thus, purebred is not used for commercial purposes.

9.4. BACKYARD BREEDING

Form the terms itself, backyard breeding occurs at the backyard. This type of breeding usually carried out by family who may have small poultry farm for own purposes. There is no commercial value present through this type of breeding.

9.5. INTERSPECIFIC HYBRIDIZATION

In interspecific hybridization, firstly it can be divided and defined into two parts; interspecific and hybridization. Interspecific defined as different species while hybridization defined as a thing made by combination of two different elements to produce new species and appears to facilitate evolution and adaptation among animals. Therefore, interspecific hybridization means different species are mated together to produce progeny. This progeny is improved by combination of valuable traits such as better growth and flesh quality, disease resistance and increase environmental tolerances. The success of inter-specific hybridization can be variable and depend on the genetic structure, crossing patterns, gamete compatibility and gene flow patterns of the parental species. Inter-specific hybridization plays a significant role in increasing aquaculture production. Therefore, it is successfully applied in carp's production to produce carps with high resistant against environmental stress. Common female carp having two pairs of barbels are interspecifically breed with male blunt snout bream without barbels to form a new crucian carp-like homodiploid progeny without barbels. The genome of the crucian carp-like homodiploid fish contains a common carp, and the results indicate the existence of gene flow between common carp and crucian carp under natural conditions. Diploid crucian carp might originate from the crosses between a female common carp interspecifically breed with a male blunt snout bream. Besides carp, a mule is a hybrid of a male donkey and a female horse, while cattle is a cross between a cow and a yak and yakalo derived from a breeding between a yak and a buffalo.

9.6. CROSS BREEDING

A breeding program is known as a cross-breed when organism with purebred parents of two different breeds, varieties, or populations are mated. Cross-

breed is carried out to produce hybrid vigor progeny that share the traits of both parents. Cross breeding is preferred when health and viability of a particular organism is crucial. Therefore, a proper and detailed Cross-breed program is developed so that inferior quality is not being further produce and its dilute the undesirable gene in the pure breed line. Furthermore, in Cross-breed itself, in case one of the parents of the domestic animal are unknown, the term mixed breed is referred rather than Cross-breed. While out Cross-breeding is another type of Cross breeding which is referred when a purebred is breed within the breed to increase the genetic diversity. In Cross breeding and inbreed breeding that bring in the common deleterious recessive phenotype can be reduce within species. In cattle, purebred females which is smaller than the bull and purebred bull which is often big in size is crossed to produce progeny that has trait from both parents. In addition, in sheep, Cross breeding is specifically used to tailor in production of lambs. However, in Llamas, through cross breeding, the results are unpredictable as sometimes it may display characteristics form the parents and sometimes from the grandparents.

CHAPTER 10

MODERN ANIMAL BREEDING

CONTENTS

In the traditional breeding, the breeding of the livestock relies on the principle of selective breeding. We aware that in this method genetic improvement is brought to increase the frequency of advantageous alleles of many loci that are rarely identified. In these methods, genes cannot be moved from distant sources like different species or genera due to the reproductive barrier. However, development of molecular biology and technology has given rise in modern breeding by overcome the breeding barriers between different species or genera. In modern animal breeding, most of the initial process involved in the laboratory. It includes modification, selection, and isolation. Involvement of biotechnology approach are includes gene transfer, in-vitro production, cloning and sexing of embryos. Therefore, even though modern breeding is an application of in-vitro techniques, production of the embryo is still possible. For example, sheep, cattle and pig's embryos are cloned through nuclear transfer. This can be successfully done due to efficient in-vitro systems, technology to stored embryo's, high throughput protocols such as DNA hybridization, amplification, culture media for growth and embryo's storage, and embryos of most species can be sexed in a non-damaging way with specific male antibodies. Through modern breeding, the transgenic embryo has been produced for chicken, fish, and cattle. In addition, even specific genes can be targeted for expression in specific tissues because of the technology that available can provide a better understanding of the genes of study and provide the ability to select which genes need to be introduce. For example, genes that influencing animal growth, genes that regulated through environmental stress, gene function to produce meat, milk or egg composition and gene involved in animal disease resistance can be identified. With current technology, the genome of domestic animals can be easily sequenced and mapped. Therefore, genotyping by sequencing has become an important part in genomics-assisted breeding programs.

Food producing animals such as cows, pigs, goats, chicken and other poultry species that are consuming genetically engineered crops or feed that contains genetically engineered ingredients may be labeled as a genetically modified organism by certain agencies. However, products derived from genetically modified diet fed such as milk, meat and eggs somehow is unable to be identified and labelled as genetically modified food. Alternatively, opportunities for modern animal breeding has become more challenging because they require greater biological efficiency at a reduced cost and consumer acceptance on this technology. Consequently, all these new technologies able to change livestock breeding drastically in the next decade.

10.1. CLONING LIVESTOCK

Cloning is a complex process that allow copy, inheritance and transfer of a genetic material from a donor to a recipient through artificial techniques. The progeny derived from cloning is genetically identical to their ancestor. It is used to multiply and improve an animal characteristic. In another terms, it is also known as production of replica copies of an individual. In animal, embryonic cloning has the potential to increase food supply by increasing the production of animals with desirable traits. Livestock species that has successfully cloned animals are cattle, swine, sheep, and goats. Farmers can easily introduce positive characteristics such as enhancing high protein in milk production, more meat with lesser bone, disease resistance and more specifically improved genetic traits into their livestock. The reason why livestock are being produced by this method is because livestock breeding through traditional way is time and labor consuming. In fact, in traditional mating the purpose to produce many good offspring sometimes are unachievable, in addition there is also high risk of infection or transmission of disease.

10.2. MALE GERM CELL TRANSPLANTATION

In livestock animals, breeding male germ cell transplantation application, is applied to produce offspring from sterile male. This is done by male germline stem cells transplantation that is collected from a donor animal's testes to the testes of an infertile recipient. The transplanted male germline stem cells which injected into seminiferous tubules will migrate from the lumen to relocate at the basement of the membrane and will start to colonize the recipient's testis to produce sperm. Now the sterile animal can produce male genetic material. The male germ cell transplantation can be carried out within species or between species. This procedure has been successfully applied to domestic livestock such as chicken, fish, goat and in species where embryonic stem cell and nuclear transplantation has limitation. Furthermore, if large scale of testis stem cell culture is possible, male germ cell transplantation can also be expanded towards artificial insemination and animal cloning. Therefore, transplantation of male germ cell is a valuable approach for livestock breeding in animal that has suppress male fertility.

10.3. ARTIFICIAL INSEMINATION

Daily, male animals produce millions of sperms in its testes. A sperm contains autosomes and half X and Y chromosome. Therefore, male animal can fertilize female partner regularly to produce offspring. For example, a bull can produce about 0.2 ml semen containing 10 million motile sperms which can fertile around 500 cows. However, natural fertilization unlikely able to produce the best progeny. It is because the ovulation period for all female of particular animal are not similar and hardly achievable and sometimes it will takes time.

In artificial insemination, number of females to be fertilized can be increased and the best progeny can be produced. Artificial insemination is involved with collection of semen from adult male through ejaculation and preserved it in a frozen form. The stored semen is diluted and further analyzed under microscope in terms of sperm motility. In addition, one can also identify the X and Y chromosomes to determine the progenies sex. This is done by using a fluorescence dye where the sex chromosome X and Y is stained with different intensities. In the later stage, these two chromosomes can be separated by using an instrument known as fluorescent activated cell sorter (FACS). Stained sperms which present in the form of suspension are converted into microdroplet using FACS. Each microdroplet consists of a single sperm cell which is then passes through a laser beam. As the fluorescence dye passes through the laser beam, the fluorescence dye is measured electronically, microdroplets of different intensities are deflected into separate collection tubes.

The diluted and separated sperms can be used within a few days or cryopreserved at -196°C by using liquid nitrogen for future use. The cryopreserve semen can be easily transported across the states or countries, therefore artificial insemination are able to apply globally. Thus, cryopreserved semen of a single male is capable of inseminating thousands of female animals. This frozen semen is use to inject the uteri of a fertile female. As mentioned above, to expect all females to ovulate at the same time and to identify the sexual heat has become difficult, therefore in an economical production of offspring breeding natural processes are becoming hard. But with artificial insemination technology and process, each female animal that reached the ovulation can be fertilized with the collected semen.

In addition, to get the most impact from the artificial insemination, farm management may use hormones to manipulate estrogen/progesterone in female animals. This will synchronize the ovulation period in all-female

at same time. A healthy ovum which being produced in ovary possesses autosomes cell and one sex chromosome identify as X. Once this is achieved, all the female animals can be fertilized together through artificial insemination. This approach will be cost saving, easy and simple. It can be apply in livestock and dairy industry. In addition, artificial insemination makes it possible to rapidly produce many offspring from a genetically excellent male and through this technique disease spreading can be prevented too.

10.4. EMBRYO TRANSFER

Embryo transfer is a technology where a donor animal is being fertilized, and her embryo are being removed to be implanted or transferred to a recipient animal who is known as a surrogate mother. This technology can also be incorporate with multiple ovulation through hormonal treatment. Since multiple ovulation is possible, isolation of the immature oocytes from follicles can be done as not all oocytes are able to become matured completely. The collected immature oocytes are then incubated *in vitro* for maturation. In addition, multiple ova can produce multiple embryos and these multiple embryos can be frozen and stored. When the time is suitable, multiple embryos can be transferred to multiple surrogate mother which lead to production of multiple progeny at same time. Therefore, sometimes this type of multiple embryo transfer is also known as multiple ovulation embryo transfer. This is economically very valuable for the breeder, as more embryos can be taken away from a single female animal into multiple female surrogate animals. For example, in embryo transfer breeding of cattle, breeder could quickly multiply the top genetics of the females with the best genetics of the male. In addition, females with less genetic quality can function as a surrogate mother for the rest of the embryos. This resulting of an overall high genetic quality of the entire livestock. In this breeding technology, the embryos can be frozen and stored, so that they can be widely distributed for future use. Embryo transfer has been carried out for many years mostly in cow, sheep, goat, and deer via the vagina-cervix-uterus route. However, in pigs due to the physiology of the reproductive tract, it is particularly difficult to carry out through similar route. Besides that, in this technique, disease transmission can be minimized as transportation of lively animal stock can be reduce and ability to disseminate desirable genes from superior female animals from various species such as cow, sheep, goats, and pigs are achievable. In addition, embryo transfer technology able to capitalize maximum number of

embryos from a genetically superior progeny in shortest time. Furthermore, passive immunity is passed on the offspring for a better survivability in a stressful environment despite the embryo being 100% genetically different from the surrogate mother and valuable production such as high volume of milk and meat will be produced. Globally, it was reported in 2012 that 699,586 bovine embryos were collected from super-ovulated cows. This is slowly being used by Agricultural Development Corporation farms and selected individual farms. However, embryo transfer needs some high level specialized technical personnel in-charge for flushing and embryo collection and good management practices and observation skills of the surrogate mother are crucial. This involves managing body condition and providing proper nutrients including minerals which is important to produce healthy embryo donors and progeny. A breeder needs to spend money to purchase all the necessary equipment and proper station to carry out all the processes hygienically. Breeder need to keep in mind that embryo transfer is not apply on rare breeds.

Selection of each donor female is one of the most important decisions in embryo transfer. Female donor should be from a superior genetic worth and marketability to justify the embryo transfer costs. Making decisions should be made by considering the genetic worth and economic value of potential calves. The reproductive potential of a donor female must also be acceptable. The ideal female donor will has regular estrous cycles which begin at a young age, routinely conceives with not more than two breeding's, maintains a 365-days or less calving interval, calves without any difficulty, free from reproductive abnormalities and disease, and has no conformational or known genetic defects. It is because successful embryo transfer programs requires highly trained technicians and someone that diligent to perform these services. However, embryos may not be marketable unless they have proper documentation. Some breed associations require reported records of embryo removal dates or other calves information that derived from embryo transfer. Technicians should complete certificates of embryo recovery, freezing, or transfer as appropriate. This information is needed to perform an eligible registration in the breed registry.

10.5. IN VITRO FERTILIZATION TECHNOLOGY

The term *in vitro* means outside, therefore in-vitro fertilization means fertilization which occurs outside of a body which to be specified, outside the uterus and artificially. By IVF technology, animal breeding has become more

productive as healthier and productive offspring are produced compared to animal production through normal course. For example, a normal animal produces about 4–5 offspring's through natural reproduction, whereas through IVF technology the same animal can produce 50–80 offspring's. The IVF technology not only can produce large number of animals but also can improve the desired gene pool. This technology involves with eggs isolation from ovaries of a female donor, and left for maturity in an incubator. The eggs are fertilized by semen obtained from superior male in the test tubes. The fertilized eggs in the form of embryo are then implanted in reproductive tract of other recipient female which acts as foster mother or surrogate mothers. They are used only to serve as animal incubator and to deliver offspring's after normal gestation period. The surrogate mothers do not contribute in the genetic make-up. Furthermore, in vitro fertilization technology can also further develop into in-vitro fertilization-embryo. This can be done through superovulation procedure. It is carried out by collecting multiple ova in a form of small microdroplets. Each microdroplet comprises of about 10 oocytes. The microdroplets are grown in culture medium which supplemented with media that can facilitate the penetration of sperms into oocytes. One dose of sperm which consists of about one million sperms per ml of medium is used for fertilization. Once fertilized, the fertilized zygotes are incubated into agar until it reaches the blastocyst stage before implanting them in the oviduct which is the perfect environment for early development of an embryo.

