Research and commercialization of GM crops in the world and in Europe, plum ´HoneySweet´ resistant to PPV.

Jaroslav Polák[1], Clive James[2]

[1]Crop Research Institute, Division of Plant Health, Dept. of Virology, Drnovská 507, 16106 Prague, Czech Republic

[2]ISAAA Center, 417 Bradfield Hall, Cornell University, Ithaca NY 14853, U.S.A.

EFPP EUROPEAN FOUNDATION FOR PLANT PATHOLOGY

IPM 2.0 Towards future-proof crop protection in Europe

Research on genetically engineered plants started in seventies of last century, e.g. Redei (1975) and continued in broad extent in agricultural crops in eighties (e.g. Colwell et al., 1985; Hoy et al., 1985; Gould, 1988). Field testing of genetically modified crops was established for the first time in the U.S. (NRC, 1989). Commercionalization of Biotech/GM (Biotech) crops started in 1995 (cotton, company Monsanto; potato, company Syngenta). A 94-fold increase from 1.7 million hectares in 1996 to 160 million hectares in 2011 (16 years) makes Biotech crops the fastest adopted crop technology in the history of agriculture (Fig. 1).

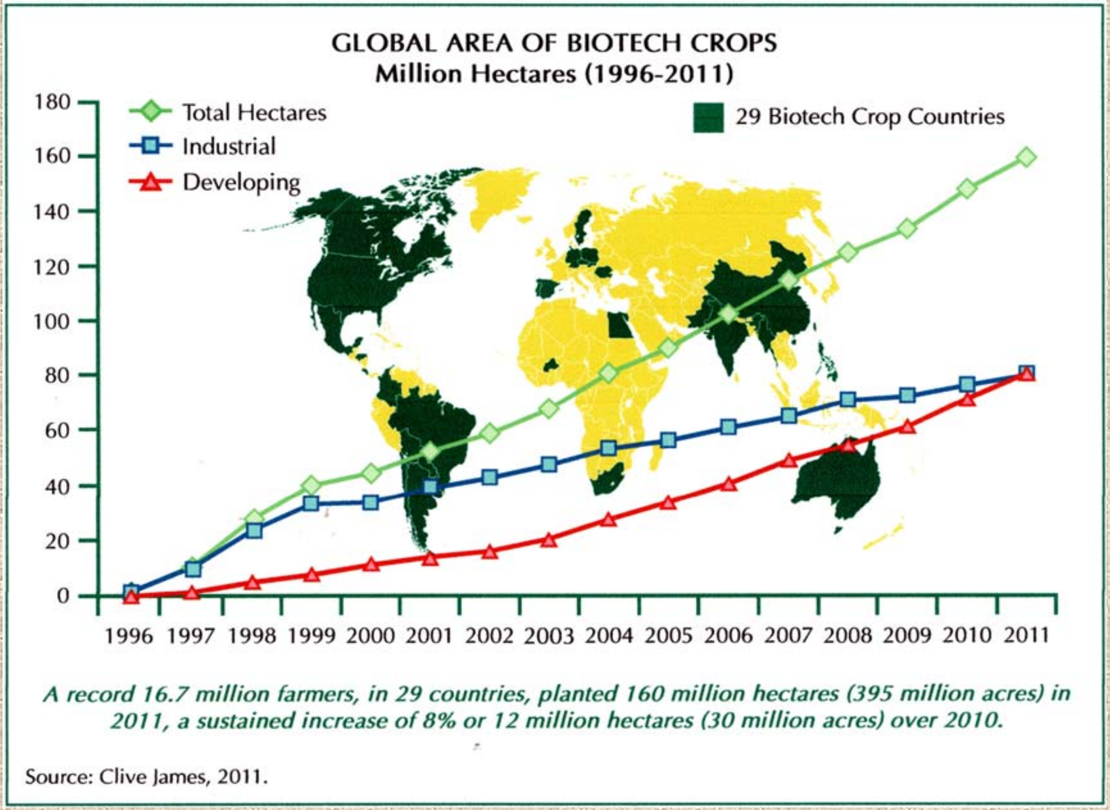

Fig. 1. Global area of biotech crops

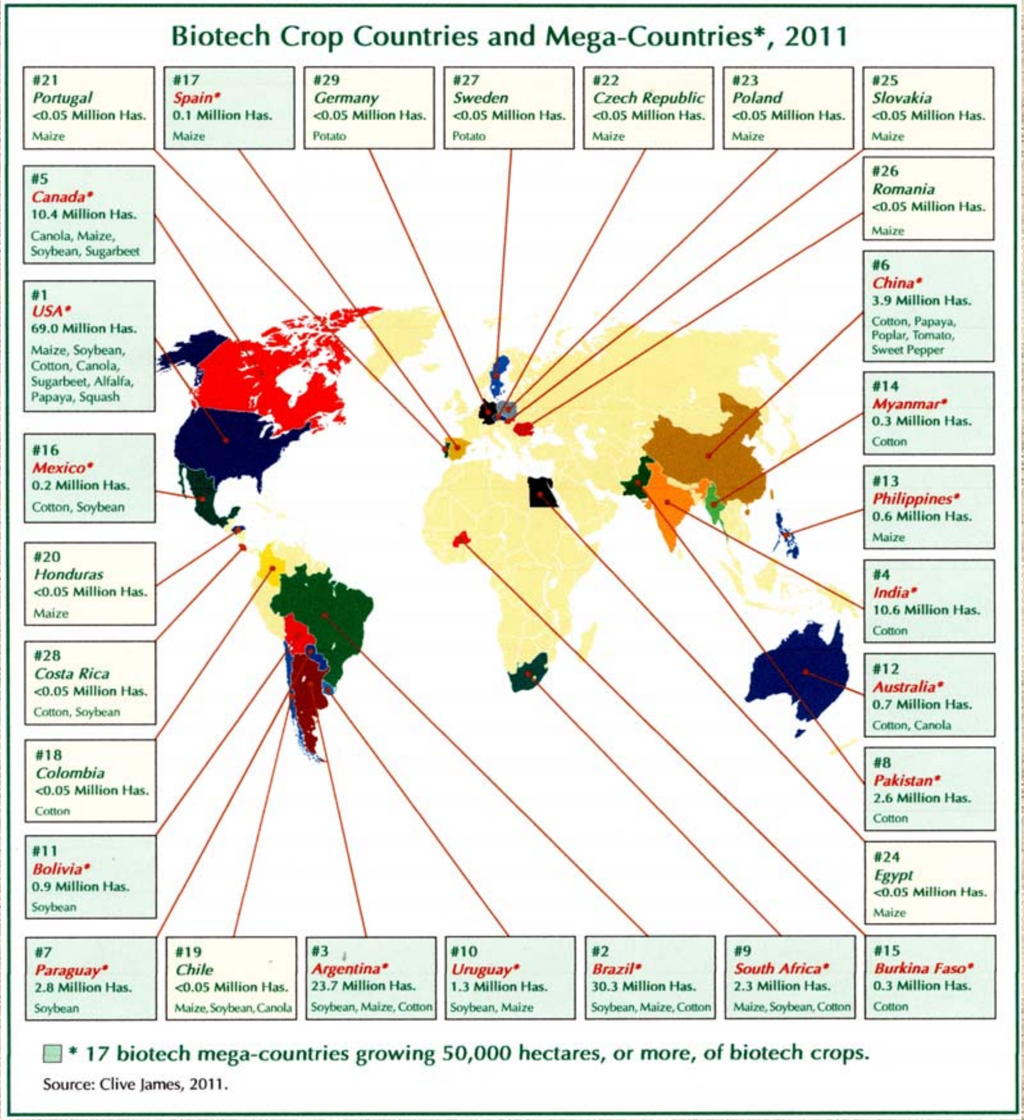

Fig. 2. Global map of biotech crop countries and mega-countries in 2011

The International Service for the Acquisition of Agri-biotech Applications (ISAAA) is every year publishing „Global status of commerialized Biotech/GM crops". The last one was published as ISAAA Brief No. 43 in 2011 (James C., 2011). The objective of this Brief is to provide information and knowledge to the scientific community and society on Biotech/GM crops and its contribution to global food, feet, fiber and fuel security, to sustainable agriculture.

Of the 29 countries planting Biotech crops in 2011 (Fig. 2), 19 were developing countries and 10 were industrial countries. 60% of the world´s population live in countries planting Biotech crops.

The US is the lead producer of Biotech crops with 69 million hectares. US is followed by Brazil with 30.3 mil. ha, Argentina (23.7 mil. ha), India (10.6 mil. ha), and Canada (10.4 mil. ha). Developing countries grew close to 50% of global Biotech crops. Another five countries, China, Paraguay, Pakistan, South Africa, and Uruguay each grew more than 1 million hectares. Biotech soybean remains with 75.4 million hectares (47% of global Biotech area) the dominant crop, followed by Biotech maize with 51 million hectares, Biotech cotton 24.7 mil. ha, and Biotech canola 8.2 mil. ha.

