Future uses of biotechnologies in plant improvement. Monsanto's work in wheat transformation

Johanson P.F., Laudoyer O.

in

Di Fonzo N. (ed.), Kaan F. (ed.), Nachit M. (ed.).
Durum wheat quality in the Mediterranean region

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 22

1995
pages 221-226

Future uses of biotechnologies in plant improvement Monsanto's work in wheat transformation

P.F. JOHANSON
MONSANTO AGRICULTURE
ST. LOUIS, MISSOURI
USA

O. LAUDOYER
HYBRITECH EUROPE SNC
LESCAR
FRANCE

SUMMARY - Several quality traits in wheat can be improved by genetic transformation. New genes are introduced into wheat cells using a particle gun. Microscopic pellets of gold or tungsten are bathed in DNA and fired through cell walls. Anthocyanin, as a marker, was used to successfully develop systems for transformation and regeneration in wheat.

Key words: Genetic transformation, particle gun, anthocyanin, marker.

RESUME - *"Utilisations futures des biotechnologies en amélioration végétale. Les travaux de Monsanto pour la transformation du blé". Plusieurs caractères de qualité du blé peuvent être améliorés par transformation génétique. De nouveaux gènes sont introduits dans les cellules de blé en utilisant un canon à particules. Des balles microscopiques d'or ou de tungstène sont baignées dans l'ADN et bombardées à travers les parois cellulaires. L'anthocyanine, un marqueur, a été utilisée avec succès pour mettre au point des systèmes pour la transformation et la régénération chez le blé.*

Mots-clés : *Transformation génétique, canon à particules, anthocyanine, marqueur*

Introduction

The science of biotechnology offers a variety of improvements to tomorrow's wheat farmers, food processors, and consumers. Today's researchers are developing and perfecting the systems that will be used to introduce genetic improvements into wheat, traits such as resistance to virus, fungal disease and insect pests. Several quality characteristics in wheat can also be improved by genetic transformation. Ultimately, it may be possible to modify the quality of wheat to produce a bread with an extended shelf life without the addition of preservatives.

One of the leaders in this biotechnology research is Monsanto, an agricultural company located in St. Louis, Missouri. For more than a decade researchers at this facility have developed and tested some of genetically improved crops. In 1985, Monsanto and Coop de Pau created a joint-venture "HybriTech Europe" to develop the technology of hybrid wheat in Europe.

Monsanto researchers are developing and testing:

(i) Cotton, potato and corn plants with resistance to specific insect pests, such as lepidopters for cotton and corn (ball and bud worm and european corn borer respectively), or coleopters for potato (Colorado potato beetle), using *Bacillus thuringiensis.*

(ii) Soybean, canola and corn plants that tolerate Roundup herbicides.

(iii) Tomato plants that can ripe longer on the vine, providing improved flavour.

(iv) Potato and wheat plants that can resist to virus, such as PVY and PLRV (Potato Leaf Roll Virus) for potato and WSMV (Wheat Streak Mosaic Virus) for wheat.

Monsanto researchers are also leading biotechnology efforts in wheat. In the spring of 1992, researchers at the University of Florida, in collaboration with scientists at Monsanto, introduced new genes into fertile wheat plants for the first time. This paper will give you a depth look at the research in transformation and regeneration that is going on at Monsanto.

Particle gun

New genes are introduced into wheat cells using this device called particle gun (Fig. 1). In this process, microscopic pellets of gold or tungsten are bathed in DNA (or genetic material) and literally *fired* through cell walls (Fig. 2). As each tiny *bullet* passes through a cell, some of the DNA is left behind. The DNA from the pellet mixes with the DNA of the cell, thereby changing the genetic makeup of that cell.

Monsanto's researchers are coating the particles with two kinds of DNA, a selectable marker gene (glyphosate, Prialaphase, Methotrexate) and a non-selectable marker gene (GUS, Anthocyanin and Luciferase). Many of the cells accept one of the genes or the other, but some accept both genes.