10.6. EMBRYONIC STEM CELL

Embryonic Stem cells are derived from embryo. The cell is isolated specifically from the Inner Cell Mass (ICM) of embryo. It able to maintain stable chromosomal content and has potential to differentiate into 200 cells type. It is often use to regenerate severely dysfunctional myocardium and as an immune privilege of these cells. There are 2 main advantages that can be clearly seen by using embryonic stem (ES) cell technology, first a reduced cost in producing a transgenic animal as the costs for normal breeding or other techniques can cost millions but by embryonic cell approach the cost can be reduced by using fewer genetically valuable embryos. Secondly, embryonic cells allow new genes to be inserted or remove deleterious genes from the genome. This approach can be used for milk and meat production, increase disease resistant and growth rate with improved carcass composition and enhance reproductivity. Animal production through embryonic stem cells

has more success rate than other cloning technique. For example, buffalo calves has been produced through embryonic stem cells.

10.7. QUADRIPARENTAL HYBRIDS

From the term itself, quadriparental hybrids refer to four parents that are used to produce a single progeny. In quadriparental hybrids, embryos of two different species of livestock are fused. This resulted in the formation of a single embryo which finally developed into a normal healthy animal.

MALE (SPECIES A) x FEMALE (SPECIES A) = EMBRYO A (blastula stage)
MALE (SPECIES B) x FEMALE (SPECIES B) = EMBRYO B (blastula stage)

EMBRYONIC CELL A (with removed membrane) FUSED EMBRYONIC CELL B = EMBRYO C (blastocyst) (new membrane formation)

EMBRYO C are then transferred into the uterus of a foster mother who is condition by a sterile male. This will enhance the implantation of the embryo as the foster mother are in a suitable condition to receive an embryo. A hybrid goat and sheep named GEEP is one of an example that is being produce through quadriparental hybrids.

10.8. NUCLEAR TRANSPLANTATION

In these days nuclear transplantation is also known as somatic nuclear transfer. Basically, in nuclear transplantation, the nucleus of a donor cell is inserted into a target cell that its nucleus is been removed. In detail, nuclei from a morula stage embryo are transferred to an unfertilized oocyte. This involve with the removal of the metaphase II chromosomes processes. However, the original morula stage embryo is more differentiated than the one-cell stage embryo. Therefore, the resulting nuclear transfer embryo is remodeled and reprogrammed to behave as nuclei of an one-stage embryo. The potential application of breeding through nuclear transfer is that this genetically identical individual's production will reduce the number of animals that need to be analyze for post-production and marketing. For example, Dolly is the best result of nuclear transplantation breeding and recently reconstituted sheep embryos has been produced through electrofusion nuclear transplantation.

10.9. GENE TRANSFER

The other approach in modern breeding is known as targeted gene transfer that involves transfer of genes at homologous sites in the host genome. It is done just to replace the wild-type of mutant genes. Targeted gene transfer is possible because the homologous DNA sequences are present at the targeted site. Cells that can be used for gene transfer is embryonic stem cells. Gene transfer usually mediated through a vector or virus that carries the desired gene from foreign origin. After transferring the targeted gene in the specific cell, the cells can multiply and introduced to blastocyst through microinjection. It is then transferred into uterus of a surrogate mother. In gene transfer, the cells that are inserted with the desired gene is detected by marker gene that express antibiotic resistance compound. The offspring are analyzed for the presence of the desired gene prior crossing it with the normal breed.

10.10. MOLECULAR GENETICS

Molecular genetics approach is widely being used in animal breeding. The success of this usage is due to the availability of the genetic linkage map, knowledge of genomic sequence, expression and regulation of the gene pool. In molecular genetics, once the desired gene is identified it can be incorporated with physical map of chromosome, linkage map or genetic map to determine the insight of the gene. Besides that, even the unknown genes or several genes that regulate some genetic traits which scattered around the genome that associated with genetic diseases or any other traits can be identified. This molecular genetic studies on livestock can be done through the helps of molecular markers. The terms molecular markers refer to a fragment of DNA that is located within the genome representing a specific characteristic. In molecular genetics, by using molecular markers, one can identify the total number of genes, one can also understand the behavior and the expression of the gene that leads to a possible alteration of the gene structure. Moreover, the information can be expanded towards identifying the related protein sequences through amino acid sequencing. The use of molecular markers is to define the genetic makeup (genotype) and to predict the performance of an animal trait in animal breeding. There is various type of molecular markers which will be discussed below.

One of them are known as marker-assisted selection (MAS). In MAS, animal performance is evaluated through DNA linkage equilibrium with a quantitative trait locus (QTL). MAS facilitates the improvement of the desirable traits by exploiting the existing genetic diversity. The molecular

genetics technology has the potential to move polygenic QTL data for breeding purposes across animals. However, after successful production of transgenic animals, some appropriate breeding methods should be followed to multiply transgenic herd.

Besides MAS and QTL, markers that can provide a valuable tool for quantifying the genetic and geographic variation is known as allozyme markers. This allozyme marker is an enzyme variant based maker that presence due to allelic differences. The pattern can be easily visualized using protein electrophoresis system. It is because, during mutations, replacement of amino acids on the genetic leads to a change in the protein composition. These changes resolved as an allele on electrophoretic gel.

Mitochondrial DNA can also be used as a molecular marker due to its properties. It is an extrachromosomal genome presence in the mitochondria cell that located outside the nucleus and it is solely inherited from the mother. Since it has a high evolutionary rate than nuclear genome, this marker is very suitable to identify the similarity between and within a species.

To identify specific gene for a disease, one can use restriction fragment length polymorphism (RFLP) marker as it allows one to map the whole genome. In case, technical simplicity is needed by the breeders, random amplification of polymorphic DNA (RAPD) can be considered as it only requires single, short oligonucleotide primer eventhough, it could amplify random sequences from a complex DNA. While, amplified fragment length polymorphism (AFLP) can be used effectively to analyzed genetic variation below the species level. It is an ideal molecular marker for population genetics, genome typing, polymorphisms, evaluation and characterization of animal genetic resource studies. Using microsatellites as a marker allows identification of certain quantitative trait that is affected by many unknown genes.

Single nucleotide polymorphisms (SNPs) are heritable single- base pair variations that occur throughout genome. SNPs comprise the most common form of genetic variation, with some estimates SNPs in a given genome. SNP genotyping plays a central role in characterizing populations, identifying disease traits in animals and identifying genes responsibility for advantageous animal's traits. Therefore, single nucleotide polymorphism (SNP) marker is identified as the third generation of molecular markers than above as it will be able to detect sequence polymorphism caused by a single nucleotide mutation at a specific locus in the DNA sequence.

The latest marker technology in hand is refer as DNA barcoding markers. It is a short DNA sequence derived from a region of a genome that is used to identify an animal species. This marker can be conveniently used to identified species for new breeding and provides a quick identification of animal genetic diversity .

The future prospect in molecular genetics are lies in the application of high throughput DNA microarray (DNA chip) technology which could revolutionize animal breeding in the next millennium. In microarray DNA, cDNA or oligonucleotides are collected in a form of spot. These spots which are known as probe is hybridize with specific nucleic acid sequence known as targets that labeled with fluorescent dye. The core principle behind in the microarray is hybridization between the complementary nucleotides through hydrogen bond formation. The tighter the non-covalent bonding of base pairs, the better the complement it is. Then the level of the intensity is compared on different location of the base pairs. The signal strength depends on the amount of the target sequence bound to the probe. This approach is applied to analyze the host-pathogen interaction so that better understanding on the molecular basis and the crosstalk between the pathogen and the infected animal is obtained. This approach was successfully applied in bovine species specifically focused on growth, reproduction and milk production of the animal.

10.10.1. Genome Engineering

Genome engineering is a developed tools to make a precise and targeted changes on the genome of a living cells. Recent approach to target genome modification enable breeders to generate mutations by introducing double-stranded breaks to activate the repair pathways and manipulate gene function. In genome engineering, CRISPR system Cas9 Nuclease, CRISPR RNA, and trans-activating crRNA has been adapted and this system has revolutionizing genome engineering. Cas9 Nuclease is a double-stranded DNA nuclease and a central component of CRISPR (clustered regularly interspaced short palindromic repeat)-based immunity. CRISPR genomic loci are transcribed and processed into guide RNAs that is incorporated into Cas9 Nuclease. Guide RNAs direct the Cas9 nuclease to its target by complementary base pairing. Cas9 nuclease can be easily programmed for target specificity by supplying guide RNAs to almost any sequence. In vitro, Cas9 permits flexibility and specificity. It allows introduction of double-stranded breaks approximately at 20 bp recognition sequences specific to the target of a non-variable scaffold DNA sequence. In animals, genome editing is performed

by expressing Cas9 Nuclease and guide RNA which is known as a single-guide RNA (sgRNA) from the DNA constructs which derived from plasmid or virus, or through supplying RNA encoding Cas9 nuclease and sgRNA or by introducing RNA programmed Cas9 nuclease directly. sgRNA can be introduced directly through microinjection or transfection (viruses) or indirectly by sgRNA expression plasmid under a promoter. However, through direct introduction of Cas9/sgRNA complexes had further simplifies and had been reported to increase the mutagenic activity and reduce off-target editing events. Modifications of Cas9-target sites can be achieved by supplying a homologous repair template in addition to sgRNA and Cas9. Homologous repair templates are plasmids containing regions of homology surrounding of the target sequence that are altered to have desired mutations or knock-in. The homologous region is amplified from genomic DNA and then cloned into plasmid backbone. In later stage, further modification is introduced by using site-directed mutagenesis. Generation of sgRNA can be achieved through in vitro transcription from plasmid templates or PCR products. Targeted double-stranded breaks can be repaired by non-homologous end joining (NHEJ), knocking out gene function and enabling large-scale deletions, or through homology-directed repair (HDR) in the presence of HDR as a template, enabling targeted insertions or substitutions. In the presence of a donor template with homology to the target locus, the HDR pathway may operate to allow precise mutations. In the absence of a template, NHEJ is activated, resulting in insertions and/or deletions which disrupt the target locus. Therefore, CRISPR/Cas9 gene editing is becoming popular in the field of genome editing.

10.10.2. Agent

Retrovirus is a type of agent that is used to transfer any genes or recombinant gene into any cells while microinjection is a type of tool that function to transfer DNA solution, recombinant DNA, sperm, fertilized oocytes, zygotes, and embryos.

10.10.2.1. Retrovirus

The retrovirus is an efficient vector that able to transfect various cell including animal cells at high efficiency. It is because it consists of proviral

sequence that can accommodate the desired gene in the chromosome stably. The genome of the retrovirus vector is range from 7 to 10 kb single-stranded RNA containing long terminal repeats (LTR) on both ends and flanked with *rev*, *gag*, *pol* and other regulatory genes which are required for viral function. During infection, it will incorporate both sequences into the target cells. Retrovirus vector contains viral and promoter sequences such as CMV to enhance expression of the desired gene in the targeted cells. Hence, the delivered genes will have high success rate to produce transgenic cells/ animals. Prior using the retrovirus for transferring the desired gene, the retrovirus itself need to be cultured to determine the colony forming unit per ml and the competency of the virus to perform the gene transfer.

10.10.2.2. Microinjection

Microinjection is a technique that involve with delivering foreign DNA into a living cell through a micropipette. A glass micropipette is used to keep the whole process sterilized. The glass micropipette is heated until the glass becomes somewhat liquified. The liquified region are quickly stretched to forms a very fine tip with an about 0.5 mm diameter. The tip will resemble an injection needle which function for DNA delivery. This delivery processes are done under a powerful microscope. During microinjection, all the equipment and tools such as the cells to be microinjected are placed in a container while the holding pipette is placed in the field of microscopic view. The holding pipette will hold the target cell at the tip during suction. The tip of the micropipette is used to penetrate the membrane cell. The content of the needle is delivered smoothly into the cytoplasm until the needle becomes empty. A single injection into one cell should be finished within 5 seconds. Microinjection is one of the best methods for producing transgenic fish and extensively used across various fields to deliver material intracellularly. Sometimes electroporation technique is used in replacement of microinjection.The novel gene is introduced into early embryo through microinjection because pronuclei are not easily visible in the nucleus of oocyte. The introduced gene will replicates during the development of embryo in in-vitro and the developing embryo is collected at different developmental stages.