The only 0.114507 million hectares of Biotech crops were planted in Europe. Six EU countries, Spain, Portugal, Czechia, Poland, Slovakia, and Romania planted 114490 ha of Bt maize. Sweden (15) and Germany (2) planted 17 ha of Biotech starch potato cv. ´Amflora´. Spain as the first European country is with 97326 ha of Bt maize on the 17. place. In the Czech Republic the Bt maize was grown on 5091 ha in 2011.

Biotech crops are strongly contributing to food security. This was achieved from 1996 to 2010 (in five years) by: increasing crop production and value by US 78 billion dollars, providing a better environment, by saving 443 million kg a.i. of pesticides; in 2010 alone reducing CO_2 emissions by 19 billion kg, equivalent to taking 9 million cars off the road, saving 91 million hectares of land, and helping 15 million farmers, the poorest people in the world. From the point of food security European policy is going against humanity in the world.

Important example of benefit of Biotech crop for good health of mankind, children is Golden Rice. Among cereals, rice has the highest energy and food yield but lucks essential amino acids and vitamins needed for normal body functions. It lacks beta carotene, the precursor of Vitamin A. Vitamin A deficiency (VAD) is a nutritional problem in the developing world afflicting 127 million people and 25% of pre-school children. Currently around 250,000 to 500,000 become blind annually, 67% of whom die within a month, or around 6,000 deaths of children a day, equivalent to 2.2 million per year. Around three billion people are dependent on rice for their caloric intake, and many cannot afford other foods containing Vitamin A or supplements. Golden Rice offers a practical Biotech crop remedy that provides cost-effective and efficient protection against VAD.

In 1984, Dr. Peter Jennings, a rice breeder at International Rice Research Institute (IRRI), conceived the Golden Rice initiative. The Rockefeller Foundation funded a research program conducted by Prof. Ingo Potrykus and Dr. Peter Beyer. They conducted the rice transformation to develop the first genetically modified rice that producted beta carotene. In 2000 the first Golden Rice was developed, then beta carotene content was low at 1.6 to 1.8 µg/g, but it proved the functionality of the genes in rice. Rice variety Cocodrie was developed by Syngenta in 2004 that contained 6 to 8 µg/g beta carotene, and designated as Golden Rice 1. In 2005, Golden Rice 2 was developed by Syngenta, that produced up to 36.7 µg/g beta carotene.

In 2005, the Bill and Melinda Gates Foundation provided funding for a collaborative project on Engineering rice for high beta-carotene, Vitamin E, protein, enhanced iron and zinc bioavailability. It is expected that Golden Rice will be released in the Philippines and Bangladesh this year (in 2012), followed by India, Indonesia and Vietnam. Whereas VAD is estimated to affect 33% of individuals in South East Asia, corresponding figures for iron deficiency (anemia) is 57%, and 71% for zinc deficiency. Rice germplasm with the GR2G event is now being crossed with rice lines having a high content of zinc and iron to pyramid the three benefits.

A lot of horticultural transgenic crops are under development and are beginning to be commercialized. Impressive progress is in Biotech vegetable projects which include tomato, potato, cabbage, brassica, cauliflower, bean, sweet pepper, chili, zuccini, squash, eggplant, cucumber, carrot, and sweet corn. US vegetables farmers are benefiting from growing transgenic squash cultivars resistant to *Zuccini yellow mosaic virus, Watermelon mosaic virus,* and *Cucumber mosaic virus,* which were deregulated and commercialized last decade in U.S.A. Not only in the Czech Republic, but in many countries of Europe we need Biotech cultivars of squash, cucumber, and zuccini (Fig. 3), resistant to mentioned viruses and other pathogens.

Fig. 3. Malformed fruit of summer squash cv. ‘Zelená’, plant naturally infected with ZYMV.

Bt-sweet corn was accepted in the fresh market in the United States, transgenic Bt-eggplant was bred to reduce pesticide use in Asia. In South Africa transgenic Bt-potato resistant to potato tuber moth (PTM), *Phthorimaea operculella* was developed and field trials were conducted between 2001 and 2007. Bt-potato provided excellent control of PTM. Biotech potato cultivars resistant to *Potato virus Y*, and *Potato leafroll virus* are developed.

