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## Food Sovereignty and Natural Resources in Archipelago Region



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## THE ROLE OF PLANT BIOTECHNOLOGY ON LOCAL FOOD SOVEREIGNTY OF MALUKU

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### Abstract

Maluku is an archipelago province consist of 559 Islands, four big islands and the rest small islands. Total area of Maluku is 658.148,48 km<sup>2</sup>, where 92.4 percent is sea and 7.6 percent land. Local food crops are non cereal food crops namely cassava, sweet potato, yam, cocoyam, banana, plantain, Fiji banana, breadfruit and sago palm. Dusun is one of the local technology of cropping system that already established before the Portuguese (1511) and the Dutch (1602) arrived in Ambon and Banda. Dawson is a sustainable agricultural system of mixed crops of perennial and annual crops. It is ecologically sound, economically viable, socially just and humane. Dusun is the embryo of the present LEISA (Low External Input Sustainable Agriculture) and Green Economics. Biotechnology simple means applying biotechnology knowledge benefit to local food crops and cropping system. The main role isto develop elite crop propagates and to improve crop cultivars. The conventional propagation technique is already established, no need for tissue culture methods except for sago palm (protocol is available). Genetic engineering technology is used to transfer important genes for improving the local varieties. The important genes are the optimistic genes, antisenescence genes, stress tolerance genes and glutenin genes. From the genotypes, genes, and methods availability cassava is one of the tuber crops are ready to transform genetically. The genes available are the glutenin genes, ipt genes, and cod A genes. The genetic modified (GM) cassava transformed with these 3 genes will be highly productive, highly resistant to abiotic stress and produce cassava flour which is similar to wheat flour. At present, Indonesia imported 5 million tons of wheat a year equaled 1.5 billion US dollars. This GM cassava benefits not only the farmers, the Maluku and the nations but also the whole world.

### INTRODUCTION

Indonesia is a maritime and the island country. Consists of 17.580 islands, oceans 3.2 million km<sup>2</sup>, 1.9 km<sup>2</sup> of land, the long beach of Sabang until Merauke 8.000/km (1/5 circumference of the earth). There are 33 provinces where 7 provinces is the island province namely: Maluku, North Maluku, North Sulawesi, West Nusa Tenggara, East Nusa Tenggara, Bangka Belitung and Riau Islands.

Maluku is geographically located between 2°30'-9°S and 124°-136°E. An area of 712,480 km<sup>2</sup> where 658,331.52 km<sup>2</sup> water (92.4%) and land area of 54,148.48 km<sup>2</sup> (7.6%). Consists of 1430 islands, four large islands namely: Seram (18,625 km<sup>2</sup>), Buru (9,000 km<sup>2</sup>), Yamdena (5,085 km<sup>2</sup>), Wetar (3,624 km<sup>2</sup>) remaining are small islands (≤2,000 km<sup>2</sup>). Maluku is administratively divided into 2 municipalities, 7 districts, 90 subdistricts, and 956 villages. The population of 1,533,506 inhabitants with the lowest density of 10 people/km<sup>2</sup> (Maluku Tenggara Barat) and highest in the city of Ambon 879 people/km<sup>2</sup> and Tual 228 person/km<sup>2</sup> (Salampessy, 2012; Norimarna, 2012).

Although the area is ecologically highly vulnerable to climate change and disasters, the islands are very rich in land and sea biotic diversity. Terrestrial habitats and marine habitats are synergistic, mutually enriching and maintain a stable habitat. Local food and farming systems that have been adapted and sustainable in the islands are known as local wisdom. Local wisdom has lasted for generations it has been scientifically proven true, only the local residents are not able to explain it in scientific terms. Therefore the development of local food crops should be started from the knowledge of the local crop, and then growth habitat, the way of cultivation, processing and supply of food (from land to table), so as to know the exact problem (right problem hunting).

Maluku local food is carbohydrates derived from the tubers and trees instead of rice. Government's past mistake is to make rice as a national food. The food needs of rice per capita in year increased from 86 kg in 1950 to 139.6 kg in the current 2012. Gratitude government has realized the mistake and pulled out of Food Act, which stated there in diversification efforts. Let's help the government in this effort with a biotechnology a touch with local food crops. This means applying biotechnology touch at the local food crops to meet human needs. The role of biotechnology in agriculture are : (1) Produce a high quality plant propagules, (2) Breeding superior cultivar. Understanding habitat and sustainable agriculture of Maluku will be discussed first before discuss the role of biotechnology.

### **Plant habitat**

Maluku archipelago is are consisting of the islands large and small. Each island or archipelago has a special environment that produces flora, fauna and diverse culture among the islands.