Non-regenerable cells expressing anthocyanin

Before developing a transformation system in embryogenic cells (cells that can reproduce themselves into plants) researchers first tested the different markers in non-regenerative cells. With the selectable marker of glyphosate tolerance genes, one of the non-selectable markers used was anthocyanin, a regulatory gene derived from corn, or maize.

Anthocyanin is particularly advantageous when using a particle system because cells expressing the new genes are a dark red in pigment and are easily visible and countable. Anthocyanin markers allow visualization of transgenic tissues from beginning trough developmental stages without sacrificing tissues. Fig. 3 shows anthocyanin expressing in individual cells after 48 hours of bombardment under a microscope.

Glyphosate is introduced to the material, and only the cells that are tolerant continue to grow. Non-tolerant cells cease growth after 7-10 days. This allows the researchers to select for glyphosate tolerance (hence a selectable marker).

After 4-5 weeks, it is possible to see anthocyanin expressing in glyphosate resistant calli (red). This calli are growing on 3mM of concentrated glyphosate. And after 8 weeks, although all the calli must have the glyphosate resistant genes to continue to grow, only the calli that contains both genes can grow and express anthocyanin (red) (Fig. 4).

Non-regenerable cells expressing GUS

Another marker that was tested was GUS (β-glucuronidase), which is derived from a bacterium. Although not as readily visible as anthocyanin-expressing cells, cells expressing GUS turn blue with the application of a substance called X-gluc. Non-regenerative wheat cells have been bombarded with glyphosate tolerant genes and GUS. The blue dots are cells that are expressing GUS. After a certain period of time after bombardment, usually 3-7 days, the cells are exposed to glyphosate, only the resistant cells continue to develop calli. The glyphosate resistant calli are exposed to the X-gluc to see which ones also express GUS the blue ones (approximately 50 percent of the glyphosate-tolerant calli turn blue, representing calli with both genes, glyphosate tolerance and GUS) (Fig. 5).

Tissue culture

While some Monsanto researchers were developing the transformation system, other were working to perfect a tissue culture system in wheat that could support regenerable, transformed material. Tissue cultures starts with an immature wheat embryo derived from the wheat seed, approximately 12-15 days after pollination. This organized structure, or ex-plant, is able to reproduce into a new plant. In the 7th day of tissue culture, the embryo is showing the development of embryogenic callus. This embryogenic callus was derived from the immature embryo. It is transferred into another petri dish to grow on its own. From the callus grow shoots, and, eventually, whole new wheat plantlets. With a strong tissue culture system for wheat in place, researchers could begin to transform regenerable cells (Fig. 6).

Embryogenic cells expressing anthocyanin

We will now more explore the work with the regenerable cells with anthocyanin as a visible marker. Immature embryos with desired calli are ready for bombardment with the particle gun. Forty-eight hours after bombardment, the expression of anthocyanin genes can be seen in the embryogenic calli (red colour). Shoots begin to develop on the calli, anthocyanin expressing in the shoots can be seen (red) (Fig. 7). On an older plant, the anthocyanin can be seen expressing at the tip and the mid-rib of the leaf. Even in the regenerated plantlet, anthocyanin expression can be seen.

Anthocyanin, as a marker, was used to successfully develop systems for transformation and regeneration for wheat. Ironically, its easily visible expression, which makes it such a desirable marker, is an undesirable trait to carry forward in this research. Why? Well, no farmer would willingly buy wheat with red leaves.

Embryogenic cells expressing glyphosate tolerance and GUS

For that reason, Monsanto researchers continued their research with glyphosate resistance and GUS in regenerable cells.

The embryogenic calli are arranged for bombardment with the particle gun. The embryos will be bombarded with both the glyphosate resistant genes and GUS marker genes. After 48 hours, a portion of the bombarded tissue is exposed to X-gluc and the expression of the GUS genes can be seen (blue dots) (Fig. 8).

The calli are exposed to glyphosate and only the resistant calli continue to grow. When you treat that calli with X-gluc, you can easily see the expression of the GUS genes too. Glyphosate resistant calli develop shoots (Fig. 9).