Genetic engineering has the potential to revolutionize fruit tree breeding. The development of transgenic fruit cultivars is in progress. Papaya resistant to *Papaya mosaic virus* is grown in U.S.A. and China. Biotech grapevine resistant to viral, bacterial, fungal disease with abiotic stress tolerance and health benefits is developed in South Africa. The scab (*Venturia inaequalis)* resistance and fire blight (*Erwinia amylovora)* resistance genes were introduced in apple using cisgenic approach in Switzerland. A large number of transgenic clones of apple and pear with clearly improved rooting ability were obtained in Sweden. Expression of thaumatin II gene in apple, pear, and strawberry cultivars conducted in Russia resulted in sweetness improvement and enhanced resistance of strawberry plants against *Botrytis cinerea.* Avocado was transformed in South Africa to obtain resistance against pathogens. Biotech banana cultivars are developed for 17 years in Belgium, Uganda, South Africa and will solve the biggest problem in banana production, Fusarium wilt disease, caused by *Fusarium oxysporum f.sp. cubense.* Topics include also banana drought resistance. Biotech banana, apple, pear, and strawberry cultivars are under the development.

Plum pox virus is causal agent of economically most important disease of stone fruits (Fig. 4). Plum (*Prunus domestica* L), clone C5 transformed with the *Plum pox virus* (PPV) coat protein (CP) was obtained by Scorza et al. (1994). Clone C5 (cv. ´HoneySweet´ at present) was proved to be highly resistant to PPV under glasshouse conditions (Ravelonandro et al., 1997). Field tests were conducted in Poland (Malinowski et al., 1998), Romania (Ravelonandro et al., 2002; Zagrai et al., 2008), Czech Republic (Polák et al., 2005), and in Spain (Malinowski et al., 2006). The all experiments confirmed the resistance of clone C5 to PPV infection.

Fig. 4. PPV symptoms on plum fruits cv. Domácí švestka.

A trial (Fig. 5) of high and permanent infection pressure of PPV-Rec alone and in combinations with *Prune dwarf virus* (PDV), and *Apple chlorotic leafspot virus* (ACLSV) was initiated in the Czech Republic and partial results were published (Polák et al., 2008a; 2008b).

Fig. 5. Plantation of ´HoneySweet´ trees in CR in 2011

´HoneySweet´ plum has been evaluated for eleven years (2002-2012) in a regulated field trial in the Czech Republic for resistance to PPV, *Prune dwarf virus* (PDV), and *Apple chlorotic leaf spot virus* (ACLSV), all serious diseases of plum. Even under high and permanent infection pressure produced through grafting, PPV has only been detected in ´HoneySweet´ trees in several leaves and fruits situated close to the point of inoculum grafting. The lack of infection spread in ´HoneySweet´ demonstrates its high level of PPV resistance. Coinfections of PPV with PDV and/or ACLSV had practically no influence on the quantity and quality of ´HoneySweet´ fruit which are large (Fig. 6), sweet, and of high eating quality. In many respects they are superior to fruit of the well-known cultivar ´Stanley´.

Fig. 6. Fruits of plum cv. ´HoneySweet´ - bottom row, fruits of plum cv. ´Jojo´ - upper row.

Many fruit growers and fruit tree nurseries the Czech Republic are supportive of the deregulation of ´HoneySweet´ plum to help improve plum production and control the spread of PPV. It is absolutely necessary to change the wrong European policy and to exploit the benefits of Biotech crops in Europe, too.

A result of over the past 20 years an international research is development of ´HoneySweet´ plum highly resistant to PPV. Moreover PPV is not possible to transmit on cv. ´HoneySweet´ by aphids. The regulatory process in the U.S.A. for 'HoneySweet' was successfully completed in 2010. The strong international cooperation between public sector scientists in Europe and the U.S.A., and the approval of 'HoneySweet' in the U.S.A. warrants the submission of 'HoneySweet' for regulatory consideration in the EU. The ability to grow ´HoneySweet´ plum in the Czech Republic would contribute to the viability of plum production by Czech growers and support the producers of products that depend upon a supply of plums including producers of plum brandy. The cultivation of 'HoneySweet' in the Czech Republic and other European countries would represent a unique opportunity to establish PPV free orchards and to grow high quality fruits for the benefit of growers and consumers.