According to use the ability of the flora there are distinguished between plants, crops and weeds. Crop are plants that are known to benefit humans. Weeds are plants that interfere with crop growth also called crop pests. With the rapid advancement of science and technology all plants will be useful for humans, on more difference between crop and weed.

Plant is the only organism in the world that can use sunlight to produce carbohydrate and oxygen from carbon dioxide and water base, such as the following equation :

Carbon dioxide + water + light + solar plant → carbohydrate+oxygen  
↳ life in the biosphere.

We are aware of that equation to maintain the availability of water, carbon dioxide, sunlight, and growing plants for the sustainability of life in the biosphere. Hence the flurry of activity in the world now associated with the term "Green", both from the biological, social, economic, political and cultural.

Plants are the basis of the food chain. Vegetation types in one place determine the type of fauna and culture. Plant growth depending on location, altitude, and latitude that determines the type of soil and climate. Abiotic environment (soil and climate) and biotic (predators and parasites) that determines the type of flora and fauna. Natural selection in Darwin's theory is known as: "Struggle for existence and survival of the fittest. Habitat is a product of natural selection means the adjustment (to fit) plant species on the surrounding environment to form a stable plant community unit called habitat.

Adaptability depends on their reproductive ability and the ability of the gene mutation. Reproduction depends on flowering, fertilization, and viable seeds (propagule). Type of plant that produces seeds that many have a high adaptability than plants do not produce seeds. Gene mutation means gene alterations of characters to suit the surrounding environment. Gene changes cause changes in physiology and morphology. Katang-katang (*Ipomoea pescapre*) that grows on the edge of the beach has a high salinity tolerance than its close relatives sweet potato (*Ipomoea batatas*) and water spinach (*Ipomoea aquatica*). Katang-Katang genes tolerant to high salt levels, if these genes can be transferred to the kangkung, then a long the coasting of Indonesia will have available kangkung (vegetables) ready for harvest all the long year.

### **Dusun: Sustainable Agriculture and Economic of Maluku**

Sustainable agriculture is a low external input agriculture. Stability productivity, social stability and environmental and biodiversity conservation (Altiere, 1978). Many terms are used for sustainable agriculture as: "Biological Agriculture, Ecological Agriculture, Regenerative Agriculture, LISA (Low Input Sustainable Agriculture), LEISA (Low External Input Sustainable Agriculture), Precision Agriculture, (Berry, 1977; Fried, 1981).



Simple terms of GIPS (1987) on sustainable agriculture is: "Sustainable Agriculture is an agricultural system that is ecologically sound economic viable, socially just and humane". Sustainable Green Revolution (Evergreen Revolution) is the term for a system of sustainable agriculture. At this time in addition to the term "Evergreen Revolution" is also a term we often hear "Green Economy" (Green Economy). Green economy is a sustainable economic system that incorporates external inputs into the production system. Green Economic according Tesorino (2006) should be ecological, holistic, sustainable, diverse and balance, and social justice. Not easy to calculate External input in the form of ecological and social input so the companies that cause environmental pollutions should pay for CER (Certified Emission Reduction) and all companies should provide funds for Community development and community empowerment known as CSR (Corporate Social Responsibility).

Dusun is a local multiple cropping system of farmer consist of annual and perennial food crops, perennial horticulture, and industrial crops. Dusun has low external input, ecological Table, Economic sustainable, Social human, and conservation of biodiversity. Maluku children and the younger generation is not aware of it evened destroys the Dusun for short term interests such as coal mining in East Kalimantan province. Dusun of nutmeg and cloves in Ambon and Banda was caused Maluku is known as "Spice Islands". Christoffel's Columbus discovery of America in 1492 due to stray in search of Spice Island. Also the arrival of the Portuguese (1511), The Netherlands (1605) and England (1621). To master the "Spice Island" Dutch does two things :

- (1) *Swap Manahata island (Manhattan, New York) owned by the Netherlands with the island of Run owned by the England in the Banda Naira.*
- (2) *Perform a "Hongi Tochten", action of logging part nutmeg and clove trees to maintain a monopoly with high prices. The selling price in the Netherlands is 200 times the purchase price in Ambon and Banda Naira. Hongi in Ambon language means noise and to chtenin the Netherlands language mean sparade (Deinum, 1948; Des Alwi, 2005).*

Farmers in Maluku are farmers and fishermen, they wave season they farmed and shady season they became fishermen. Agriculture in Maluku is non of wet rice fields and non-cereals. Netherlands gave the term "Graanloze hakbouw". Graanloze means non cereals, hakbouw = agricultural use machetes, hoes and crowbars. Non cereals food in Maluku are : tubers (gembili, yam, taro), fruits (bananas, breadfruit), and tree (sago, palm, nipa). Local government makes a mistake by turning Buru Maluku as rice barns with printing more than 4,000 hectares of rice paddies from the planned 10,000 hectares. The paddy fields not improved the

welfare of local people but improved derivative PKI disposal on the island of Buru. Buru can be a breadbasket of Maluku with non-paddy farming system that will improved the welfare of all farmers (original or trans).

