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The Development of Insect-resistant Plants through Biotechnology

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ABSTRAK

Pembentukan Tanaman Tahan Serangga Hama melalui Bioteknologi. *Sutrisno*. Sejumlah tanaman tahan serangga hama telah ditanam di seluruh dunia sejak beberapa waktu yang lalu. Pembentukan tanaman tahan serangga hama akan terus berlanjut untuk mengantisipasi timbulnya serangga hama biotipe baru dan pergeseran status serangga hama utama. Bioteknologi menawarkan alat ampuh untuk digunakan dalam pembuatan tanaman tahan serangga hama secara komplemeter dengan metode konvensional. Alat bioteknologi itu ialah kultur anter, fusi protoplas, kultur embrio, variasi somaklonal, seleksi dibantu markah, dan transformasi genetik. Pilihan tiap alat itu dapat didasarkan pada banyak alasan antara lain efisiensi, kecepatan, kepastian, dan asal gen-gen ketahanan. Tanaman tahan serangga hama dihasilkan dari pendekatan konvensional atau bioteknologi mungkin menyebabkan efek serupa dalam pembentukan serangga hama biotipe baru.

Kata kunci: Tanaman tahan serangga hama, bioteknologi

The development of insect-resistant plants has been started in 1782 since Havens published a paper about a wheat cultivar resist-ance to Hessian fly. Since then, numerous insect-resistant plants have been developed by the inter-national and national research cen-ter, private sector through conven-tional method or biotechnology. For example, International Rice Re-search Institute (IRRI) has devel-oped and released numerous rice variety resistance to brown plant-hopper, green leafhopper, rice stem borer, and rice gallmidge. Several transgenic insecticidal cultivars ob-tained through biotechnology ap-proach have also been developed, such as Bt-corn, Bt-cotton, and Bt-rice.

Many insect-resistant plants have been adopted by farmers at all over the world. Rice resistance to the rice brown planthopper and green rice leafhopper has been planted over 20 million hectare in Asia. Wheat resistant to Hessian fly are grown in the mid-western of United Stated. In 1999, Bt-plants re-sistance to insect has been planted over 8.9 million hectare mostly in United Stated (James and Krattiger, 1996). The wide spread use of insect-resistant plants may be due to the fact that those plants are the most economic, least complicated and environmentally soundest ap-proach for protecting plants from insect damage.

The risks may arise from the use of insect-resistant plants de-rived from conventional method have been well documented that includes the development of new insect biotypes, such as brown planthopper biotypes and Hessian fly biotypes. However, up to now the development of new insect bio-types that overcoming transgenic insecticidal plants derived from bio-technology approach has not been recorded. Two strategies to delay the development of new insect bio-types have been reported. The first strategy relate to insect-plant resist-ant development and the second strategy relate to their application in the field in which insect-resistant plants as one of the component of integrated pest management ap-proach.

Controversy in the use of trans-genic insecticidal plants derived from biotechnology approach is still continuing in the world, including in Indonesia. This paper was presen-ted with the aim to get better understanding to transgenic insecti-cidal plants as one of insectresistant plants.

METHOD TO DEVELOP INSECT-RESISTANT PLANTS

In breeding program for insect-resistance plants, the first step is to identify the parents or donors of resistance. These may be cultivated germplasm, landraces, weeds races, wild species, or different spe-cies. The breeding method used to develop insect-resistance plants based on the following factors: the source of donor of resistance, the efficiency and certainty of selection of progenies. If the donor of resist-ance is commercial varieties, they may be improved by pure line or mass selection or hybridized with elite germplasm. If they are land-races, weed races, or wild species, they have to be hybridized with the elite germplasm. If the donor of resistance is different species, bio-technology method may used, such as genetic transformation or proto-plast fusion. If the donors have not available, biotechnology method may be used, such as somaclonal variation for insect-resistance. If the efficiency and certainty in the selec-tion of desired traits were needed, the use of biotechnology method such as marker assisted selection and anther culture may be appro-priate.