Photo credit : MC Nishanth

CHAPTER 11

ADVANCES IN GMO/GMF

CONTENTS

In these days, research towards advancing GMO/GMF has been expanded and accelerated. It help to identify the agricultural traits to improve the world crop plant and animal in lesser time. Breeders are looking forward to uncover the association between genetic makeup and phenotypes. A genomic revolution powered by advances in technology will significantly increase the level at which we are able to obtain and analyze data for better understanding to improve the whole agriculture ecosystem with less defects.

11.1. SAFETY

Generally, consumers consider that conventional foods are safe to be eaten. Conventional food is an example of food that is being produced through normal planting and breeding procedure and processes. However, even in the novel varieties of organisms that is used to produce food by traditional breeding methods some of the characteristics of organisms may be altered through natural processes, either in a positive or a negative way but this is always consider as safe by many of us. Anything beyond that, for example growing in the laboratory or in the building under different lights spectrum and without soils are sometimes may considered unsafe by some of us. That is the reason why national food authorities may not called upon to examine the securities of such conventional foods as it is considered safe.

Most national authorities consider that specific assessments are necessary for all GM foods. Specific systems have been set up for rigorous evaluation of GM organisms and GM foods relative to both human health and the environment. The WHO Department of Food Safety aims at assisting national authorities in the identification of foods that is subject to risk assessment and to recommend appropriate safety assessment.

The safety assessment of GM foods generally focuses on:

(a) toxicity properties
(b) potential to provoke an allergic reaction
(c) specific components thought to have nutritional
(d) the stability of the inserted gene
(e) nutritional effects associated with genetic modification
(f) any unintended effects which could result from the gene insertion

Bioengineered genetically modified organisms (GMOs) testing is required following legislative action in countries producing GMO. Kits that detect GMO-specific DNA sequences in seed, grain, plant, tissues

and processed foods and ingredients are available. The kit will utilize the real-time PCR assay that offers both qualitative and quantitative with high sensitivity and specificity results. It will also perfectly function at very low concentrations.

11.2. RISK

The potential risk that has been identified is the ability to provoke an allergic reaction. Usually, an allergic reaction can be provoked during the gene transfer from commonly allergenic organisms to a non-allergic organism. Unless significant data is provided to prove that the protein product of the transferred gene is not allergenic. Protocols for the testing of GM foods have been evaluated by the Food and Agriculture Organization of the United Nations (FAO) and WHO.

However, no allergic effects have been found in the currently available GM foods. While the risk of the gene transfer may happen if the transferred genetic material adversely affects human health and this is happen only if the gene is derived from an animal cells or bacteria and in other hand if antibiotic resistance genes were used as a markers during producing GMOs.

Normally, once we obtained a GM plant, what worries most of the people is the outcrossing event. Outcrossing is the migration of genes from GM plants into conventional crops or to the wild species. It is because it may led to an indirect effect on food safety and food security in the future if uncontrolable outcrossing happens. Cases have been reported where GM crops that was approved for animal feed or industrial use were detected at low levels in the products intended for human consumption. Therefore, several countries have adopted strategies to reduce outcrossing by having a clear field separation between GM crops and conventional crops/poultry farming.

Environmental risk assessments covers both the GMO and the potential receiving environment. The assessment process includes evaluation of the GMO characteristics and its effect and stability to the environment and also vice versa. The assessment also includes unintended effects towards the environment due to the insertion of the new gene in a particular plant or animal. Issues include the capability of the GMO to escape and accidentally introduce the engineered genes into wild populations through outcrossing, the persistence of the inserted gene after the being harvested, the susceptibility of non-target organisms such as insects which are not happen to be a pests to the desirable gene product, the stability of the inserted gene

in the environment and the changes in the biodiversity spectrum will all be assessed thoroughly.

11.3. PUBLIC CONCERN

The way governments have regulated GM foods varies among countries. In some countries, GM foods are not yet regulated. Countries which have legislation in place focus primarily on an assessment of risks on consumer health. Countries which have regulatory provisions for GM foods usually regulate GMOs based on health and environmental risks, as well as control- and trade-related issues. Intellectual property rights are likely an important element for GM foods. FAO/WHO expert consultation in 2003 has considered potential problems of the technology gap and the unbalanced distribution of GMO benefits and risks between developed and developing countries. The problem can be reduced through the existence of intellectual property rights and patenting that places an advantage on the strongholds of scientific and technological expertise.

11.4. PROTOCOLS

There are various protocol initiated in hand with increasing popularity in GMO production and usage. Two main protocol that was initiated is known as Cartagena and Nagoya Protocol. The Cartagena Protocol is an international agreement that aims to ensure living modified organisms through modern biotechnology is being handle and transported safely as it may have adverse effects on biological diversity, and human health. It was adopted on January 29 in the year 2000, and entered into a force on September 11, 2003. It establishes an advance informed agreement (AIA) procedure which includes a scientific risk assessment prior to the transboundary movement of LMOs. Till date, there are now 168 Parties signed this Protocol. Malaysia signed the Protocol in 2002 and was ratified as a party in 2003. The other protocol is known as the Nagoya Protocol. The Nagoya Protocol refers to the access of genetic resources fairly and have an equitable sharing of benefits on the utilization of biological diversity. They agree in contributing to the conservation and sustainable use of biodiversity for a development of human well-being. It provides a transparent legal framework for an effective implementation between the provider and recipient of the genetic users. Utilization of this protocol includes research and development on the genetic or biochemical composition of genetic resources, as well as subsequent applications and commercialization. The Nagoya Protocol on access to

genetic material was adopted on 29 October 2010 in Nagoya, Japan and entered into force on 12 October 2014.

11.5. CONSORTIUM

World Health Organization (WHO) has been taking an active role, primarily for two reasons in relation to GMO and GMF is 1) public health from the point of positive effect- an increase in the nutrient content of foods, decreased allergenicity and more efficient and/or sustainable food production; and 2) public health from the point of negative effect- based on the need to examine the potential negative effects on human health of the consumption of food produced through genetic modification. Modern technologies should be thoroughly evaluated if they are into constitute a novel improvement in the way a food is being produced.

WHO, together with (Food and Agriculture Organization) FAO, has convened several non-expert consultations on the evaluation of GM foods and provide technical advice for the Codex Alimentarius Commission which was fed into the Codex Guidelines on the safety assessment of GM foods. WHO will keep close collaboration with FAO and other international bodies on the safety of GM foods from the view of public health and protection So far, WHO have not detected any health damage in people who have consumed GM foods that has been approved by authorities.

Corporation and governments will look forward to use advance system to identify plant and animal species in real time by respecting the security within and across countries. In Indian Council of Agricultural Research (ICAR) has documented genetic enhancement as a major option to bridge the demand and supply gap under normal situation as well as under projected scenarios of increased frequency and stress intensity. Centre for Environmental Risk Assessment (CERA-ILSI) has come up with many programs such as training and safety assessment canvas so that sustainable network of trained experts who able to communicate with the breeders efficiently.

Uganda Biotechnology and Biosafety Consortium (UBBC) is consist of different peoples from various background such as policymakers, scientists, private sector leaders, civil society organization leaders and government officers. The objective of this consortium is for a common cause of advancing the role of biotechnology in improving livelihoods of the people in Uganda.

The Asia Pacific Consortium on Agricultural Biotechnology (APCoAB), was established in 2003. Their mission is to harness the benefits of agricultural biotechnology for human and animal welfare through the application of latest scientific technologies while safeguarding the environment for the advancement of society in the Asia-Pacific Region. They engaged in research and development, commercialization and human resource development in agricultural biotechnology. APCoAB also consider the environmental safety in the Asia-Pacific region due to the GMO/GMF. This consortium facilitates and promote public awareness on the important issues of intellectual property, biosafety, risk assessment and regulatory procedures, concerning GMO/GMF.

Besides focusing on the material, itself, MIMOS Berhad in Malaysia has embarked on a cloud infrastructure platform known as Mi-Cloud that is nondependent on any specific cloud hardware or software which is used in GMO/GMF advancement. The platform will help existing operational and infrastructure to be offered to the end user over the internet in a simple, flexible way with low cost-effective licensing and easy integration with other system and provide a secured benefit.

11.6. NEXT GENERATION OF FARMERS

There is group of young people that educate people who want farming to make a difference in the world. According to the US Department of Agriculture, the number of farmers aged 25 to 34 increased to 2.2% between 2007 and 2012. Just the second time in the last century that young farmers has grown and all of these new young farmers, 69% have college degrees. They believe that agriculture and growing food are one of the most direct and meaningful ways to reduce environmental footprint.

11.7. NEXT LEVEL OF GMO/GMF

Genetic modified plants have been modified through insertion of single or a few genes which capable to express desirable traits. Developing crops for example with improved phosphate uptake are relevant for sustainable agricultural production and it is critical to prevent environmental pollution caused by phosphate leaching. Besides that, several encoding genes that encodes for phosphate uptake can be cloned for maximize phosphate uptake in selected agriculture plant. For example, Phosphate transporter (PHTs) have important roles in Pi acquisition, allocation, and signal transduction. There

are several phosphate transporters, such as PHT1, PHT2, PHT3, PHT4. The other phosphate transporters such as the putative sulfate transporter family gene OsSULTR3;3 and SULTR-like transporter named SPDT (SULTR-like phosphorus distribution transporter). OsSULTR3;3 is reported to be involved in grain phytate and phosphorus metabolism while SPDT is expressed in the xylem region of both enlarged and diffused vascular bundles in the nodes and have been confirmed as a plasma membrane that localized Pi transporter. These genes can be used to produce successful clone or trait. Complete description of the clones should be documented and uploaded in the server to be access by farmers and researchers. This will ease them in selecting the preferable clones to be planted on their land. Each clone should embed with internal scanner devices where the progress of the clone can be monitored remotely.

11.8. DATA

Big data has been a popular term being mentioned for the last few years. It is a platform that able to bring useful insight of data through data collection from multiple sources. Big data is moving agriculture into a different track. The big data platform can collect, store and analyze massive amount of data derived from multiple value chain. Many researchers involve in big data having thoughts that big data can revolutionize the food production from seeding to harvesting. Big-data can be used to analyze varieties of soil, plant, and climate. Analyzing crops across farms helps to identify diseases that could ruin a potential harvest. The challenges and opportunities of data is immense in certain country like India which have big land, population, varieties of crops, languages and farming methods. However, the real potential is when this data from multiple large farms is collected, grouped and analyzed in real time valuable information can be extracted. Precision agriculture aids farmers in tailored and effective water management, helping in production, improving economic efficiency and minimizing waste and environmental impact. Recent progress in Big Data, advanced analytics capabilities and agri-robotics such as aerial imagery, sensors, and sophisticated local weather forecasts have truly transform the agri-landscape and thus holds promise for increasing global agricultural productivity over the next few decades. Digital agriculture is another method that is used to digitalize, visualize, design, monitor and control the relevant agricultural objects and farming processes according to agricultural needs by using digital technology. These days it is necessary for precision agriculture to

acquire the farmland information from the soil, to the plant, and to climate region to make an accurate farming decision in real time. The big agriculture companies are being challenged because new technologies give farmers the power to know what to plant, when and how to plant, and what crop inputs to use to maximize harvests and profitability. In the past, innovation was concentrated only in companies with the largest R&D budget, high volume products, or the one with most market share but the trend has changed with new technology the crop genetics, required a scale and infrastructure that are cost-prohibitive for big as well as small companies. Through big data, one can test varieties of genetics, crop inputs, and conditions across hundreds of fields, soils, and climates. They can conduct field-plot trials in real-time conditions over thousands of acres. This gives farmers information to optimize planting starting from the individual seed in specific fields with specific soil and climate conditions. For crop protection suppliers, this means that their products can be applied at different dose rates within a single field. Farmers can combine their digital data to calculate valuable information such as highest yield for a particular variety for a specific soil types and specific fertilizer. Therefore, researchers have also need to use the similar method to obtained data and result so that comparison and further utilization of the data can be applied successfully in the field by farmers.

In the traditional way, large agriculture companies and researchers may not be able to make the shift to supply farmers with both the best crop traits and the best information for farm inputs. Innovation and digitalization leds to the creation of new methods and tools. Farmers need accurate weather forecasts and input factors to increase their productivity in the farm. Therefore, by optimizing input factors such as soil nutrients, soil humidity, plant and root growth will help farmers to achieve their productivity. The use of granular and analytical data, capability to integrate various sources of information such as weather, soil content and plant characteristics will help in increasing crop yield and optimizing resource usage with low cost. Since, climate change, extreme weather events, and complex plant growth will demand proactive measures to adopt Big Data information. Big Data and advanced analytics are streamlining crop production by finding the core determinants of process performance and action to continually improve the accuracy, quality, and the production yield through a systematic plantation technique. It will provide significant information for an accurate decision making which is already being used for optimizing production schedules in certain country. We should also look at establishing a systematic mechanism to capture the data that could offer additional value-creation opportunities.