With the X-gluc, the researcher can test shoots to see that they still express the GUS gene; in that case, they turn blue. The glyphosate resistant, GUS positive genes develop into plantlets. Again, to test the plants for the GUS gene, small pieces of leaf are treated with the X-gluc and they do turn blue. The glyphosate resistant, GUS positive plantlets are potted in soil and transferred to a climate-controlled growth chamber. These plants are fertile (Fig. 10).

Conclusion

Developing transformation and regeneration systems is just a first step in a long process. Next year, researchers hope to test some of these wheat plants in outdoor field conditions. At the same time, the company is identifying problems in wheat and looking for the gene solutions. Only when researchers begin to introduce desirable traits into wheat plants will these systems truly be tested.

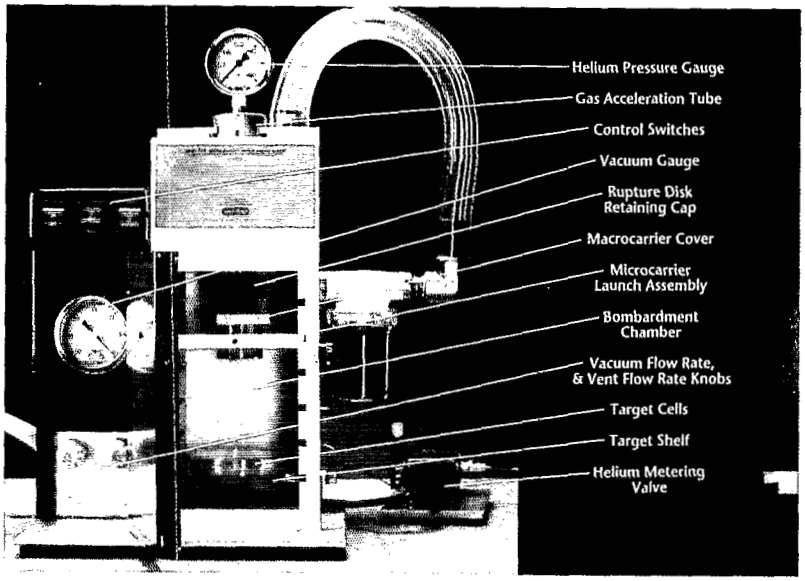

Fig. 1. Particle gun.

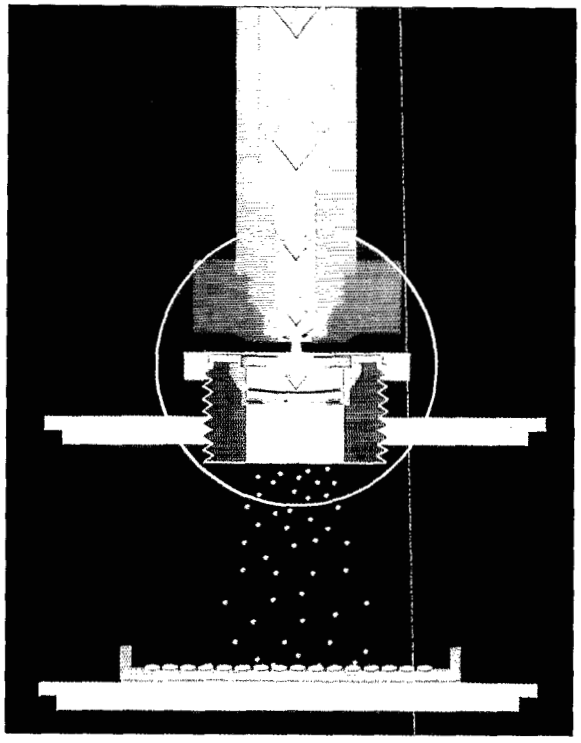

Fig. 2. Bombardment.

Fig. 3. Anthocyanin expressing in individual cells after 48 hours of bombardment under a microscope.