The method to develop insect-resistant plant can be group into two, i.e. conventional and bio-technology. The conventional ap-proaches use the whole living or-ganism, while biotechnology ap-proaches use cells or biomolecules to develop insect-resistant plants. The conventional method includes pure line selection, mass selection, hybridization (pedigree, bulk, single seed descent, backcross, recurrent selection). While biotechnology ap-proach includes protoplast fusion, somaclonal variation, molecular as-sisted selection, and genetic trans-formation (Collin and Edwards, 1998; Mujeeb-Kazi and Sitch, 1989). For anther culture and embryo cul-ture, at the beginning they used or-ganism of F1 plants to produce pol-len grain and wild and cultivated species to produce embryo. How-ever, since in the following steps they may be using cells culture, so we consider the anther culture and embryo culture as a biotechnology approach. A short brief about bio-technology approach is as follows.

Anther Culture

Anther or pollen grain could be cultured *in vitro* using artificial medium. On the artificial medium anther may form callus, shoot, root, and finally the entire plants. All the plant are haploid. This approach is possible to speed up the formation of homozygous population of insect-resistant plants.

Embryo Culture

Wild species are often more resistant to insects. Wide hybridization to transfer genes conferring insect-resistant from wild species to cultivated plants will produce abnormal interspecific hybrid em-bryos. The embryo can be rescued by culturing it on the nutrient me-dium to generate the entire plants. Several genes for insect resistance have been transferred from wild to cultivated germplasm (Table 1)

Protoplast Fusion

Protoplasts are plant cells that could be isolated by digested the wall enzimatically. The traits of resistant to the pest may be present in the one of two species that cannot be hybridized sexually. The two species may be formed hybrids through protoplasts fusion. The protoplasts may be cultured on arti-ficial medium and some protoplasts will grow into entire plants. The plants may be carried the resistant traits.

Somaclonal Variation

Somaclonal variation may be used to select insect-resistant vari-ants. Insect-resistant somaclones can be selected through the follow-ing steps (1) calli or cell suspension derived from high yielding variety were grown for several or long term cycles, (2) the long-term cell lines were regenerated into plants, and (3) the regenerated plants were evaluated against target insects.

About 2000 plantlets of sugar cane were evaluated for resistance to the sugar cane borer under arti-ficial infestation as well as natural infestation in field plots. Some somaclones showed resistance to sugar cane borer. The same method has been used to obtain somaclones of sorghum showed resistance to the fall armyworm.

Marker Assisted Selection

Nucleic acid probe, antibodies, or enzymes may be used as a marker assisted selection in breed-ing program. Those probes may be used to determine the genetic con-stitutions of plants or plant extracts, includes the present of resistant traits. The precise information pro-vided by this method will increase the speed and certainty of selection progeny carry resistance traits in conventional plant breeding. The probes that tight linkage to the re-sistance traits have been identified (Table 2).

Genetic Transformation

Genetic transformation refers to the introduction of cloned DNA seg-ments or genes from plants, bac-teria, or animals into a new genetic background. The DNA segments or genes introduced into the proto-plast or cell may confer resistance to insect. The genes may be de-livered to the protoplast or cell by using one of the following method: Agrobacterium, biolistic, electro-poration, PEG, vortex, and micro-injection. Several resistant plants have been developed through gen-etic transformation (Table 3).

STRATEGIES TO DEVELOP INSECT-RESISTANT PLANTS

Table 1. Genes conferring insect resistance transferred from wild to cultivated species

Recipient	Alien donor	Trait
Bread wheat	*Secale cereale*	Resistance to greenbug
	S. cereale	Resistance to Hessian fly
	Aegilops squarrosa	Resistance to Hessian fly
Rice	*Oryza officinalis*	Resistance to BPH
	O. officinalis	Resistance to WBPH
	O. australiensis	Resistance to BPH
	O. minuta	Resistance to BPH
Peanut	*Arachis monticola*	Resistance to chewing insect
Lettuce	*Lactusa virusa*	Resistance to Aphids
Cotton	*Gossypium armourianum*	Boll weevil, leafworm, bollworm

Source: Mujeeb-Kazi and Sitch (1989)