Rapid proliferation of mobile technologies could let farmers to improve productivity by understanding plant needs and preferences of farmers. It also has the potential to change the agri-business that offer new products and services thus developing sustainable revenue streams. With innovation hand in hand some advance practices on data use are crucial. There is a need to formulate a mathematical model not only from a single plantation but from a different plantation area. Researchers and farmers must simplify and standardize big data through a wide data management strategy as Big Data able to deliver the next revolution of farming strategies. With innovation, technology as a toolkit will allows one to speed up the process of converting data into information to decision making. However, innovation, technology, and big data can't convert information into knowledge without human intelligence. Going forward, the biggest innovations lies in the ability to convert information into knowledge.

11.9. TECHNOLOGY

Technologies have changed the ways farmers and breeders work towards consumer acceptation. Consumers become more demanding on information and needs of product satisfaction. The advancement of technology has led to a greater productivity gain. It changes the conventional practices and improved integration and automation of the whole agriculture environment. Technologies help in plant disease management, plant productivity, development of robotics and sensors, utilization of cloud computing and internet of thing (IoT). The key objective is to drive the agriculture farming forward with advanced rebranding to increase the productivity and acceptance of GMO and GMF.

Plant diseases and pest causes considerable crop losses and threaten to current global food security. The disease and pest have traditionally been fought with chemical pesticides which later spread throughout the environment and become hazardous to our health and other beneficial organism. Therefore, latest technologies such as genetic modification plant that able to protect themselves from the disease and pest are replacing traditional pesticides.

As we know, RNA is a common molecule in nature that degrades rapidly rather than building up in the environment. A new approach on the efficacy of environmentally friendly RNA-based vaccines that protect plants from diseases and pests are recently being considered. The challenge in developing RNA-based vaccines to protect plants has leads to the production

of RNA molecules. The method utilizes the RNA amplification system of bacteriophage and the RNA production in bacterial cells. This involves vaccinating plants against pathogens with double-stranded RNA molecules that can be sprayed directly on the leaves. This vaccine triggers a mechanism known as RNA interference which is an innate defense mechanism in plants and animal against pathogens. The vaccine can be targeted to the chosen pathogen using RNA molecules which share similar sequence identity with the pest's genes. This means that the double-stranded RNA molecules do not affect the expression of other plant genes, but only target the genes of the plant disease or pest by preventing their expression. This new method will enable the efficient production of RNA based vaccines and promote the development and adoption of RNA based plant protection approach. However, double-stranded RNA molecules that has been produced through chemical synthesis as a drug molecule is an expensive approach for plant protection.

With climate change, it is become hard to increase crop productivity. Heatwave has cause major crop failures. Heat will not only reduce the amount of the harvested crop but also the quality of the crop itself. For example, in wheat, the effect of intense heat includes reduction in the protein content while in chickpeas the plant become sterile. Therefore, heat tolerant crop will be the next millennial crop worldwide. This is done by identifying natural variations in crop genetic collections, varieties that can stand heat and carry out photosynthesis at high temperature and variety that can pollinate under heat. Finally testing of those materials for heat tolerance at various regions is carried out. Successful varieties able to grow well in heathy environment and feed millions of people globally.

Agriculture industries have started to investigate using robots to tackle complex farming. The key idea is to ensure robots to become more autonomous, flexible and cooperative. Eventually, robots will interacts with one another and work side by side with farmers and breeders. These robots will cost less and will have greater range of capabilities. Autonomous robots improve the accuracy of routine tasks by reducing the effort and time required for each task in the farming. Autonomous robots can perform inspection and provide real-time data to the farmers and breeders. Efficiency of the agriculture processes will be improved as errors can be minimized through the collaboration of the robots with human. Herd can be monitored efficiently by robotics compared to human. Besides that, robots and drones can map the whole agriculture land, identify new agriculture land or new

plant or animal species. In addition, usage of sensors to detect each new plant and usage of motion sensors in animal can be used to maximize the productivity of these products.

Furthermore, even agriculture industry can adopt to cloud computing. Cloud computing ensure that all data and program are stored and being accessed over the internet instead of papers or hard drive. When automation being integrated in farming, cloud computing is a path for a positive changes in agriculture as loss of data will not be faced. This will also allow data sharing across other farmers and breeders not only within country but also across country. The availability of cheaper and fastest technology allows cloud computing accessible by many. Additionally, the increase of the internet speed that allows files or data to be downloaded in lesser time is another advantage that cloud computing can provide.

Artificial intelligence (AI) has been advancing and transforming agriculture activities. AI will actively improve the process and strategies in agriculture. The GMO and GMF production may combine with AI to ensure that the preparation, breeding programme, predictive analytics and the automation process is performed efficiently.

Finally, the internet of things (IoT) is being adopt in many industries as well as in agriculture. This technology enables communication between facility, machines, and human. IoT is simple and will connect and integrate every device over the internet and allow everyone and everything to communicate together. By incorporating big data, robots, sensors,softwares and human, it has the potential to create smart farming that would control the entire farming process. For example, farming facilities that has been equipped with semi-automated control are able to decentralize the whole farming processes. Animal can be identified by radio frequency identification codes and workstation know where and what that animal is feeding. The IoT gained importance due to the availability of the internet, both wired and wireless. This is coupled with decline in cost and allow faster decision making and better productivity.

11.10. SYSTEM

Standard global agriculture system that is being applied till date is no longer sustainable as competition on natural resources and biodiversity are increasing due to changes in the climate and population growth. Hence, the need for food and the challenges to feed every single people are increasing as well. Therefore, transition for a sustainable farming systems and smarter

food system are crucial so that food derived from agriculture products can be produced from less land, water, and energy. In addition, the food produce from agriculture product must exhibit increased vitamin and nutrient content that can improve public health. Besides that, smarter system will help in reducing CO_2 emission by lesser tilling, reduction of farmers exposure towards chemical contacts by avoiding usage of pesticide, insecticide and herbicide and lesser food waste due to discoloration, bruises spot, accumulation of carcinogenic compound. Despite using more pesticides to control insects and pest, passive monitoring system on crops can be used to identify pests and fungus in an agricultural setting. By specifically targeting the fields or rows that are in risk, farmers can reduce costs and environmental impact associated with pesticides usage. A good system will also enhance customer satisfaction which in hand will increase farmer's income. Therefore, development and deploy of high-density planting to maximize yield potential, popularizing the system and the mechanism and harvesting is the way to move forward.

CHAPTER 12

GMO TO GMF DATABASE

CONTENTS

Genetically modified (GM) foods are foods that derived from organisms whose genetic material (DNA) has been modified in a way that does not occur naturally, but through the introduction of a gene from a similar/ different organism. Currently, available GM foods are mostly from plants, but in the future foods derived from GM microorganisms or GM animals are likely to be introduced in the market. Most existing genetically modified crops have been developed to improve yield, through the introduction of resistant to plant diseases or increased tolerance to herbicides. In the next decade, genetic modification could be aimed at altering the nutrient content of food, reducing its allergenic potential, or improving the efficiency of food production systems. All GM foods need to be assessed before being allowed on the market. FAO/WHO Codex guidelines exist for risk analysis of GM food.

12.1. ALFALFA

Genetically engineered alfalfa consists of a gene that makes it resistant to the herbicide Roundup. This allows farmers to use herbicide to kill the weeds without damaging the growing alfalfa. Cultivation of genetically engineered alfalfa was approved in 2011.

12.2. APPLE

According to the United States, significant increase of apple grown are thrown away due to browning and bruises. The waste occurs during post-harvesting. By using biotechnology, an enzyme that responsible for browning are silenced. Apple DNA that function to produce polyphenol oxidase (PPO), the enzyme that causes the flesh to turn brown are silenced. This approach has reduce food waste that occurs throughout the supply chain. The genetically modified apple will remain fresh for much longer than conventional apple. In addition, the modified apple will not brown or

rotten as a regular apple. Furthermore, a major advantage is that apples will no longer need to be washed in antioxidants solutions to restore their natural taste. Natural and nutritious content in GMO apple will remain.

12.3. BANANA

Banana can be found in various colors and sizes. Certain bananas can be eaten raw and some need to be cooked. They also vary in nutritional value. However, consumers will only notice one variety of banana in the supermarkets and the entire global commercial banana industry depends on sweet, seedless Cavendish. The Cavendish banana is existing 95% of all banana that sold commercially making it more convenient to eat. In these days commercial banana industry relies almost totally on the Cavendish because marketing only one variety/clones makes harvesting, packaging, and transporting cost-effective and delivers a uniform product to consumers. However, if something goes wrong, the whole batch will be spoiled. Main problem that is faced by banana industry is the infected disease caused by *Fusarium oxysporum*, *Mycosphaerella fijiensi,* etc. These diseases have emerged to threaten the world entire banana crop industry. To overcome the problem, mutation breeding using tissue culture to developed banana resistant to fungus is being produced. Thousands of plantlets is irradiated with doses of gamma rays to initiate a random mutation. Once mutation is done, screening through-out all the plantlet to identified plantlet that could lead disease resistant is further selected. An orange super banana is genetically engineered to produce beta-carotene.

12.4. BEAN

Beans are a type of crop that is liked by many people over the world. In certain country, it is the main vegetable source of protein and iron. Genetically modified (GM) bean resistant to the golden mosaic virus is produced to beat the worst infecting disease in beans. In the field trials, the GMO beans crop are not infected even with the presence of whitefly. Whitefly is a type of insect that transmits the mosaic virus. The transgenic bean presents economic and environmental advantages such as reduced waste, good harvest, and reduced agrochemicals applications. The transgenic seeds are multiplied through breeding processes. In addition, virus resistant bean is also produced through production of small RNA fragments which responsible for the activation of its defense mechanism against the golden mosaic virus. Through this technology, there won't be any formation of new protein in the plants and consequently no allergenicity and toxicity risks is appeared. Furthermore, the RNA fragments can cause resistant to several variations of the same virus.

12.5. BREAD

In bread, an enzyme that enhance the rising and strengthen the dough and prolong its freshness is made from genetically engineering approach.

12.6. BRINJAL

Bt Brinjal is an example of a GM crop. It was produced by insertion of crystal protein gene (Cry1Ac) taken from the soil bacterium *Bacillus thuringiensis* which is often known as Bt. Insertion of this gene allow brinjal to become resistant against fruit and shoot borer (FSB) disease. This biotechnologically produced brinjal is only sprayed twice through-out the

growth while in the conventional variety it was sprayed for at least 18 times. An increase production of more than six million hectares are achieved with Bt brinjal in certain countries and more than 100-fold are achieved globally. Bt Brinjal contributes to more sustainable crop production system and provide resilient responses to the challenges environment. These traits increase the production rate, insect and disease resistance, herbicide tolerance and at the same time increases food quality.

12.7. CANOLA

GM canola was approved in 1996, 2003 and 2006.

12.8. CASSAVA

Beta Carotene enriched cassava.

12.9. CHEESE

In cheese production, chymosin is used. This chymosin enzyme is genetically engineered.

12.10. CHICKPEA

Chickpea is the second largest cultivated grain food legume in the world. It was grown about 11.5 million hectares. Genomes of 90 chickpeas and its wild-type variety have been sequenced and this data was used to improved grains, high yield and quality, greater drought tolerance and disease resistant. About 28 269 genes of chickpea was identified and from there millions of genetic markers and low diversity genome regions were detected. Through this approach, by using good genes and incorporating it with wild genes in breeding, development of superior varieties was generated, and it has contributed more income, better nutrition and utilize dryland efficiently.

12.11. CITRUS FRUITS

In citrus fruit, bacteria cause citrus canker disease. It includes brown spots on leave and its fruit. The spots are usually surrounded by a yellow halo and can be seen on both side of the leaf. GM citrus able to overcome this disease.

12.12. COCOA

In cocoa, crown gall disease is a very popular disease and it is caused by bacteria. The symptoms appear as roundish rough-surfaced galls at or near the soil line or on roots and lower stems. The galls are first appears as creamed colored or greenish and basically, it will later turn brown or black. GM cocoa is basically will become resistant to this disease.

12.13. CORN/MAIZE

GM varieties have definite advantages over conventional corn. GM corn has enhanced grain quality and reduction of human exposure to mycotoxins which are produced by fungus that can cause disease and death in humans and animals. Genetically engineered corn had 5.6 to 24.5% higher yield than similar nonengineered varieties and biomass decomposition was found to be higher in GMO variety. GMO variety corn also capable to be grown within minimal area land with high yield production. Besides that, a new zinc-enriched corn variety was also being released. This type of corn is very important to be consumed by human population who is affected by zinc deficiency. By eating this fortified corn, impaired growth and development, respiratory infections, diarrheal disease and general weakening of immune system due to zinc deficiency can be overcome. BIO-MSN01 contains 36%

more zinc on average than other corn, people who feed this corn will obtained 5 time more zinc than traditional corn varieties. Additionally, BIO-MSN01 also can produce high yield, tolerant to several corn disease such as rust, turcicum leaf blight, and grey leaf spot and can be grown between 0 to 1400 m above the sea level. Besides enriching in zinc, corn is also developed to be enriched in provitamin A which eventually helps in preventing blindness in children who consume the corn. Corn are also genetically modified to produce an insecticide known as BT protein that kills certain insect pests. The gene added to corn from the soil bacterium *Bacillus thuringiensis* (Bt) which known to possess an insecticidal effect. The transformed corn will be able to withstand stem borers which known to reduce corn production by an average of 13%. In 2003, herbicide-tolerant increase yield as well as income for most of the farmers as lesser insecticides investment was required. Furthermore, modify corn that resistant to the herbicide glyphosate is also important so that it can survive upon herbicide application that use to kill weeds. Cornflakes derived from genetically modified corn are considered as genetically modified food.