Cultivation system in Maluku has specific characters compared with the monoculture farming system. Dusun in Ambon, Banda, Tobelo, Galela after establishment it will be a shelter for the birds and mamalsof Wallaceae. Therefore Dusun in Maluku can be called "agrohortlife", short for agronomy, horticulture, and wildlife. Agronomic crops such as nutmeg, cloves, coconut and horticultural crops such as langsung, kacang, kuini, and wildlife such as betet, cockatoos, parrots, mamoa, bats, kusu, etc.

The function of Dusun needs to be improved with included the reingermplasm conservation. Canary nut, Fiji banana, and various flora of Wallace can be used as the main component of the Dusun.

### ***In Vitro* Clonal Propagation at Local Food Crops**

Propagation of plants consists of generative propagation (seeds) and vegetative propagation. Seeds are the generative propagules and cuttings are the vegetative propagules. The objectives of propagation is to produce high quality propagules. In vitro propagation is a clonal propagation (vegetative).

In vitro propagation produced micropropagules or in vitro propagules that can be done through several pathways of regeneration as follows:

- (1) explants → axillary bud → plantlets
- (2) explants → callus/nodules → adventitious buds → plantlets
- (3) explants → callus → somatic embryo → plantlets / synthetic seed
- (4) explants → somatic embryo → plantlets / synthetic seed

Micropropagule consist of plantlets and seed synthetic. Plantlets and synthetic seeds with a high degree of uniformity is lines regeneration without callus (1) and (4).

Food crops in Maluku such as tuber crops, bananas, and sago are vegetatively propagated by tillers (banana, sago, taro), stem cuttings (cassava, sweet potato), tuber cuttings (yams) and tubers (gembili). Conventional propagation of food crops is well established on need for in vitro propagation, except for research and in vitro conservation that can be done at the university there in Maluku.

Banana sand sago if you want to be propagated in vitro, the protocol was available. Bananas through axillary bud sand adventitious buds, while sago through hembryogenesis. The advantage of in vitro propagation is fast, can be produced in large number sand disease free, and equal to the parent plant and uniform. In vitro propagation through callus often produces some clonal variability. In certain plants such as

bananas omoclonal variability easily separated morphology at seedling stage, but this can't be applied to oil palm. Propagules produced through tissue culture is a quality propagules. Quality means uniform genotype and disease free. Horticultural crops that require high quality propagules derives generally from tissue culture.

Propagules produced through tissue culture is far more expensive than many other vegetative propagules. Tissue culture requires specialized equipment, skilled labor, and high production costs. Wattimena (1992), calculated the cost of the production of potato micropropagules consist of electricity cost of 82.6%, the labor cost of production 15.6%, and chemicals 1.1% of the total production cost.

### **Breeding varieties through biotechnology methods**

Breeding of improved varieties ranging from the conventional methods, cellular and molecular methods. Selection of breeding method or combination of methods is determined by 3 factors :

- (1) The objective of breeding: if the objective was to increase yield, quality of yield, plant resistance to abiotic and biotic stresses, bio industry, biopharmaca, etc.
- (2) Sources of the gene to be transferred from the donor parents to the recipient parents. Is the source gene from plant itself or from other plants (species, genus, family), or from other organisms or synthetic genes
- (3) The biological characteristics of plants. Nature of flowering, pollination, fertility, seed viability, regeneration ability of in vitro culture.

Conventional plant breeding methods depending on the nature of pollination. Self pollinated plants are mass selection (pedigree, single seed descent, etc.), breeding method of plants is crosspollinated (backcrossing, hybridization of pure line, etc.). Biotechnology breeding consists of a cellular method (of somaclonal diversity, somatic hybridization, haploidization) and molecular methods (genetic engineering, and molecular markers).

The good molecular methods to date is transformation the do recipient plants with the help of *Agrobacterium tumefaciens* in tissue culture media. Only plants can be regenerated through tissue culture methods that can use cellular and genetic transformation. Indonesia lately greatly affected by the seller if sophisticated and exposure molecular genetic tools that said tissue culture is not needed. Genetic transformation can be performed in planta. To days transgenic crops such as soybean, corn, wheat, potatoes, cotton etc is still transformed in vitro. There has been no release yet of in plantatransgenic plants.

Plant breedingsince the green revolutiontill to date is always the cooperation between experts of plant breeding and plantphysiologist. Plantphysiologists determine the ideal typeplants (idiotype) are productive

and plant breeding expert finding and transfer ring genes necessary. The good cooperation between biologists and biochemists in the breeding of the IR64 "Golden Rice" (GR) (Protrykus, 2003).