Biotechnologies and biotechnological, syn. genetically modified (GM) crops are the only possibility of mankind to survive under the conditions when the world population is growing all the time. Biotech crops have an enormous potential for contributing to the Millenium Development Goals (MDG) of reducing poverty by 50% by 2015. 3 milliards of people from 9 milliards supposed to live in the world in 2050 it means the one third of total population will hungered in case that GM crops will not be grown.

European policy in relation to GM crops is scandal. In October 2011, forty one leading Swedish biological scientists in a strongly-worded open letter to politicians and environmentalists asked to revise European legislation to allow society to benefit from Biotech crops. A contingent of scientists from the United Kingdom endorsed the Swedish petition. I would like to ask scientists present on the 10th conference of European Foundation for Plant Pathology in Wageningen 2012, to join Swedish an UK scientists.

Acknowledgements

This work was supported by grants of Ministry of Agriculture, CR, No. QI101A123, and No. 0002700604. Authors are in debt to Jiban Kumar-Kundu, PhD., and Prof. Dr. Boris Krška for cooperation and to Mrs M. Ducháčová and J. Pívalová for technical assistance.

References

Colwell R.K., Norse E.A., Pimentel D., Sharples F.E., Simberloff D., 1985. Genetic engineering in agriculture. Science 229: 111-114.

James C., 2011. Global status of commercialized Biotech/GM crops: 2011. ISAAA Brief No. 43.

Gould F., 1988. Evolutionary biology and genetically engineered crops. BioScience 38: 26.

Hoy C.W., Feldman J., Gould F., Kennedy G.G., Reed G., Wymann J.A. 1985. Naturqally occurringbiological control in genetically engineered crops. In: Conservation biological control, Barbosa P., Ed., Academic Press, New York.

Malinowski, T., Zawadzka, B., Ravelonandro, M., Scorza, R., 1998. Preliminary report on the apparent breaking of resistance of a transgenic plum by chip-bud inoculation of Plum pox virus PPV-S. Acta Virol. 42: 241-243.

Malinowski, T., Cambra, M., Capote, N., Zawadzka, B., Gorris, M.T., Scorza, R., Ravelonandro, M. 2006. Field trials of plum clones transformed with the Plum pox virus coat protein (PPV-CP) gene. Plant Dis. 90:1012-1018.

NRC (National Research Council) 1989. Field testing genetically modified organisms: Framework for Decision. Natioanl Academic Press, Washington, D.C.

Polák J., Pívalová J., Jokeš M., Svoboda J., Scorza R., Ravelonandro M., 2005. Preliminary results of interactions of Plum pox virus (PPV), Prune dwarf virus (PDV), and Apple chlorotic leafspot virus (ACLSV) with transgenic plants of plum *Prunus domestica*, clone C5 grown in an open field. Phytopathol. Pol. 36: 115-122.

Polák J., Pívalová J., Kumar-Kundu J., Jokeš M., Scorza R., Ravelonandro M., 2008a. Behaviour of transgenic Plum pox virus-resistant *Prunus domestica* L. clone C5 grown in the open field under a high and permanent infection pressure of the PPV-Rec strain. . J. Plant Pathol. 90 (Suppl. 1): S1.33-S1.36.

Polák J., Kumar-Kundu J., Pívalová J., Scorza R., Ravelonandro M., 2008b. Interactions of Plum pox virus strain Rec with Apple chlorotic leaf spot and Prune dwarf viruses in field growing transgenic plum *Prunus domestica* L., clone C. Plant Protect. Sci. 44: 1-5

Ravelonandro M., Scorza R., Bachelier J.C., Labbone G., Lery L., Damsteegt V., Callahan A.M., Dunez J., 1997. Resistance of transgenic plums (*Prunus domestica* L.) to Plum pox virus infection. Plant Dis. 81: 1231-1235.

Ravelonandro M., Scorza R., Minoiu N., Zagrai I., Platon I., 2002. Field tests of transgenic plums in Romania. Plant´s Health (Spec. ed.): 16-18.

Redei G.P., 1975. Arabidopsis as a genetic tool. Annual Rev. Genet. 9: 111.

Scorza R., Ravelonandro M., Callahan A.M., Cordts J.M., Fuchs M., Dunez J., Gonsalvez D., 1994. Transgenic plums (Prunus domestica L.) express the plum pox virus coat protein gene. Plant Cell. Rep. 14: 18-22.

Zagrai I., Ravelonandro M., Scorza R., Minoiu N., Zagrai L. 2008. Field release of transgenic plums in Romania. Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Animal Science and Biotechnologies. Vol. 65/2008, pg. 358-365.