12.14. CORDYCEPS

Cordyceps militaris has been used in traditional medicines due to its broad pharmacological components such as Cordycepin, Adenosine, Mannitol, Cordy polysaccarid, Superoxide, and Dismutse. It may help treat heart and kidney disease and some diseases caused by viruses. The fungus is also known to lessen the diabetes, sexual dysfunction, function as antioxidant and anticancer. Through usage of technologies, the fungus was successfully breed to produce more with lesser price.

12.15. COWPEA

Cowpea is an important crop as it provides food and cash for farmers and fodder for livestock. Two varieties IT99K-573–2-1 and IT98K-205–8 are improved varieties that mature in 60 days, high yield, can be grown in the drier regions and are also resistant to Striga. In addition, it also contains 20% protein that provides a good option to tackle malnutrition in certain countries. These physical quality characteristic of the varieties such as color and bigger size were appealing and liked by consumers.

12.16. CROOKNECK SQUASH

Crookneck Squash is a cultivar of *Cucurbita pepo*. It is a bushy type of plant and its skin is yellow in color. It has a yellowish color and a distinctive curved stem-end or also known as crooked neck. It is harvested immature. Its quality degrades as the squash reaches full maturity. The yellow crookneck and straight-neck yellow squash are genetically engineered to protect the squash against viruses.

12.17. FLAX

Flax is consumed both by human and animal. It is a type of food and fiber crop cultivated in cooler regions. Flax are genetically modified for Sulfonylurea herbicide tolerance purposely for plantation related concern. It allows the synthesis of essential amino acids in the presence of sulfonylurea herbicides. It is genetically engineered to be antibiotic resistant and nopaline synthesis for research purposes.

12.18. MELON

A GM Melon was engineered for delaying the senescence and ripening. It is produced for commercial purposes. In addition, there is engineered melon that is antibiotic resistant that is developed for research purposes.

12.19. OIL PALM

In Malaysia the main focused on oil palm trait is to commence high yielding oil palms that allow more production of oil and to be Ganoderma tolerance. Oil palm selected using molecular breeding emphasis on desirable agronomic trait such as height increment, oil yield components, compactness, and drought tolerance will deliver higher yield without increasing the hectarage of the field located in the coastal and inland area. The genetic information of the oil palm genome of about 1.8 billion chemical units that make up the genetic code of the oil palm was sequenced, assembled and annotated. This knowledge was applied to local oil palm populations along with genetic testing and the best seedlings were planted to identify the parental palms for seed production. About 80 000 seedlings were analyzed and selected palms showed potential to produce at least 15% more oil. Under good growth conditions, the potential yield from the Genome Select palms can go above 11MT oil/ha across all environments.

12.20. POTATO

Potatoes are an important agricultural product and their susceptibility to blight disease highlights the challenges in producing potatoes in a sustainable way with minimizing the effects on the environment. Breeding from the wild relatives are laborious and slow and by the time a gene is successfully introduced into a cultivated variety, the infected potato plant will become detrimental. With new insight, GM technology was utilized to tip the evolutionary balance in favor of potatoes against the late blight. Through this, an alternative approach besides using chemicals were found. Hence, approach spraying a potato crop for 10–15 times or up to 25 times was reduced. Potato that is genetically modified to resist bruising and to produce less chemical that can cause cancer are developed. Potatoes DNA was altered to produce less acrylamide that is suspected to be carcinogenic to human and potato with high level of folate to ensure reduction of normal fatal neurological was developed.

12.21. PAPAYA

In papaya, Anthracnose is a type of diseases that caused by fungi. Usually, it appears small and in irregular yellow, brown or black spots. The color of the infected part darkens as it ages. The disease can also produce cankers petiole and no stems formation that cause severe defoliation. In addition, there are also delayed ripening problem. The virus resistant papaya variety that can able to produce more papaya than the normal variety was developed. Papaya with ringspot virus resistant was developed through genetic engineering

to enhance the productivity. The reason of this innovation was due to the problem faced by the Hawaii papaya industry in the early 1900.

12.22. PINEAPPLE

In pineapple, 3 to 4-month-old crown are most susceptible to Phytophthora heart rot disease. Once infected with fungi the color of the heartleaves will change to yellow or light coppery brown. Further infection will lead to wilting and deterioration of the heartleaves. GM pineapple will become resistant to this diease.

12.23. PEARL MILLET

Pearl millet bred contains large amounts of zinc and iron. Bread derived from enhanced pearl millet with folate, is very important nutrient for fatal neurological development.

12.24. PLUM

The GM plum, called c5, is genetically altered to resist the mutation of the Plum Pox Virus among stone fruit trees. This virus is the most serious virus causing disease of stone fruit, with the potential to devastate stone fruit production.

12.25. RICE

Current global population dependent on rice as a source of carbohydrate. It is important staple food for the people who live in tropic region. The

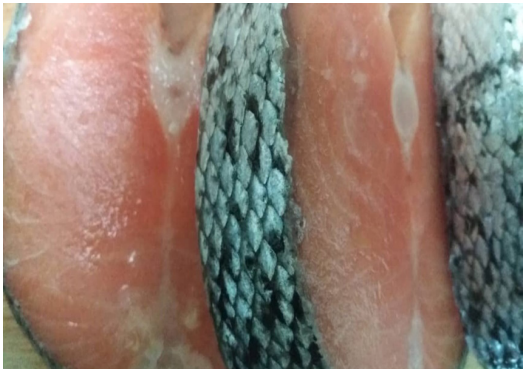
common disease that infects rice is sheath blight disease which cause by fungi. Sheath blight disease is similar to stem rot disease. Initial lesions symptoms usually can be noticed on the sheaths of lower leave that near to the water line. This disease can be found during late tilling or early internode elongation stage. Swarna-Sub 1 is a flood-tolerant rice variety which produce by inserting flood tolerance gene. The seeds belong to this variety capable to produce 140 000 tons of seed covering over millions of hectares. UKMRC-2 and UKMRC-8, a new variety of high-quality white rice that is durable was produce through an innovation process involving cross-breeding of wild rice species (Oryza rufifpogon) and local rice species (Oryza sativa). This breeding was done with control pollination. They believe that 20% gene can be increased in the local varieties through breeding with wild rice species, as wild rice varieties have potential in enhancing features such as resistance to flooding, disease, pest infestation and increase yielding. UKMRC-2 was found to produce 12 tonnes of white rice per hectare and resistant to disease like karah leaf. The UKMRC-2 seeds can survived well even though submerged in flood for about two to three weeks, it produces quality long rice with 3.5 mm. In addition, UKMRC-8 able to give yield about 14 tonnes of 3.68 mm white rice per hectare with low crushed rice. It can also survive well even after being submerged in flood. Both UKMRC-2 and 8 has low starch, easy to be planted, durable and resistant to disease. Another variety that tolerant to flood contain SUB1 gene. It capable to recovered yield up to 4.5 tons of the global average rice yield per hectare. The popular variety known as Golden Rice is a biofortified rice (GR2E) which increased with provitamin A. This type of rice will contribute to reduce vitamin A deficiency in human population. A single plate that serve Golden Rice could provide half the required daily intake of pro-vitamin A for a 1 to 3-year-old child. Iron content is normally found in the endosperm tissues that make up the white rice. Rice that enhance with iron are made through modern breeding rather than conventional breeding. It is because in conventional breeding it is hard to identified wild-type rice that contain high amount of iron. In rice, there are a compound known as nicotianamine that help to take up iron from the soil. This compound is only switched on during an event where the content of iron is lesser in the soil. Through genetic engineering, nicotianamine is switched on completedly so that iron level is boosted in the rice grain. Another notable example is rice with increased folate and zinc content. This rice clearly has a health benefit for consumer as well as the farmers. There are around 44 varieties of newly improved rice developed by various countries such as Philippines, Bangladesh, Myanmar, Nigeria,

Tanzania, Cambodia, India, Vietnam, Indonesia, Mozambique, and Rwanda. However, International Rice Research Institute (IRRI) has released about thousands of improved rice varieties across 78 countries. All this varieties are salt tolerant variety, flood tolerant variety, and nutritional improved variety

12.26. SALAD

Developing high quality, nutritious and sustainable salad is important. Smaller, tougher leaves with lots of small cells packed closely showed a lasting condition. Through breeding program, salad plant that possess the favorable genetic material are selectively breed by using lesser water during the growth. This approach produces longer shelf life with reduce water consumption.

12.27. SALMON



Salmon are being fished approximately 1.5 million tonnes from Atlantic. While in UK, salmon farming contributes around half billion pounds for their economy. Therefore, the development to improve the quality of the stock and its resistance to disease are crucial for salmon breeders. Scientist

has researched new technology that will help in selecting the best fish for breeding. A chip that loaded with hundreds of DNA pieces single nucleotide polymorphisms (SNPs) of salmon genetic code will allow breeders to detect fish with the best genes. It capable to detect variations in the genetic code of each individual fish. These variations make it possible to identify genes that are linked to desirable physical traits such as growth and resistance to diseases. By taking a small amount of DNA from the salmon fin, salmon breeders could carry out test to identify the best salmon with the best characteristics to be breed. In another hand, since the content of omega-3 fatty acids lesser in most salmon, increasing the content through its feed will increase the total content of the omega-3 fatty acids. For that, genetically modified *Camelina sativa* with algae gene that produce more oil which rich in omega-3-fatty acid are usually used to feed salmon. This will together increase the health benefits of the consumer.

12.28. SOY

Soy is the most tremendously genetically modified food in the world. Genetically modified soy was first approved in 2010. It is modified to have a high level of oleic acid, which is naturally found in olive oil. Oleic acid is a monounsaturated omega-9 fatty acid that lower LDL cholesterol a type of "bad" cholesterol present in blood. Soy also being modified to be resistant to the glyphosate herbicide which is used to kill weeds.

12.29. SUGAR BEET

Sugar beet are often used for sugar production. It is an important part of crop rotation cycle. However, sugar beet plants are susceptible to Rhizomania which turns the bulbous taproot into many small roots, making the crop difficult and costly to be processable into a product. It is also susceptible to the beet leaf curl virus, which causes crinkling and stunting of the leaves. Therefore, sugar beets were engineered for glyphosate herbicide resistant. Genetically modified sugar beet is officially deregulated in 2012.

12.30. SUGARCANE

Drought tolerance sugarcane had become popular for planting as a food crop among many farmers. These sugarcanes can contribute to resilience and sustainability in facing climate changes.

12.31. SWEET PEPPER

The coat protein (CP) gene was transformed into sweet pepper. Safety assessments of genetically modified (GM) plants showed no genotoxicity either in vitro or in vivo. In addition, no abnormality was detected in animal feeding studies. These demonstrate that the CMV-resistant sweet pepper is comparable to the non-GM in terms of food safety.

12.32. TOMATO

The best-flavored tomato is derived from essential nutrients like amino acids and omega-3 fats. These nutrient-indicate flavor compounds are not depending on the sugar content to make a tomato sweet. Therefore, the best-flavored tomato that is hard to cultivate *Maglia Rosa* is crossed breed with

FLA. 8059 which has good shelf life and disease resistant. The progeny plant tasted good and grew very well.

12.33. TROUT

Trout infected with bacterial cold-water disease which caused by *Flavobacterium psychrophilum*. This pathogen caused death, affect the growth and yield of trout. However, disease resistant trout showed a higher survival of disease resistant and allow smaller number of trout harboring the pathogen in the trout tissues compared to the non-resistant trout.

12.34. VEGETABLE OIL

Vegetable oil derived from the seed is used mainly for human consumption. Small improvement within a seed is made to convert important sugars into oil. This is done by stacking and appropriate combination of genes that can produce an additive effect on seed oil content and oil yield.

12.35. WHEAT

A gene controlling shape and size of spikelet's in wheat was isolated. This will help breeders to deliver higher yield. The genetic mechanism that control the shape of spikelet's also function for the floral architecture development. This approach have improved almost 1.6% yield increase that is need for growing world population. There are also wheat varieties that resistant to the rust disease. Zinc-enriched wheat will also help in preventing blindness in children.