Breeding of GR rice begins with studying biosynthesis the pathways from B Carotene started from :



Biggest competitions in the biosynthesis of organic compounds are being GGPP precursor. Therefore how to induce the formation of Bcarotene from GGPP precursor and how Bcarotene was expressed in rice endosperm. Obtained the necessary genes comprising the gene *psy* (phytoene synthetase), *PDS* gene (phytoene desaturase), *LCY* gene (lycopene) and gene *CRT1* (carotenoid biosynthesis). So that these genes are expressed in the endosperm, specific promoters required *GT1* (endosperm specific glutelin promoter).

Lessons learned on the Golden Rice are:

- (1) In the food sovereignty, improved crop varieties not only to productivity but also nutrition and food security (through bio-fortification)
- (2) The breeding of crops to improve food nutrition requires good cooperation between various biologists.

The breeding method of rice of the Green Revolution Rice and Golden Rice will serve as guidelines do improve the tuber crops cultivars in the Maluku (cassava, sweet potato, gembili, yams, taro), as well as fruit and trees.

### **Improved Crop Production and Product Quality Tubers Based on Plant Metabolism (Plant Physiology)**

1. The increase in production with an increase in "Source"

Plants produce carbohydrates in the leaves and transported to the parts that require storage. The organ where the carbohydrates are produced in plant physiology called "Source" and the storage section called "Sink". At this sink carbohydrate crops commonly harvested in grains or tubers. With the increased production of crops done by increasing the capacity of the source and sink such as cereal crops are only increasing our cereal root crops.

On cereal crops increased production should be coupled with an increase in the source and sink. Due to the limited sink capacity of cereals. The rice grain endosperm is covered by dead skin (caryopsis), so the grain can't be belogged, when it's full it can not be filled again. So the rice and beans are called "sink limited" crops (sink limitation).

On root crops the eye bulb or tubers are non-stematic tissue. Sink capability is not limited to how much any carbohydrate that comes from the source can be accommodated. That's why one mukibat

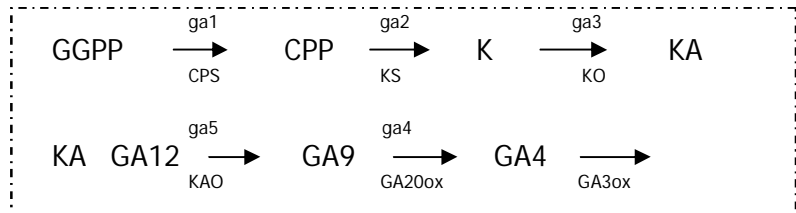


cassava tree (cassava root stock + scion of rubber cassava) can produce tubers 50-100 kg. On tubers (cassava, sweet potato, gembili, taro) crop yield improvement by increasing the ability of the source and the amount of green leaf.

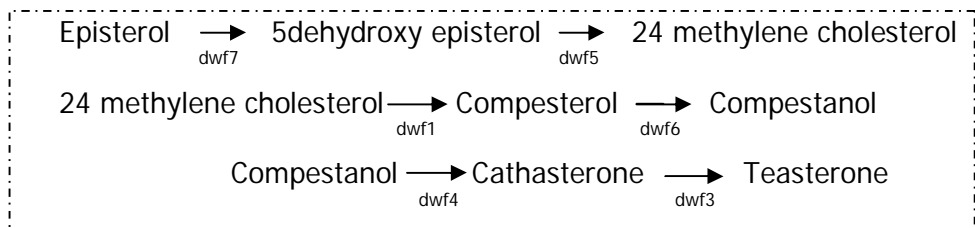
a) Improved source can be done include :

- Prevent leaf aging (senescence)  
In aging there is an increase in leaf ABA formation with decreased production of cytokines. This can be prevented by gene IPT (isopentenyl transferase) that produce cytokines IP (isopentenyl adenine). This gene can be isolated from *Agrobacterium tumefaciens* or from *Arabidopsis thaliana* (At). According Kakimoto (2001) there are 9 genes in AtIPT (IPT1 At-At IPT9) similar to that IPT gene from *A.tumefaciens*. So that this gene is only expressed in leaf senescence then given "senescence specific promoter" (SAG 12). When transformed with gene constructs SAG12-IPT then an expression of cytokines IP only on leaves will turn yellow, this will result all leaves remain green. The SAG12-IPT gene will be a new breakthrough in the increased production of tropical root crops.
- Internodes Shortening / Dwarfed plant  
Mean shortening or dwarfed plants are shortening the internodes without affecting the number of nodes and leaves of plants. Plant height is determined by the number of nodes and internodes length. Internodes longer this affected by GA'S. Apply plant growth retardants (Cycocel, CCC ancymidol, paclobutrazol, uniconazole) will inhibit biosynthesis of GA'S. Cycocel and CCC inhibits cyclization and GGPP (geranyl geranyl diphosphate) into CPP (copalyl diphosphate), where as ancymidol, paclobutrazol, and uniconazole inhibit oxidation of entkaurene into kaurenic acid genes that produce enzyme for GA'S biosynthesis can be altered known as dwarf mutants. In the *Arabidopsis thaliana* in addition to dwarf mutants of GA'S biosynthesis there is also dwarfed mutants brassinosteroid biosynthesis. Here is a dwarf mutant of *Arabidopsis thaliana* and brassinosteroid dwarf mutants :

