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KP4: when wheat uses a killer protein for self-defense

KP4. Is it a detergent? Or a fuel additive? Nope. It is a protein. But not just any old protein. KP4 stands for 'Killer Protein 4'. Not to worry though, the protein with this forbidding name can only pride itself on being a threat to certain mushrooms but it has indirectly become the object of biotechnological and economic debate.

In November 2001, the Swiss Federal Office of the Environment, Forests and Landscape (OFEFP) rejected the request made by researchers of the Swiss Federal Institute of Technology in Zurich (ETHZ) to plant a trial field of genetically modified wheat. The gene of a virus had been introduced into the wheat so that it in turn would produce a protein called DP4. This protein was intended to protect the wheat against wheat rot – a disease caused by mildew called Tilletia tritici. The main object of this fundamental research was to test the resistance of genetically modified wheat under field conditions. The controversial decision taken by the OFEFP revived the debate on transgenic plants. Yet, ironically, this example has reminded us that transgenic organisms have existed in nature long before man intervened.

Most unappetizing mushrooms

For some people, the word mushroom brings to mind a mouth-watering dish of chanterelles, ceps or morels. There are other varieties however of no gastronomic interest and which - unlike the ones just mentioned - consist of a single cell. Yeast, used in baking or brewing beer, is one such mushroom, as is mildew. In this category are found the Ustilago and the Tilletia families, which group species that attack wheat, barley, maize (corn) and oats, amongst others.

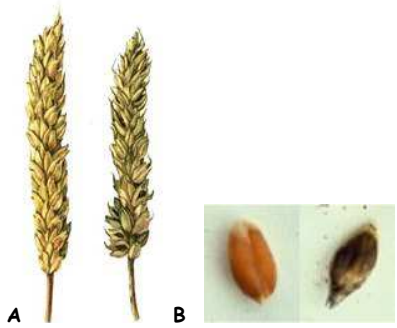

Fig.1 ***A.*** *A healthy ear of wheat on the left and one infected by rot on the right,* ***B.*** *A healthy grain of wheat on the left and an infected one on the right.*

The mildew – which spreads through the seed – cannot be detected before the plants are in bud. So it is the contaminated seed which propagates the infection. The slightest Tilletia tritici infection can have enormous economic repercussions. Diseased wheat smells and tastes

of rotten fish. You only need a few contaminated ears of wheat to ruin a whole field. What is more, the chemical origin of the stench (trimethylamine) is toxic. Effective chemical treatments exist of course but they must not be used for seed which is for human or animal consumption. This means special care has to be taken in the transport of grain to avoid propagation of the infection. The disease, practically unknown in Switzerland now, still causes extensive damage in underdeveloped countries.

Mushrooms make a deal with viruses

Ustilago maydis is a mushroom with a special attribute. It can be infected by viruses – which in itself is not unusual – but only by certain viruses. To be more precise, these are strains of virus each of which produces a different KP protein. Every KP protein has specific properties with specific functions; whereas a contaminated mushroom is immune to its own KP, the same KP is toxic for uncontaminated Ustilago maydis or infected ones that produce another KP protein (for example KP6). In other words, this mushroom uses a transgene to produce a different strain of protein which will enable it to survive in the midst of other types of mildew and at their cost, and all this without the help of man!

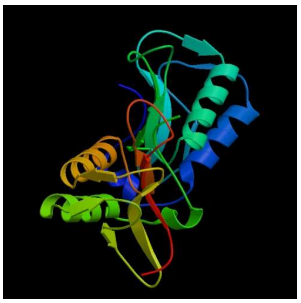

Fig.2 *Tridimensional structure of KP4*

After it has been produced and secreted by the mushroom, KP4 binds to the surface of other mildews it comes into contact with. It then acts rather like a 'cellular cork' by blocking the calcium canals, which are kinds of 'holes' in the cells' membrane through which calcium penetrates into the cell. The resulting lack of calcium – which plays a very important role in many cellular mechanisms – will rapidly inhibit the growth and multiplication of mildew.

Such a fuss for so small a field

KP proteins are also toxic for the Tilletia family of mildews. By introducing into wheat cells, the viral gene which holds the recipe for producing KP4, the ETHZ team hoped to make wheat resistant to Tilletia tritici. Their scheme was to plant 8m2 of modified wheat in the middle of a field of 90m2 to test the resistance of the wheat in completely natural conditions.

In spite of all the security measures proposed, the plan was rejected, the main reason being that it was not possible to estimate the potential risks to man and the environment.

Yet the ETHZ team had demonstrated that KP4 is harmless for a variety of organisms, from bacteria to human cells. It may however cause allergies, which anyway is the case for all proteins produced in large quantities, and in any organism. To avoid such a risk, it is essential to adjust the production of a transgenic protein adequately. In this case, one of the reasons the trials were refused seems to have been the lack of data regarding the concentration of the protein in different parts of the plant.

Other reasons put forward had little to do with the KP4 protein, as for example the one which weighted most in the balance against the experiment. This was the introduction into wheat of a bacterial gene which produced a protein already used in medicine as a resistance against ampicillin, an antibiotic. This is common practise in the production of transgenic organisms. As and when they are produced, the organisms that have incorporated the desired foreign gene (in this case the one for KP4) appear. The ones which have not can be eliminated forthwith. The experts are uneasy about the possible risk of the resistant gene spreading to other pathological bacteria and as a result making treatment by ampicillin ineffective. It would be quite possible to avoid the use of these resistant genes so it is a source of constant wonder that researchers persist in this technique thereby laying themselves wide open to criticism every time. Whatever happens, you can bet your bottom dollar you will be hearing more about KP4!

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For further information

Illustrations:

- Fig.1A, Source: http://www.inpv.edu.dz/manuel/7-Grandes%20cultures.htm
- Fig.1B, Adaptation: http://www.syngenta-agro.fr/synfront/index.aspx?idpage=48&idt=32
- Fig.2, Source: PDB ID : 1KPT, Gu, F., Khimani, A., Rane, S.G., Flurkey, W.H., Bozarth, R.F., Smith, T.J., Structure and function of a virally encoded fungal toxin from Ustilago maydis: a fungal and mammalian Ca2+ channel inhibitor. *Structure* **3** pp.805 (1995)

At UniProtKB/Swiss-Prot:

- KP4 killer toxin, Ustilago maydis P4 virus: Q90121

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