12.36. WINE

Mostly wine is a type of fermented product. It is being produced through genetically engineered yeast.

12.37. WATERMELON

Normally, each seed contains a complete set of DNAs to make a new plant. A new watermelon plant is produced when one watermelon flower is fertilized by the pollen from a different watermelon plant. This watermelon plant will contain new seeds to produce a new plant. However, to produce seedless plant, each produced seed will be exposed to a certain chemical. This chemical will give twice as much DNA than a normal seed. These seeds will grow into a watermelon vines and if it is fertilized by a normal pollen the new seeds will grow into unusual vine because it has 1 and 1/2 set of DNAs. Therefore, in this watermelon progeny normal seed will not being formed fully even if it is formed the seeds will look like white seeds that is unfertilized and cannot being used to produce new plant.

12.38. ZUCCHINI

Zucchini is known as a summer squash. It has been genetically modified to protect the squash against viruses. In zucchini, two envelope proteins are present that protect the vegetable from virus attacks. Research is also being done to create genetically modified zucchinis which are resistant to fungus and fungal infections however it is yet to be marketed.

CHAPTER 13

COUNTRIES

CONTENTS

Food often has societal and historical connections and, sometimes they, may have religious importance. Depending on the countries, people often have different attitudes towards GMO food. Modification of food and food production may evoke a negative responses among consumers, especially if there is an absence of risk communication, risk assessment efforts and cost/benefit evaluations. Let see how each country are engaging with GMO/GMF.

13.1. AUSTRALIA

Started to engage by creating heat resistant variations in crops.

13.2. BANGLADESH

Bangladesh started to grew biotech crops. They are the first in the region to grow GM crops. They approved Bt brinjal/eggplant in October 2013.

13.3. CANADA

The government of Canada has approved the sale of Provitamin A biofortified Rice GR2E (Golden Rice) in their country. Health Canada is responsible for assessing their people`s health and safety of their food products including GM food. The Canadian Food Inspection Agency (CFIA) is responsible for the environmental assessment, import permission and registration of different GM food products including plants, animal feeds and animal feed ingredients. Under the Novel Foods Regulations, pre-market assessment is required before genetically modified (GM) food can be sold in Canada.

13.4. CHINA

China has established a safety supervision system that covers the complete chain of GM products, including research, production, and trading. They also allow GMO material such as GMO wheat from Australia to be tested in China. The Ministry of Agriculture and the Ministry of Health are the authorities that responsible for GM food regulation. China was the first country to commercialize a transgenic crop in the early 1900s with the introduction of virus resistant tobacco. They also participate to grow biotech crops such as Bt cotton. Starting from April 2004, GM crops and products intended for food use are required to go through safety assessment before they

can be imported into Mainland of China. China also have been particularly active in utilizing new gene editing technology to modify animals.

13.5. COLOMBIA

In Colombia, many of their people affected by zinc deficiency. Therefore, they allow fortified maize with more zinc to be planted in their country.

13.6. CZECH REPUBLIC

The country follows a scientific approach towards agribiotech. Bt corn is currently planted in their country. They have also revised legislation for the farmer who planting GMO plant.

13.7. FRANCE

Majority people opposed to GM products while the livestock industry using GM soybean as their livestock feed. GMO status in France are only at laboratory stage.

13.8. INDIA

India has emphasized the important of genetically engineered crops in bridging the demand and supply gap for food need in the future. The government has approved field trials of many transgenic. They also showed interest on rice variety that can stand flooding. Besides that, Indian government allows field trials for GM mustard and brinjal. In addition, they also concentrating in growing GMO, India also looking into improving the biosafety, risk assessment, and management of the GMO crops. This proved that India has help its farmers to face the threat of climates.

13.9. INDONESIA

In Indonesia, GM sugarcane and corn will be commercialized soon.

13.10. KENYA

Kenya has the requisite capacity for GM crop research and regulation. Kenya has the technology to produce adequate food for their people through GMO. They have approved GMO maize that able to stand stem borers.

13.11. MALAYSIA

In Malaysia, so far, no GM crop being approved however many research on GM are actively being carry out. In 2013, research on GM papaya was allowed and currently, it is at the confined trials. Malaysia also agree that research on GMO and ethics should go hand in hand. Hence bioethics should be an integral part in GM to ensure individual rights are not violated and health, environment, and biodiversity are preserved.

13.12. MOZAMBIQUE

Started to carry out research on GM crops at the end of year 2014.

13.13. MYANMAR

Myanmar recognized the widespread cultivation of Bt cotton variety. This has double the cotton production and increase country income.

13.14. NETHERLANDS

In Netherlands stringent regulations and threats from biotech prevent GM crop trials and commercial cultivation in the country. Their government also use pragmatic approach towards GE imports products.

13.15. NEW DELHI

They have been given access to produce climate-smart rice varieties which can tolerant to flood.

13.16. NEW ZEALAND

The Food Standards Australia New Zealand (FSANZ) is responsible for conducting safety assessment on GM food in New Zealand. Starting from May 1999, all GM foods are required to go through a safety assessment with FSANZ before they are allowed to sell it in the market.

13.17. PHILIPPINES

Already develop a GMO rice and papaya. In 2009, the Philippines produced tons of milk fish through better feeding and rearing methods. Besides that, they also grows biotech corn that resistant to insect and herbicide since 2003.

13.18. SERBIA

Ban import, production or commercial growing GM crops.

13.19. SINGAPORE

Singapore have no barriers on GM products, they even have revised the Biosafety Guidelines Research in 2013.

13.20. SPAIN

Completely involve in GM crops planting and imports.

13.21. THAILAND

They are the world top rice producer who produce varieties of rice.

13.22. UNITES STATES

In 2015, the first genome edited crop, a herbicide-resistant rape-seed was planted on the fields. The GMO crops continues to led the production and the annual increase for the past five years. United States government requested producers and developers of GM food to consult with the Food and Drug Administration (FDA) before placing GMO/GMF in the market. So far, all GM food produced in the US has complete their consultation with FDA. In 1994, the transgenic Flavor Saver tomato was approved by FDA. The modification allowed the tomato to delay ripening after picking. The U.S. Food and Drug Administration (FDA) in the United responsible for ensuring safety and wholesomeness of food (except meat and poultry, which are regulated by the U.S. Department of Agriculture (USDA)), including GM food, in the United States. On the other hand, the Environmental Protection Agency (EPA) would have the responsibility for food safety when the GM plant that produce the food derived from products of any pesticidal substances. If the GM plant would be grown in the open environment, it must be further assessed by USDA and/or EPA for any environmental implications. In 1992, FDA issued its policy statement regarding the regulation of GM food. In 1994, FDA concluded that the first GM food, the Flavr Savr™ tomato, was as safe as other commercial varieties of tomato after a thorough safety and nutritional evaluation. Following the decision, all GM food produce in the US has consulted with the FDA before commercialization.

CHAPTER 14

GENETICALLY MODIFIED ORGANISM/FOOD COMPANIES

CONTENTS

14.1. AGRICULTURAL BIOTECH RESEARCH INSTITUTE OF IRAN

Agricultural Biotech Research Institute of Iran (ABRII) was established in 1999. It was aimed to utilize and develop novel agricultural technologies to solve agricultural problems. This establishment had contributed to the country's food security, enhancing food safety, protecting its natural resources and ecosystem, producing knowledge and wealth, and promoting national self-sufficiency in their agricultural products. Their experts ensured that research has a measurable impact on the various sections of agricultural biotechnology. Basically, ABRII focused on genetic engineering and biosafety, tissue culture and gene transformation, system biology, microbial technology, molecular physiology, and nanotechnology. ABRII has gained a reputation as one of the most prominent and effective research institutes both within the country and more widely in the region. To date, ABRII has many branches at West and Northwest Region at Tabriz, Central-Region Branch at Isfahan, Northern-Region Branch at Rasht and Northeast & East Region Branch at Mashhad.

14.2. AGRITOPE INC.

Agritope Inc. is an oregano-based agricultural biotechnology company that specialized in fruit and vegetable crops. They also offer grapevine plant propagation and disease screening and elimination program. The company is utilizing its patented ethylene control technology to develop a wide variety of fruits and vegetables that are resistant to the decaying effects of ethylene and novel variety of flowers. Agritope also conducts research, development and commercialization program in plant functional genomics. Agritope developed new plant varieties containing increased levels of naturally phytochemicals. Agritopels MetaGene^{TM} Metabolic Genomics Technology facilitates the identification of specific genes that regulate the levels of phytochemicals such as carotenoids, lycopene, flavonoids, isoflavones, vitamins, folic acid and various elements and minerals.

14.3. BASF

BASF creates chemistry for a sustainable future. Their portfolio ranges from chemicals, products performance and crop protection. They combine economic success with environmental protection and social responsibility.

Through research and innovation, BASF support in nearly every industry in meeting the current and future needs of society. To develop solutions for the affected farmers, BASF team will start a research project with the following features: offering broad protection on numerous crops, long-term effect, and environmental compatibility. In Malaysia, BASF has been operating for more than 25 years. BASF (Malaysia) Sdn. Bhd. was incorporated in 1989. They offer innovative solutions for agriculture industry.

14.4. MS TECHNOLOGIES LLC

MS Technologies™ is dedicated to leading the way in traits development, technology, and soybean genetic innovations to improve the future of farming. Their portfolio offers a variety of herbicide-tolerant and insect-resistant traits, including wide access to additional traits and technologies through collaborative agreements with other parties. Growers can rely on MS Technologies™ to lead the way for farming trends and technologies change and because MS Technologies™ keeps the principles of farming a priority, the expectations for higher yields can be assured. Through collaborations with other parties, they have been leveraged to develop new technologies with the support of growers as they enter the next era of farming. They strive to cultivate traits that help to achieve higher yields while maintaining their commitment to the principles of farming.

14.5. BAYER CROPSCIENCE LTD

Bayer is a Life Science company with more than 150-year history and their core competencies are in agriculture sector. The growing and aging of world population requires an adequate supply of food and improved medical care. With their innovative products, they contribute in finding solutions to some of the major challenges. With life expectancy continuing to rise, Bayer able to improve quality of life for growing population by focusing their research and development activities on preventing, alleviating and treating diseases in agriculture. They are also making an important contribution to provides a reliable supply of high-quality food, feed, and plant-based raw materials. This is in line with their objective to operate sustainably and addressing social and ethical responsibilities. To lead the way in innovation and to develop holistic solutions, Bayer aim to build up on their expertise in the integration of seed technology with chemical and biological crop protection. In doing so, they support farmers with improved and innovative solutions tailored to specific local requirements. Innovative technologies are increasingly being

applied in research and development to enhance their portfolio in bringing better agriculture. Some examples include new breeding technologies to improve yields or computational life sciences for the collection, processing, and analysis of extensive research and development data as the basis for faster and more focused development.

14.6. SYNGENTA

In these days, most of us are facing problem on how to feed the rising population sustainably. Syngenta helps humanity to face their challenges in easier way. Their world-class science and innovative crop solutions transform cropping methods to enable millions of growers to make better use of available resources. They believe in Good Growth Plan they align their corporate governance with international standards and practice to help people in achieving their objective and create value to society by their strong environment and social performance. They channel their finding through usage of sustainable natural resources, healthy ecosystems and by thriving rural communities. In addition, they manage to solve most of the problem through the eyes of growers and by applying world-class science they develop crop protection products and high-quality seeds. Their innovation has helped farmers to overcome problems with weeds, insect, disease and climate change. Therefore, farmers trusted Syngenta to produce healthy, premium crops and minimize the use of precious natural resources.

14.7. BEJO ZADEN BV (NETHERLANDS)

Bejo Zaden is a leading company in breeding and production of vegetable varieties and seeds for present and future. This is because they believe the best vegetable seeds will promises a healthy and flavorful harvest. They have expanded their invention towards organic farming that shaped by interaction between growers, dealers, and supply chain partners. They build long-term relationships with customers rather than going for short term relationship. Bejo Zaden are always in close communication with growers, exchanging views and listening to their concerns every day. By keeping ear to the ground, they can provide better support and advice real and lasting crop results in real time. Therefore, Bejo Zaden has invested considerably in research by applying modern breeding techniques and new technologies that goes right down to the DNA level. Bejo Zaden, ensure that their approach will protects natural resources and ensure growers harvest their product from healthy and fertile soil.

14.8. CENTRAL INSTITUTE FOR COTTON RESEARCH

Central Institute for Cotton Research (CICR) is one of the world's largest cotton gene banks with an array of 11,345 accessions of all the four cultivated species. CICR is the only country in the world that cultivates all the four species

- *G. ossypium hirsutum*;
- *G. barbadense*;
- G. *arboretum;* and
- G. *herbaceum*.