- GA'S dwarf mutant (Sponsel Dan Hedden, 2004)



- Brassinosteroid dwarf mutant (Sunghwa Choe, 2004)



**Information :**

- Gai-1 = gen dwarf orthologos with mutant ga1
- Ga, dwf = dwarf mutant
- GGPP = Geranyl Geranyl diP
- CPP = Copalyl diP
- K = Kaurene,
- KA = Karenoid acid
- CPS = Copalyl Synthetase,
- KO = Kaurene Oxidase
- KS = Kaurene Synthetase
- KAO = Kaurenoic Acid Oxidase
- GA20ox, GA3ox = (GA20, GA3) Oxidase
- GA12, GA9, GA4 are the types of GAS
- Knownin plants : 136GA'S (GA1 -GA136)
- High GA activity are : GA1, GA3, GA4, GA5, GA6, GA7

Structure and function of the "dwarf genes" can be traced in Ref Genomics (structure and function) of *Arabidopsis thaliana*. In the wheat crop also contained genes RHT (reduce height) is not a response to gibberellin used as a source of genes to produce a wheat crop that is stocky and short. Short internode of plants without reducing the amount of leaf could allocate carbohydrates for internode elongation into storage carbohydrate in the form of tubers.

- b) Increase production by increasing starch content :  
 Carbohydrates produce through photosynthesis by Calvin cycles not only use as starch but too many organic substances needed by plants. The organic substances harvested by tubers is mainly starch.

By molecular biotechnology method the carbohydrates produced by photosynthesis can be directed to increase starch biosynthesis simple starch biosynthesis as follow :

Triose-P  $\Rightarrow$  F1,6P(fructosa 1.6-biphosphate)  $\Rightarrow$  F6P6(fructose-6-phosphate)  $\Rightarrow$  G6P (glucose-6-phosphate)  $\Rightarrow$  G1P (glucose-1-phosphate)  $\Rightarrow$  ADP glucose starch

According to Heldt (2005) a crucial step in the formation of starch is :

- Formation of fructose-1,6-bisphosphate (F1,6BP) of triose-phosphate by the enzyme fructose bisphosphate adolosa (FBA).
- Formation of ADP glucose from glucose-1-phosphate (G1P) by the enzyme ADP-glucose-pyrophosphatase with carrying two genes that produce FBA and ADPGPP on strong constitutive promoter (CAMV35S and RVBQ2) will get a higher starch content. Barry et al (1998) filed a patent application for an increase in starch transgenic plants with genes FDA (FBA).

c) Improving the quality of starch tubers

Quality in question is made of flour of tubers has visco-elastic properties similar to wheat flour. This property is needed for food product soft flour. Viscoelasticity contained in wheat is not found in other plant carbohydrates. There is a protein in wheat called gluten prolamins. Prolamins consist of albumin, globulin and gluten (Osborne, 1924).

Gluten is a pseudo polymer from glutenin and gliadin. Pseudopolymer was tied by disulfide bonds (-S-) that exist in the amino acid cysteine ( $\text{HS-CH}_2\text{CHNH}_2\text{COOH}$ ), the more cysteine residue the better properties visco-elastic of gluten. Gluten is composed of 10 percent high molecular weight glutenin (HMWG) and 40 percent low molecular weight glutenin (LMWG), and 50 percent of gliadin (Payne and Lawrence *in* Dekova, 2005).

Ben Mousa *et al* (2004) has succeeded in transferring gluten genes (LMWG) in Kennebec potato cultivar seed PBI 101.3 vector with patatin promoter and the NOS terminator. Potato tubers of transgenic Kennebec has starch visco-elasticity three times higher than non-transgenic Kennebec.