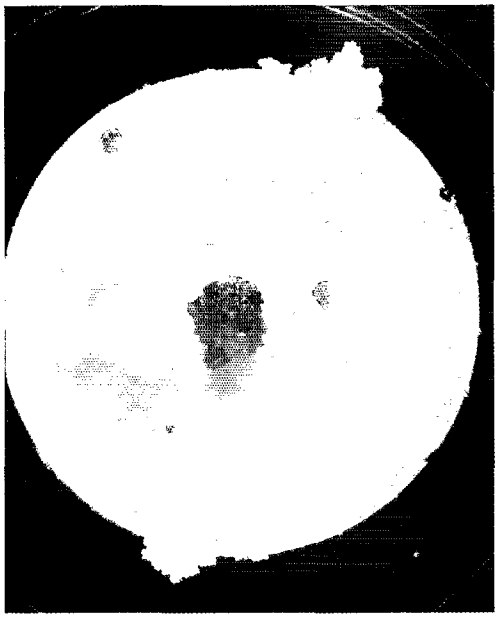

Fig. 4. Calli expressing anthocyanin (red).

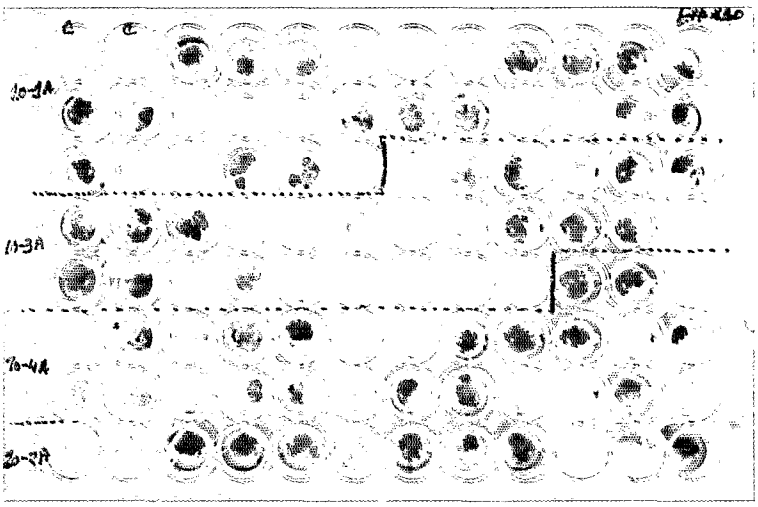

Fig. 5. 50% of the glyphosate tolerant calli turn blue, representing calli with both genes (glyphosate tolerance and GUS).

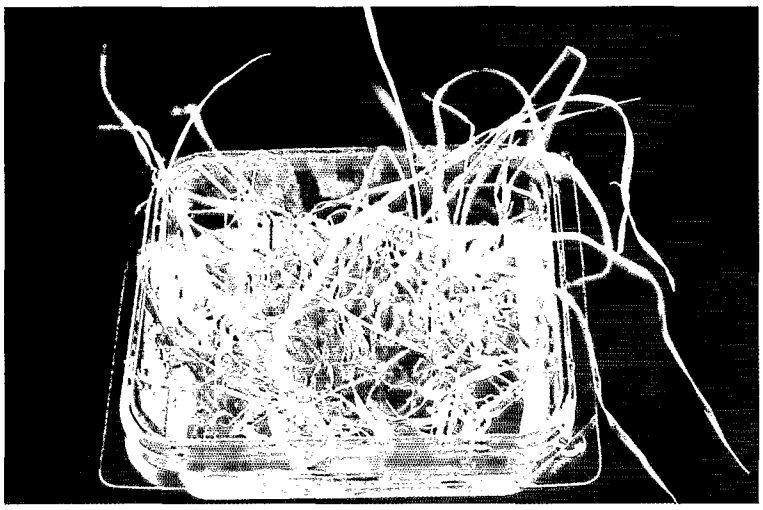

Fig. 6. Shoots growing from the calli.