The gene bank has a wide range of economically important quality traits that can be sourced and pyramided into cultivars with resistance, cultivars that can stand biotic and abiotic stresses and cultivars that are suitable for specific agro-eco zones in the country. The gene bank provides a powerful opportunity to combat the challenges posed by climate change uncertainties. The recent techniques of fiber evaluation in the field provide new opportunities for plant breeders. The National Centre for Cotton Genetic Resources allow CICR to maintained 11,345 accessions of the four cultivated species of *Gossypium* (*G. hirsutum* – 8265, *G. barbadense* – 305, *G.arboreum* – 1936, *G. herbaceum* – 565) and 26 wild species, 193 perennials, 6 races of *G.arboreum* and 7 races of *G. hirsutum*, 1 race of *G. barbadense*, 1 race of *G. herbaceum* and 32 derivatives are maintained in their species garden. Germplasm lines were screened and number of lines with good agronomic traits, superior fiber quality and resistance to pests and diseases were identified and distributed to the end users.

14.9. RUSSIAN ACADEMY OF SCIENCES

The Academy represents Israel in nearly all the scientific unions of the International Council for Science (ICSU), and it is a founding member of All European Academies, the Association of Academies and Societies of Sciences in Asia (AASSA), the Global Network of Science Academies (IAP) and the Inter-Academy Council. The establishment of Israeli academic centers in Egypt and China is among the major multi- and bi-national initiatives in which the Academy has played a leading role. They are also actively involve with natural science research.

14.10. CENTRO DE TECNOLOGIA CANAVIEIRA (CTC)

CTC has the most complete sugarcane germplasm bank. They carry out breeding program for region that producing sugar cane. Rigorous inoculation tests are carried out in order to select only the varieties that known to be resistant to the main diseases. They also employ advanced biotechnology by analyzing the DNA of the varieties. Modern biotechnology is also present in the genetic transformation system. CTC already produced insect resistant varieties.

14.11. CHINESE ACADEMY OF AGRICULTURAL SCIENCES

The Chinese Academy of Agricultural Sciences (CAAS) was established in 1957 headquartered in Beijing. It is a national, integrative agricultural scientific research organization that responsible for carrying out both basic and applied research, as well as focusing research into new technologies that will impact agriculture. This will overcome a broad range of challenges in agriculture. Significant of CAAS is to promote sustainable agriculture within and outside China. CAAS also extending its reach through technology exchange and cooperative research agreements with agricultural research institutions/universities domestically and internationally as well as with global non-governmental organizations.

Research and policy work at the academy cover a broad range and that involve with agriculture such as crop science, horticulture science, agriculture resources and environment, agriculture mechanization and engineering, agro-product quality, safety and processing, and agriculture information. They align their research closely with farmers, and community. So far, the academy has generated many scientific and technological advances such as new varieties of crops such as super rice, dwarf sterile wheat, insect resistant cotton, disease resistant wheat, high oil soybeans and transgenic phytase corn, livestock, and poultry. CAAS also has one long-term and 10 mid-term national gene banks for the storage of crop germplasm resources, together with 12 national crop germplasm nurseries. Overall, more than 420,000 accessions are conserved. As CAAS plays a greater role in the global scientific family, it is seeking to establish further international collaboration and large-scale cooperation in agricultural research to accelerate the pace

of innovation and make significant contributions to eliminating poverty and hunger around the world.

14.12. COTTON AND SERICULTURE DEPARTMENT (MYANMAR)

Myanmar Cotton and Sericulture Enterprise (MCSE) was established on April 1994 to strengthen the cotton sector. All the responsibilities of cotton cultivation, research, supply of chemicals, procurement of seed cotton, ginning, supply of cotton lint and by-products to state-owned cotton industries and cotton export were taken care by MCSE. The function of MCSE is to provide adequate raw material for government textile industry and to promote living standards of farmers by increasing income from cotton cultivation. They also aimed to set up allied industries of cotton by-products within the country.

14.13. DOW AGROSCIENCES, LLC

Dow AgroSciences LLC began in the 1950s as an agricultural unit. From the beginning, they have set up to discover and develop innovative solutions that can improve the way the world farms should be. They provide a variety of products and services to meet the needs of their customers. Their research with strategic partners is making a breakthrough and sustainable solutions to the industry through producing innovative hybrids and seeds varieties, crop-enhancing traits, crop protection products, advance pasture and land management, production of turf and ornamental plant and healthy oils. Dow AgroSciences has made a sustainable advancement in food production, variety, and quality. Dow AgroSciences also responsible to boost food production to cater the demand on global population food intake in limited natural resources.

14.14. DUPONT (PIONEER HI-BRED INTERNATIONAL INC.)

DowDuPont is a holding company comprises of The Dow Chemical Company and DuPont with an intention to form strong, independent, publicly traded agriculture company that will lead their respective industries through productive, science-based innovation to meet the needs of people and help to solve global challenges. DowDuPont brings together the complementary

portfolios of Dow and DuPont, two innovative, science-based companies that hold leadership positions in the agriculture. Upon completion of the intended separations, the future agriculture products companies will remain deeply committed to market-driven research and development, backed by world-class engineering capabilities that is honed to deliver differentiated products and solutions. Each of them will be committed to uphold sustainability, to use science and innovation to tackle world challenges and to maintain a safety culture. The Agriculture Division offer a complete portfolio of products and technologies, and a robust pipeline of germplasm, traits and crop protection. The combined heritage and complementary capabilities of DuPont Crop Protection, Pioneer and Dow AgroSciences will make the future intended agriculture company stronger, more competitive, and better equipped to deliver growth and value. Their agriculture innovations will help to shape the industry to respond towards important global challenges. Upon completion of the intended separations, the intended agriculture company will remain committed towards sustainable agriculture production, and intends to bring a broader suite of products, technologies, and services to the market faster. In this way, they can serve consumers better and meet their expectations with innovation and helping them to increase productivity and profitability.

14.15. EMBRAPA (BRAZIL)

The Brazilian Agricultural Research Corporation (EMBRAPA) is a state-owned research corporation affiliated with the Brazilian Ministry of Agriculture. It was established in 1973. It has been devoted to developed sustainable technologies, knowledge and technical-scientific information for Brazilian agriculture, including livestock. They have incorporated a wide area of formerly degraded *Cerrado* land into a production system. The region now accounts for nearly 50% of grain production. They have quadrupled the beef and pork supply and increased the chicken supply to 22-fold. These are some of the achievements that took Brazil from a basic food importer to one of the world's largest food producers and exporters.

14.16. FLORIGENE PTY LTD. (AUSTRALIA)

Florigene is a biotechnology company that based in Melbourne, Australia. Their main focuses are to develop novel color expressions in a range of commercial plants through genetic modification. They had isolated the gene responsible for the expression of the color blue in petunias. Florigene has developed naturally long-life and disease resistant carnations, new

morphologies of gerberas and natural color modifications of the three main cut flowers such as roses, carnations, and chrysanthemums.

14.17. FUTURAGENE GROUP

In a world that is challenged by growing population, diminishing resources and climate change, FuturaGene's technologies has addresses critical challenges of plant yield enhancement and yield protection. They harness biotechnology to ensure the security and sustainability of fiber and feed. They believe that the key to sustainable agriculture lies in continuous improvement and protection of yield and processability of crops, thus maximizing output, whilst minimizing all inputs such as land, water, and fertilizer. FuturaGene will continue its worldwide biotech activities with enhanced resources that drive its mission to be a world leader in plant genetic research and development for global biofuel, forestry and agricultural markets.

14.18. GENECTIVE S.A.

Genective is a joint venture between Group LIMAGRAIN and Group KWS that aim on the development, regulatory approval, and commercialization of GM corn. They share the vision that genetically modified organisms (GMOs) constitute an important means to face future challenges in agriculture. VILMORIN and KWS has been successfully collaborating in developing and marketing corn hybrid seeds in the US for years and thus decided to also combine their efforts in developing GM traits for corn.

14.19. INTERNATIONAL RICE RESEARCH INSTITUTE

The International Rice Research Institute (IRRI) is a dynamic and rapidly evolving organization that focus on solving complex global problems through vibrant, rice-based agri-food systems. IRRI's mission and purpose is to improve the quality of life of those who depend on rice sector. Their target ranging from the ultra-poor to those moving up the development ladder with environmental sustainable rice production systems. As the world faces multiple complex challenges such as continue population growth, scarcity of land, water, and labor limitation, rapid urbanization and climate change, technology advances and continuous evolution of the public and private sector role can be solved by simple solutions. IRRI has an opportunity to

make a profound difference over the next few critical decades based on their differentiating capacities at the intersection of research and development.

14.20. J.R. SIMPLOT CO.

J. R. Simplot Co. has been remaining their core values on respect for resources, spirit of innovation and passion for people. Simplot is a far-reaching organization in seed production, farming, animal nutrition, ranching, fertilizer manufacturing, frozen food processing, food brands, and distribution through sustainable practices. This includes leading innovations in genetics to grow healthy foods and reduce waste, constructing state-of-the-art processing facilities that reduce energy consumption and waste. They used genetic technology to suppress potato genes to create Innate™ potatoes which is a new, naturally-grown potatoes that will help growers and retailers reduce waste. Innate potatoes are created with a biotechnology process that results in improved, more sustainable crops. They're less susceptible to bruising caused by impact and pressure during harvest, enabling growers and packers to utilize a higher number of potatoes and experience less waste and rejection. These potatoes have lower levels of asparagine and sugars, providing alternative choices for consumers.

14.21. JK AGRI GENETICS LTD (INDIA)

JK Agri Genetics Ltd.(JKAL), is a leading seed company established in 1989 with its headquarters at Hyderabad, Andhra Pradesh (India). JKAL is one of the pioneers in the Indian seed industry that committed to serve farming community. JKAL is engaged in research and development, production, processing and marketing of rice, cotton, maize, pearl millet, sorghum, sunflower, castor, mustard, wheat, sorghum, fodder beet, tomato, okra, chillies, hot pepper and other vegetable seeds. All their breeding projects are long-term projects with regular flow of superior products in every mandate crop. They focus on application of biotechnology for trait introgression, molecular tools such as marker assisted breeding, fast development of inbred lines by using double haploid and development of climate-resilient genotypes. They give more emphasis on development and identification of full maturity hybrids crops, high oil content in mustard and big boll size of cotton, create more resistant hybrid towards disease, strengthening germplasm collection in all mandate crops, maintenance breeding, and seeds production.

14.22. MAHARASHTRA HYBRID SEED COMPANY (MAHYCO)

Maharashtra Hybrid Seeds Co. (Mahyco) is an agriculture company based in India. It is one of the country's major seeds producer. The company produces seeds for cotton, wheat, rice, sorghum, pearl millet, maize, oilseeds and vegetables crops. Mahyco has 21 notified research varieties and production of 115 products across 30 crop species. Mahyco has six research centers in India focusing on molecular breeding, applied genomics, crop transformation, plant virus interaction, molecular microbiology, abiotic stress tolerance and molecular entomology. Mahyco has been playing a leadership role in delivering biotechnology crops to the Indian farmer. MAHYCO believe plant biotechnology applications in the field will deliver significant economic benefits to the farmer and can play an important role in improving human health. Better crop pest and disease tolerance, fewer applications of pesticides, better nutritive value and higher yield are some of the benefits that current and future biotechnology crops can/should deliver. At Mahyco, plant biotechnology is viewed as a tool to be used in a selective manner, as an integral part of plant breeding programs. Traits of value that are difficult to breed for, or are absent in germplasm available to breeders, are additional focus of biotechnology research at Mahyco. These traits, when made available, enable breeders to incorporate them in a precise manner, eliminating unwanted traits that are retained in traditional breeding methods. Their major research areas include crop genomics, crop transformation, plant-virus interactions, microbiology, abiotic stress tolerance, molecular and conventional entomology, double haploids, mutagenesis and protein science.

14.23. METAHELIX LIFE SCIENCES PVT. LTD (INDIA)

Metahelix is an agriculture biotechnology company. They encounter agricultural crops that suffer from losses due to diseases and pests. Chemical and biological insecticides have been in use for a long time to minimize yield losses due to insect and pests. For example, rice which is the major staple crop for India and many other Asian countries also encounter various problem in the field. Yellow stem borer is the major lepidopteran pest that cause significant yield losses in rice. It is very difficult to control this pest using external pesticides since it bores into the stem and feeds on tissue and affecting the growth and productivity of the tiller. This symptom cause yield

losses eventually. Therefore, it is a vital requirement to develop transgenic rice varieties expressing appropriate proteins which could control the pest and prevent yield losses. The added benefit will reduce cost due to lesser usage of chemical insecticide spray. Therefore, focusing on developing traits and technologies for crop protection & improved productivity such as resistant rice plants and hybrid seeds are their main mission.

14.24. MONSANTO COMPANY

Monsanto, is an agriculture company which was established in 2002 after it was divested from Pharmacia Corporation. Agriculture biotechnology was firmly established as the new company's strategic research focus to improve farmers livelihood by increasing their profitability through yield enhancements. Monsanto is a global modern agriculture company. They develop products and tools to help farmers around the world to grow crops by using energy, water, and land more efficiently. They believe innovation has the potential to bring humanity's needs in balance with the resources in our planet. They are also committed to carbon neutral footprint by 2021. They believe the results of agricultural innovation is the path to environmental sustainability. That is the reason why Monsanto develop new products and technologies that will help farmers to find smart ways to use the natural resources efficiently to grow more food.