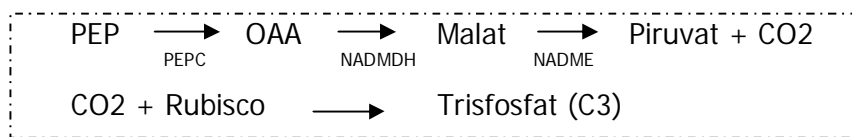
Liberty *et al* (2012) at the Faculty of Agriculture Pajajaran (UNPAD) has successfully transferred the gene Glu-IDY-10 by the method of particle bombardment with helium to Kitaake rice cultivars and await testing of the expression of gluten Gene on rice flour.

d) Increased abiotic resistance

Tuber crops have better adapted to abiotic stress than the cereal crops and legumes. Abiotic stresses that affect plant production are temperature, drought, light intensity, high, Al-toxicity, and flooding (lack of  $\text{rootO}_2$ ).

Plants to anticipate future certain climate change is facultative CAM plants with C3-CAM and C4-CAM (Wattimena, 2012). CAM plants are a very resistant to abiotic stresses, but the growth is very slow. If an facultative CAM plant expose to abiotic stress it will photosynthesis with the CAM system plants and the plants remain alive, if the plant will return to normal conditions it will turn to C3 or C4 photosynthesis. At the time of drought or flood CAM plants survive better than C3 and C4 plants.

Root crop are C3 plants so as to make the C3-CAM plants just add carboxylation and decarboxylation. Genes need to insert C3 plant to be C3-CAM are follows:



From the above scheme required only PEPC, NADMDH and NADME. NADME and NADMDH present in mitochondria, so it may be necessary to insert PEPC genes. Of the C3-CAM plant *Mesembryanthemum crystallinum* has been isolated nine genes related to C3-CAM such as PEPC, NADMDH and NADME (Cushman and Banner, 1996). Cod Agene (choline oxidase) (Alia *et.al*, 1998) or BADH gene (Betaine aldehyde dehydrogenase) (Meyer *et al.* 2000) which can accumulate glycinebetaine in plants so that the plants resistant to drought stress, temperature, light and high salinity without pleiotropic effect (Alia *et.al*, 1998).

### **GM cassava Global Prima Donna Food**

Singkong or cassava are important food for people in the tropics America, Africa, Asia though not as a major world food, as world food cassava rank five often potato. Cassava food is still considered low grade food, and food for the poor. That's why cassava plant called "subsistence crop" or "safety net crop" is a food rescue. Cassava as a crop in Indonesia rescue so has been experienced in Dean delsera (1914-1918) in Java so that cassava is called "cassava general".

Although already a lot of research on cassava tuber-based food, including the possibility of substitution of wheat flour, cassava food has not been optimized as a staple food. One way to make cassava as a staple food is genetically transforming cassava so that cassava flour will have similar properties to wheat flour. In addition to the quality of flour, cassava plants can be genetically transformed to a high starch production and resistance to biotic stresses, including irregularly climate change. Comparison of cassava tubers with other tropical tuber crops. Cassava has some following advantages :

- a) All parts of the cassava plant are useful:  
Fresh leaves can be used as a variety of vegetables, tempeh cassava leaves, meat of cassava leaves, feed and phytopharmaca.  
Tubers : a variety of fresh foods, dehydrated products (cassava, chips, pellets, tapioca, cassava), fermentation products (tape, mocav flour), hydrolysis products (fructose), beverages product and pastries. Tuber skin can be as mushroom and the remainder (Spent) can be used as food and feed.
- b) Environmental adaptation ranging from the tropics to the 25° North and South latitude. Can be cultivated on marginal soil to fertile soil and drought resistant.
- c) Stress pests and diseases
- d) Cultivation is very simple and the cost of production is cheaper when compared to cereal crops.
- e) Can be intercropped with rice, corn, sorghum, soybeans, green beans, or other earlier harvest annual crops.
- f) Long duration of harvest time from age 8 months to 20 months for starch producing clones and clones 8-12 months for fresh consumption and chips.
- g) There are diverse types (cultivars) for various needs
- Fresh consumption and chips: Mangi, Valenca, Mentega, Malang1, Malang2, Gajah, etc.
  - High starch content (over 30%): Adira4, Darul Hidayah, Malang4, Malang6, etc.
  - Fresh consumption and high starch content: Malang1, Malang2, Mangi, Mangu, Valenca, Cicurug, Gajah, etc.
- h) Cassava is clonally propagated. Clonal propagation plants produce a uniform population, stable and fast production.
- i) Cassava can be regenerated in vitro either through direct regeneration or callus phase (axillary buds, somatic embryos)
- j) Protocol for genetic transformation of cassava is available. GM cassava crops are environmentally friendly, cassava never flowers at the cultivated area in Indonesia. Cassava plants only flower and produce seed in mountainous regions in Indonesia (cold temperatures).
- k) Cassava ranks five as world food, from the tuber crops rank two after potatoes. World food crops ranking from number 1 to number 5 are successively rice, wheat, corn, potatoes and cassava.