Table 2. The probes for marker assisted selection

Crop	Trait of resistance	Genes	Probes
Rice	WBH	Wbph-1	RG146
	BPH	Bph-10(t)	RG457
	Gallmidge	Gm-2	RG476, RG776, RG 224
Mungbean	Bruchid beetle	-	PA 882
Wheat	Hessian fly	H-23	XksuH4, XksuG48(A)
		H-24	XcnlBCD 457, Xcnl CDO 482, Xksu G48 (B)

Source: Panda and Khush (1995)

Table 3. Resistant plants develop through genetic transformation

Plants	Traits
Alfalfa	Insect resistance
Cabbage	Insect resistance
Cotton	Resistance to bollworms and budworm (Bt toxin)
Eggplant	Insect resistance
Maize/Corn	Resistance to corn borer (Bt toxin)
Potato	Resistance to Colorado potato beetle (Bt toxin)
	Resistance to Potato tuber moths
Soybean	Insect resistance
Sugar cane	Insect resistance
Sweet potato	Insect resistance
Tobacco	Insect resistance
Tomato	Insect resistance
Rice	Insect resistance (Bt toxin)

Source: James (1999)

Sooner or later an insect-resist-ant plants will become susceptible due to the development of new bio-types. To anticipate the breakdown of an insect-resistant plants, various strategies should be adopted in de-veloping those plants. One strategy is to prolong the useful life of insect-resistant plants. This can be real-ized by developing durable insect-resistant plant. The durable resist-ant plant is governed by polygenes. Durable resistance is also referred to poligenic resistance or horizontal resistance. The other strategy is to develop varieties with different genes conferring insect-resistant. This may be achieved by develop-ing vertical resistant plants. The vertical resistant plant is governed by major genes. The more vertical resistant varieties with different genes is available, the more easy farmer access to new varieties to replace the previous varieties.

Durable Insect-resistant Plants

The level of resistance of dur-able insects-resistant plants is gen-erally not very high. Because of this low selection pressure, the devel-opment of new biotypes is very slow. Polygenes resistance could be obtained from the landraces. Once crossing has been made be-tween cultivated and landraces, we face to the difficulty in the selecting the desired segregants. This may be due to not all the polygenes or QTL from landraces are transferred to cultivated and the level of resist-ance is diluted. The drawback is that the screening techniques cur-rently available is only applicable to identify segregants with high level of resistance not segregants with low level of resistance.

Molecular-assisted selection offers great role to facilitate the de-velopment of durable insect-resist-ant plants. However, the first step should be done is to tagged the QTL for insect resistance with mol-ecular markers. Molecular marker-based selection will assist in the accumulation of polygenes. QTLs for resistance to rice brown plant-hopper have been tagged with RFLP markers (Alam and Cohen, 1998).

Vertical Insect-resistant Plants

Vertical insect-resistant plants are more easier to develop since major genes are more easier to transfer from one variety to others. To anticipate the emergence of new insect biotypes that over-coming resistant plant, numerous vertical insect-resistant plants with different genes should be develop through conventional or biotechnol-ogy. Numerous rice varieties with different genes resistance to BPH have been produced by IRRI.

Insect-resistant transgenic plants developed through genetic transformation currently available is generally vertical resistance with the major genes, such Bt-gene. To anticipate the development of new biotype overcoming Bt-plants, others resistant plants with different genes should be develop.

Pyramiding of major genes may be one of strategy to develop the longer useful life of resistance plants. This approach needs reli-able method to determine the pres-ent of the two genes. If bioassay method will be adopted, several insect biotypes should available for this purpose. The use of molecular markers is very reliable method to diagnose the integration of the two genes in the plants. Pyramiding of major genes may be achieved through genetic transformation, for example pyramiding the two Bt genes or combining genes encod-ing a toxin and a gene encoding a repellent.

Tissue-specific Expression

The constitutive expression of resistance genes at all time and in the whole tissue may be caused a great selection pressure that leads to the development of new insect biotypes. This problem may be solved by expressing the resistance traits in a specific tissue, or at certain growth stages, or only in response to insect feeding. Pro-moters to regulate these expression are available.

CONCLUSION

Numerous insect-resistant plants have been developed through biotechnology. The use of biotechnology tools in breeding program will continue. Advances in biotechnology will accelerate the development of insect-resistant plants. The acceptability to biotech-nology products may be greater a long with the increase in the understanding to biotechnology processes.

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