14.25. FORAGE GENETICS INTERNATIONAL

Forage Genetics International (FGI) is one of the only companies in the world that functioned to produce forage. They breed, develop and produce premier seeds and technologies that not only advance their operation but also move the whole industry to the next level. FGI has developed HarvXtra® Alfalfa trait that is the most advanced alfalfa trait on the market and the first genetically engineered alfalfa trait that was developed to maximize quality by reducing the amount of lignin in the plant compared to conventional alfalfa from similar stage of maturity. For growers, this means that they have the flexibility to produce higher-quality forage or delay harvest to maximize yield potential, depending on weather or operational needs. In addition, the Roundup Ready® Alfalfa trait delivers unsurpassed weed control during the establishment phase.

14.26. NATH BIO-GENE (I) LIMITED

Nath group is a well-diversified group that has interests in paper, seeds, agro-research plantation, biotechnologies, pharmaceuticals & chemicals. Nath Bio-Genes is amongst the pioneering seed companies in India and also the first seed company in Asia Pacific region. Founded in 1979, thirty years standing, it is amongst the most experienced seed companies in India: almost as old as the industry itself. They have a strong research and development department. They have carried out research in laboratories, irrigated land, testing facilities, and farms, all over the country. They have an excellent range of products and competitive pipeline products in major field crops, vegetable crops, and bio-stimulants. They strive to maintain good relations with farming community and build close contacts to work hand in hand with progressive farmers. Till date, they have established a network of over 15,000 farmers growing seeds from the company. An indigenous seed company is poised to play a more significant role in agricultural development, farmer prosperity and promotion of indigenously developed agriculture technologies. Under the banner of Nath Seeds, the parent organization, some of the most reputed cotton hybrids were introduced and some of them are known as Somnath and Jagannath.

14.27. NATIONAL INSTITUTE OF AGROBIOLOGICAL SCIENCES (JAPAN)

The National Institute of Agrobiological Sciences (NIAS) is the largest agricultural research institute in Japan that focused on basic life sciences. The institute focus on understanding the biological phenomena of agriculture important plants, insects, microbes, and animals to create innovative technologies, and eventually contribute to the solution of global issues such as food shortage due to rapid population growth and environmental problems due to climate change. Research activities are pursued in a 5-year cycle to intensify all efforts towards a common goal and to forge the way of new breakthrough with a direct impact on agricultural productivity. They have successfully sequenced rice genome, developed genetic recombination techniques for silkworm, and constructed genetically modified swine. Furthermore, they also focused on the utilization of the rice genome sequence to develop efficient breeding strategies, elucidated the draft sequences of silkworm and swine genomes, and carried out functional characterization studies of agronomically important genes. In addition, they challenge themselves to focus on elucidating the genome information to control the

life cycles of an organisms, to collect and to utilize the genetic resources, and to provide platform for genomics and transgenics applications in agriculture industry.

14.28. NUSEED PTY LTD

Nuseed develops plant traits of well-performing canola, sorghum and sunflower hybrid seeds. Nuseed recognizes the critical role of sunflower seeds and oils that will contribute in promoting healthier living. This have create a new potential and share added value chain. This focus allows them to dig deeper to deliver products that not only meet current grower needs, but also create more opportunity for tomorrow. They deliver hybrid seed products through innovative seed production. They drive to innovate global approach to impact at the local level by gather worldwide resources. This enables them to offer the best regional products to meet the needs of a specific area. Nuseed is committed to deliver canola with leading technology. It can contribute to high yield, profitability, and solid blackleg resistance. They also had innovated Monola®, a specialty oilseed that produces a superior and healthier frying oil option used as a sustainable alternative by major restaurants and snack companies around the world. While for sorghum, Nuseed produce better flour for human usage and deliver more nutrition to livestock.

14.29. OKANAGAN SPECIALTY FRUITS INCORPORATED

Okanagan Specialty Fruits Inc. (OSF) is a dynamic agricultural biotechnology company based in Summerland, British, Columbia and Canada. OSF specializes in developing tree fruit varieties that carries novel characteristic that benefit fruit producers and consumers through usage of biotechnology. This is to deliver innovated product for the tree fruit industry as well as consumer. OSF's flagship project objective is to develop and to commercialized non-browning Arctic® apples. Arctic® apples with non-browning trait offers valuable benefits for all members of the apple supply chain such as from growers up to consumers. Therefore, they have developed a vertically-integrated Arctic® apples with value added. With this innovation, OSF has change the way people think and experience about tree fruits. Their creative product development and commercialization expertise has opened the door towards novel, exciting, specialized fruits that support

the evolving healthy diet choices of today's consumer. To increase the public acceptance on their product, OSF takes a proactive approach such as to be transparency and open communication. They actively engage with all stakeholders and provide readily accessible information on their products and used science-based information for their concrete explanation. They are committed to improve the food they have grown through safe, cutting-edge science. They see the benefit of using every technology to develop new products, including utilizing genetic engineering and advanced molecular biology tools. They view gene amplification or suppression as a safe and proven approach to improve fruit quality and work hard to be at the forefront of the latest genetic techniques.

14.30. ORIGIN AGRITECH (CHINA)

Founded in 1997 and headquartered in the Zhong-Guan-Cun (ZGC) Life Science Park in Beijing, Origin Agritech Limited (NASDAQ GS: SEED) is China's leading agricultural biotechnology company, specializing in crop seed breeding and genetic improvement, seed production, processing, distribution, and technical services. As the first Chinese seed company with an in-house biotech research center, Origin leads the development of genetically modified (GM) technology. Now they have corn hybrids that has excellent yield, disease resistant, seed quality, rice hybrids consist of early, middle and long maturing Indica hybrid rice that suitable for planting in the upper, middle and lower stream of Yangtze River area and canola seed varieties consist of high-quality hybrids with low erucic acid and gluconate content.

14.31. PETOSEED COMPANY

Petoseed is a hybrid vegetable seed company that specializes in producing high yielding, disease resistant hybrid seed varieties.

14.32. PT PERKEBUNAN NUSANTARA XI (PERSERO)

PT Perkebunan Nusantara XI (Persero) was founded in 1996 and is based in Surabaya, Indonesia. PT Perkebunan Nusantara XI (Persero) engage in the sugar plantation business. The company produces sugar, ethanol, and molasses in the form of alcohol and methylated spirit. They also engage in the cultivation and plantation of tobacco, cacao, and coffee. In addition,

the company engage in the jute sacks business too. Currently, they have genetically modified sugar cane *Saccharum* sp. that carries drought tolerant and antibiotic resistant trait.

14.33. RENESSEN LLC (NETHERLANDS)

Renessen LLC develop and process grain with new technology that makes products for animal feed. The new technology provides important benefit to livestock industries. Currently, they have one single events on Maize-*Zea mays* L. that is Lepidopteran insect resistant and modified amino acid.

14.34. SEMINIS VEGETABLE SEEDS (CANADA)

Seminis is committed to provide growers with healthy and clean vegetable seeds. They are the world's largest developer and marketer of vegetable seeds for open-field and unheated-protected crops. They have diverse collection of germplasm. Therefore, they quickly develop new vegetable seed varieties that features the best characteristics that is needed based on regional preferences. So far Seminis Seed shown a predictable result and helps growers to produce the highest yielding and best-performing products.

14.35. STINE SEED FARM, INC (USA)

Stine Seed Company is the world's largest private seed company, and the largest independent seed company in the US. They specialized in soybean and corn genetics. Stine Seed have developed high-density corn varieties, with shorter plants that can be grown in 20-inch rows. This hybrids is better suited to narrow rows to ultra-narrow with higher populations.

14.36. TECHNOPLANT ARGENTINA

Technoplant Argentina has successfully developed genetically modified potato *Solanum tuberosum* that is resistant towards viral disease resistance.

14.37. VERDECA

Verdeca has focused on the development of traits that increase soybean adaptability and yields to address global needs and regional opportunities. Since soybeans are the fourth-largest global crop and its demand continues to increase annually together with other joint venture, Verdeca develop

and deregulates soybean varieties with next-generation agricultural technologies. Verdeca technologies helped to increase crop productivity by making more efficient and sustainable use of land and water. Verdeca has developed drought-tolerance technologies that enable soybeans to produce more efficiently by using available water resources and soybean varieties that enable soybean plants to produce more effectively by using available nitrogen and produce higher yields per hectare.

14.38. ZENECA PLANT SCIENCE

Zeneca Plant Science was a subsidiary of Zeneca Agrochemicals. This Dutch plant biotechnology is a small-to-medium enterprise (SME), that has strength in genetic modification for fungal and nematode resistance and in the production of enzymes and carbohydrates in plants. They have successfully developed GM tomato paste product, potatoes with various traits, cereal products and "low phytate" products with the ability to reduce the environmental impact of the feed by reducing the phosphate content of effluent from intensive animal husbandry.

CHAPTER 15

PLANT/ANIMAL APPS

CONTENTS

15.1. CROP NUTRIENT INTERACTIONS

- This is an Android apps.
- It requires no internet connection.
- It is used to determine appropriate nutrient levels.

15.2. PESTOZ-IDENTIFY PLANT DISEASE

- Identify the disease affecting plant/crop by uploading the pictures of the infected plant part on the app.
- The disease description and its solution can be obtained within seconds.
- Crops covered (tomato; cauliflower; cabbage; soybean; anion; banana; urad; tuar; sugarcane; sorghum; sesame; paddy; pigeon pea; maize; bindhi; cotton; beans).
- Also known as crop doctor.

15.3. PLANTNET PLANT IDENTIFICATION

- Identify plant species by uploading the species pictures.
- Not allowed for ornamental plant identification.
- Plant organ picture is more suitable.

15.4. PLANTSNAP

- Identify plants of all type.
- Powered by deep learning and artificial intelligence.
- Providing innovators with resources.
- Removing obstacles in regulatory frameworks.
- Promoting research and access to current information.

15.5. FARMING STIMULATOR 14

- Control the farm and fields to fulfill successful harvesting.
- One can able to control farm machines.

15.6. AGRI APP

- Provide complete information on crop production and protection.
- Provide a digital platform for farmers, breeders and retailer.
- Provide networking between scientist and farmers.
- Consist of videos on best practices, farming technology, and agricultural information.

15.7. ARGONAUTE

- Provide report of the cash flow.
- Allows farmers to record their expenses and income.
- Enable farmers to have records on livestock and machinery procedure.

15.8. SOIL SAMPLER

- Provide most advanced and effective way of taking soil samples.
- Navigate the user straight to the soil pick up position.
- Time Saver.
- Suitable for corn, wheat, soybean, barley and rapeseed cultivation.

15.9. DIGITAL AGRICULTURE

- Help to facilitate farmers with agriculture information timely.
- Provide platform to share and exchange agricultural information, knowledge and innovation.

15.10. CORN PLANNING CALCULATOR

- Allow farmer to calculate the correct spacing for corn planting.

15.11. GRAIN SHRINKAGE CALCULATOR

- Allow breeder to determine the level a grain shrink after the removal of the moisture from field to storage.
- It applies to common grains such as corn, wheat, soybeans, oats, barley, buckwheat, and rye.

15.12. GOAT BREEDING CALCULATOR

- Allow to calculate when doe/nanny will give birth.

15.13. SWINE BREEDING CALCULATOR

- Allow to calculate when sow will farrow.

15.14. CATTLE BREEDING CALCULATOR

- Is a gestation calculator meant to ease forward and backward date calculations.
- Allow the breeder to calculate service, return and calving dates for the cattle.

15.15. SHEEP BREEDING CALCULATOR

- Allow to calculate when the lambing due date.

15.16. MYFARM

- It is a livestock farm management application.
- Helps livestock farmers to optimize the performance of their farms and increase their productivity and profits.
- It gives the farmer the fastest, easiest and flexible decision-making tool.
- All information from the farm can be recorded including animal data, servings, deaths, revenue and expenditure.
- It also gives the farmer an alert notification.

15.17. RAISE ANIMAL FARM

- Provide information on raising livestock.
- Teaches good management program.

15.18. BREEDING WHEEL

- Allow better management of the dairy herds.
- Allow breeders to monitor the productive and reproductive status of a herd.

- Breeders can able to identify animals with reproductive problem.

15.19. POULTRY MANAGER 2.0

- A farming application that manage all aspects of poultry farming.

15.20. GENETIC ENGINEERING

- Provide easy way to learn genetic engineering skills.
- It covers almost 10 topics such as genes and chromosome, introduction to genetic engineering, regulation of gene expression, PCR and its application, recombinant DNA technology, cloning, gene therapy, electrophoresis, blotting, and gene transfer.

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INDEX

D

E

F

G

T

U

V

W