### **Genetic modified cassava**

GM cassava requires the genes that have been available, cassava clones genes carrier and gene receiver. Of genes available for increased production which has been discussed in chapter 7, only 3 genes necessary to transform GM cassava namely glutelin gene, IPT gene, and BADH gene.



a) Glutelin Gene (LMWG, glu-IDX5, glu-IDY10)

Gen-glu IDX5 and glu-IDY10 being transformed into rice by the University of Padjadjaran (Liberty *et al.* 2012) need only collaboration with UNPAD to make quality cassava flour as wheat flour.

At this time the reduction in the use made of wheat flour with cassava flour processing improvements and substitution. Quality cassava flour processing done by fermentation. Bacterial fermentation with yeast (*Saccharum cerevisiae*, *Bacillus subtilis*, etc.) or mold (*Aspergillus niger*, *Pseudomonas sacharophila*, *Rhizopus delemans* etc.). The most important fermentation is *A.niger* the super "degradable" and "digestible" (Lowe, 2011). *A. niger* has so complete hydrolyzing enzymes such as  $\alpha$ -amylase, glucoamylase and cellulase.

b) Antisenescensgene (IPT) with SAG12 promoter (SAG12-IPT)

Cassava plants at the age of 3-5 months has a dense canopy so that no weeds are growing, because there is no light under the canopy. At the start of tuber formation density of the canopy began to decrease more than 50 percent by harvest time. Theoretically, if the density of the canopy can be maintained until the time of harvest, the production will be increased twofold and did not need weeding.

Cassava crop interms of production is "dependent Source" (dependence photosynthesis resource). The greater source of photosynthate (green leaves) the greater the production of cassava. Cassava production average 20-40 tons/10 months with IPT gene production at least 40-80 tons/Ha/10 months, and with in 20 months the production could increase 80-120 tons/ha.

Giving "senescence inducible" IPT gene SAG12 promoter is only working on the leaves begin to senesce (yellow). If given the "constitutive promoter" an increase in cytokinin-IPT and this will increase the water evapotranspiration and increase water use cytokinins induce opening of leaves stomata.

The fermentation process remove toxic HCN contained in cyanogenic glucosides (linamarin) with after fermentation process occurs linamarin is hydrolysis into glucose, acetone and HCN (HCN is removed with wasted water), cassava flour became whiter and viscoelastic increased.

Rapporteur developers of fermented mofaf flour (modified cassava flour) are Dr. Achmad Subagio Magr from Jember University.

All wheat flour food can be substituted with 20 percent Mofaf without changing the structure and flavor. There are certain cakes and that can be substituted by mofaf up to 100 percent. If implemented cassava transformation with gluteligenes that wheat flour can be replaced by GM flour. The process of making GM cassava flour still has to go through the process of fermentation.

GM. Cassava flour will stop the import of wheat and flour. Indonesian wheat imports in 2011 amounted to 5.4 million tons (BPS) if the price is 300 U.S. dollars per ton means of foreign exchange for import of wheat it was 1.6 billion U.S. dollars, equivalent 1.52 trillion rupiah per year.

c) Abiotic stress resistance genes (BADH genes, genes PEPC)

Cassava plants are plants that are relatively resistant to abiotic stresses. Transformation with the BADH gene or genes PEPC can improve cassava plant resistance to abiotic stresses. BADH gene increases plant resistance to drought stress, temperature, light, high salt level without pleiotropic effect (Alia et al, 1998). PEPC genes can make C3 cassava plants to C3-CAM. Actually to be C3-CAM is required additional PEPC genes, NADMDH, and NADME. Because genes NADMDH and NADME already in C3 plants mitochondria only possibility needed PEPC gene. C3-CAM cassava plants will not die in the extreme changing climate. At the extreme abiotic environment cassava plants will perform CAM photosynthesis (dormant or growing slowly) and when the environmental conditions return to normal cassava plant will switch to C3 (fast growing).

d) Genotypes of cassava clones

There are many clones of cassava to meet various needs. Cassava genotypes used we re-genotype which can be used for fresh consumption, chips and high starch content. These cassava genotypes include Mangi, Valenca, Cicurug, Gajah, Malang1, and Malang2.

e) Transformation glutelin, IPT and BADH genes

The transformation of these genes using *Agrobacterium* or particle bombardment using a PDS 1000 He (Biorad). Selection of transformed plants can be carried out by the selection marker. Antibiotic selection markers, herbicides, non herbicides, and non antibiotics selection markers. Common antibiotic markers is nptII (neomycin phosphotransferase) and hpt (hygromycin phosphotransferase). Herbicide markers consists of pat (phosphinotricin acetyl transferase) and CP4 (EPSP synthetase). Another marker is pmi (phosphomannose isomerase), asa 2 (anthranilate synthetase), cah (cyanamide hydratase) and xylose isomerase. After tentative transformed cassava plant has been selected through markers, plant molecular has to be needed to identify true transformed cassava.