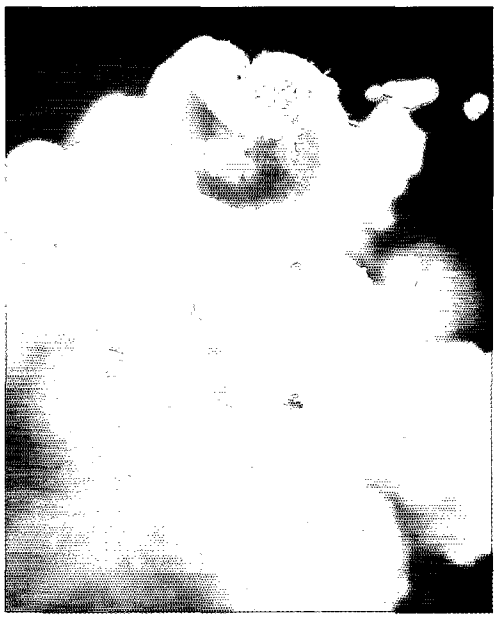

Fig. 7. Anthocyanin expressing in the shoots (red).

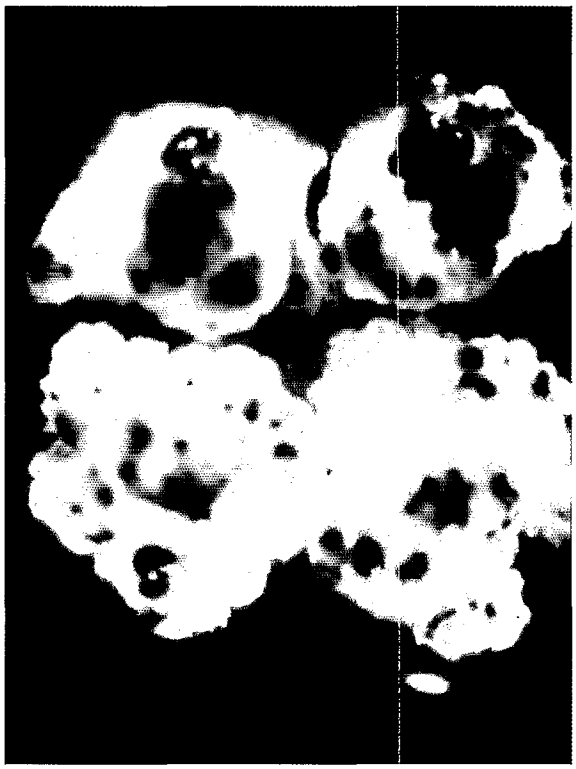

Fig. 8. Expression of the GUS gene (blue dots) in embryogenic callus 48 hours after bombardment.

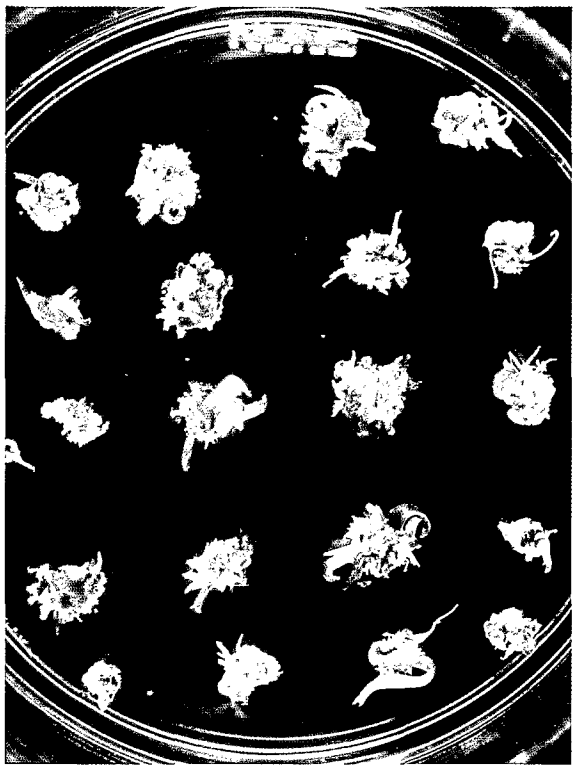

Fig. 9. Glyphosate resistant calli developing shoots.

Fig. 10. Fertile glyphosate resistant, GUS positive plants.