Transfer of these three genes has to use three different markers. Markers use different antibiotics and non herbicides to be secure the environmental and food safety.

### **Pros and Cons of Transgenic Products in Indonesia**

There is no single technology that does not have any impact. But in developing a technology always considered the advantages and disadvantages. If the advantages is greater than disadvantages of course the technology can be applied. Process improvements that technology continues to be improved to minimize the losses. That is what we experienced in the production of transgenic plants.

The 1990 to 2000 in Indonesia occurred a fierce polemic between the pros and cons of transgenic products. Overcome the confusion and polemic the Medical Sciences Commission of Indonesian Academy of Sciences on 29 September 2000 issued released: "The AIPI views about transgenic products". "AIPI feel that the differences of opinion on issues of agricultural transgenic products is very alarming because it can be a waste of an opportunity that ultimately harm the nation of Indonesia".

"Every human creations may not be perfect, so in addition to the good will there are also draw backs, as well as genetic engineering technology. Acceptance and utilization of technology is always determined after review and compare the good ness and short comings". AIPI after indepth discussion of the opinion issue deight of them: "Indonesia must be planned and directed to develop the ability of genetic engineering technology, because the technology is capable of producing a variety of innovations to support health development, preservation of food selfsufficiency and improve the ability of Indonesia in the era of globalization".

"Genetic engineering and transgenic products, should be monitored and evaluated regularly and to inform ongoing excellence and find flaws as early as possible". In Indonesia cons of genetically modified products come mainly from three agencies, namely: (1) National Consortium for Conservation and Nature Indonesia (KOPHALINDO), (2) the Indonesian Consumers Foundation (YLKI) and the Indonesian Pesticide Network (PAN).

On 28 August 2000, the three agencies held a press conference in Jakarta that reject GM crops. Their demands include: "Government to stop the processor product field testing of transgenic plants in the field and its circulation until here is a provision (regulation) is more powerful and vivid that govern the testing and distribution of crops or transgenic products in Indonesia, which was prepared through a public consultation. Governments are required stop provision of free seeds (from multi nationals) to farmers' groups or other community groups.

"Relevant Minister and Kabu log to be more selective and careful in the procurement of foreign agricultural products suspected to contain genetically modified ingredients".

"Appealing to the experts and observers agricultural food and health and environmental ethics are competent to provide academic support are honest about the potential dangers inherent in genetically modified food for Indonesian consumers". In Indonesia before were released genetically modified plants are required to conduct testing of biological safety (bio-safety) and food safety (food safety) according to the provisions of the statutes bio-safety and food Safety (KKHKP) and the supervision of the Technical Team bio-safety and food safety (TTKHKP).

There are three safe provision for biotech crops in Indonesia, namely:

- Biosafety and food safety for biotech crops grown in Indonesia and used as food and feed (corn, soybeans, potatoes, rice, etc.).
- Biological security for biotech crops grown in Indonesia, but not for food crops (cotton).
- Food safety: for biotech crops are not grown in Indonesia but only imported for food and feed (corn, soybeans).

As an expert in plant biotechnology we realize that in realizing transgenic plant varieties must qualify in biosafety testing and food safety. Because the transgenic plants produced by the youth of the nation should have the full support of the government.

The government must realize that genetic engineering expert Indonesia already has the ability to parallel with foreign experts. The government should not import transgenic corn, soybean produced abroad and grown in Indonesia. The government only facilitates getting the necessary genes, biotechnology expert of the nation will transfer these genes in Indonesian crop varieties that are adapted to the environment of Indonesia. Transgenic crop varieties from abroad are generally less adaptable to the Indonesian environment.

In 2012 this conflict transgenic plants have started to fade, this is due to :

- a) Honesty and openness in reporting research findings transgenic plants.
- b) Improvements in the process of making transgenic plants, including :
  - Transformation of the chloroplast gene is not in the cell nucleus, preventing the migration of genes to other organisms.
  - Changing the antibiotic selection markers and herbicide selection markers with non-antibiotic selection markers and non-herbicide, making food safe and secure environment.
- c) The public gets a good and correct information about genetically modified crops. At Compass broadcast 27 November 2012 said that the Director of Standards of food products FDA has allowed 11 kinds of products consisting of transgenic maize (7 cultivars), soybeans (2 cultivars), sugar cane (1 species) and one ice cream ingredients. Hopefully the FDA along with TTKHKP agency has tested food safety of 11 types of transgenic foods imported it. Because of the precautionary principle must be adhered to any transgenic products.

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