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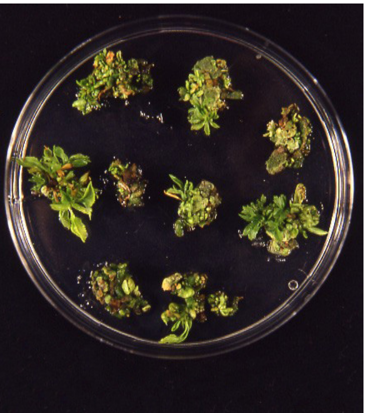

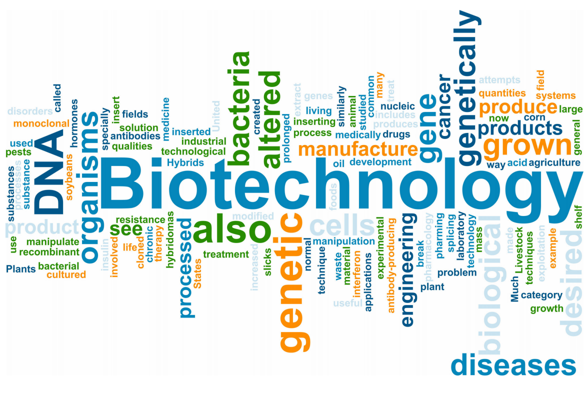

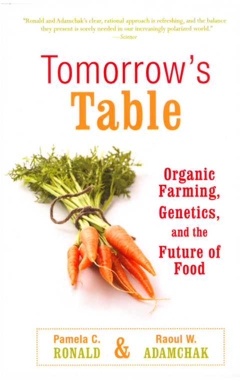

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Redaktör och ansvarig utgivare
Editor: J. Weibull

Redaktionsråd *(Editorial Council):*
Tomas Bryngelsson
Larisa Gustavsson
Per Henriksson
Roland Lyhagen
Inger Åhman

Adress *(Address)*: Sveriges Utsädesförening,
c/o Prof. Tomas Bryngelsson
Område växtförädling och bioteknik
SLU
Box 101
230 53 Alnarp

Tel. +46 40 41 51 74
Bankgiro: 485-0657

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Contact persons:
Anders Nilsson: Anders.Nilsson@slu.se
Tomas Bryngelsson: Tomas.Bryngelsson@slu.se

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Innehållsförteckning

(Contents)

Från redaktören

From the editor

Jens Weibull

– Måste vi ta till genmodifierade växter för att klara livsmedelsförsörjningen i framtiden? Hur skulle vi kunna kombinera de moderna teknikerna med mer skonsamma metoder för att få till ett mer resurshushållande jordbruk? Är det vigseln mellan ekologisk produktion och modern genteknik som är lösningen på framtidens livsmedelsförsörjning?

Dessa är några av frågorna som diskuterades på ett seminarium anordnat av KSLA i slutet av augusti 2012. Förutom forskarparet Pamela Ronald och Raoul Adamchek – makar och författare till boken *Tomorrow's table: Organic farming, genetics and the future of food* – deltog föredragshållare från en lång rad aktörer. Flera av inläggen återges i detta årets första nummer av tidskriften. Under fint moderatorskap av Annika Åhnberg diskuterades genteknikens möjligheter, eller begränsningar. Paret Ronald/Adamchek, som i sina yrkesroller representerar båda sidorna, menade att det inte råder några motsättningar mellan hållbar och resurssnål växtodling, och användning av moderna tekniker. Snarare kompletterar perspektiven varandra, menade de.

Diskussionen om den moderna biotekniken har många gånger kommit att betrakta den som det revolutionerande verktyget med möjligheten att en gång för alla lösa den globala livsmedelsförsörjningen. Så är det naturligtvis inte. Samma sak trodde man när mutationsforskningen gjorde sitt intåg eller när hybridförädlingen satte fart. Biotekniken – som här får omfamna en mängd olika och sinsemellan varierande tekniker – är bara ännu ett redskap i raden av alla de som vi kommer att behöva för att kunna hålla jämna steg mer de globala behoven. FNs livsmedelsorgan FAO gör bedömningen att vi år 2050 måste ha ökat vår jordbruksproduktion med 70 % vilket är en smått formidabel utmaning. Den borde göra det uppenbart att vi inte har råd att avhända oss alla möjligheter som står till vårt förfogande.

Men är inte den moderna biotekniken bara en fråga för den utvecklade världen? Det är ju här som de ekonomiska resurserna finns. Det är nog riktigt att det var så i den tidiga teknikutvecklingen. Men under en lång följd av år har forskare och studenter från utvecklingsländer kunnat tillgodogöra allt mer och hämta in det försprång som i-världen hade. Samtidigt har metoderna blivit alltmer tillgängliga och kostnaderna har sjunkit. FAO understödjer numera starkt användningen av resurssnåla bioteknologiska metoder och menar att en laddade GMO-debatten har överskuggat det värdefulla i de nya teknikerna. Det omfattande arbetet inom *The International Assessment of Agricultural Knowledge, Science and Technology for Development* (IAASTD)[1] underströk också vikten av att tillämpa den moderna tekniken för att komma till rätta med flera av utvecklingsländernas problem i jordbruket. Detta sagt, pekade man också på de risker som finns och kanske främst när det gäller koncentrationen av olika former av immaterialrättsligt skydd hos allt färre, och större, globala företag.

Som nyttig motvikt till de som redan frälsts av bioteknikens argument, och intellektuellt stimulerande, utgör Janne Bengtssons kritiska reflektioner. Han menar att där evolutionen redan har misslyckats kommer vi inte heller att lyckas. Dessutom finns det betydligt enklare sätt att öka jordbruksproduktionen i utvecklingsländerna. Lösningen stavas, bland annat, bättre resurshushållning och att vi ger begreppet hållbarhet en ny innebörd.

Sist i detta nummer bjuds ni på en fascinerande resa i korngenomets underbara värld. God läsning!

– Must we use genetically modified plants to cope with food insecurity in the future? How could we combine modern techniques with more gentle methods to get to a more resource-conserving agriculture? Is the marriage between organic production and modern genetic engineering thesolution to future food security? These were some

[1]http://www.unep.org/dewa/Assessments/Ecosystems/IAASTD/tabid/105853/Default.aspx/

of the issues discussed at a seminar organized by The Royal Swedish Academy of Agriculture and Forestry (KSLA) in late August 2012. Apart from Pamela Ronald and Raoul Adamchek - spouses and authors of *Tomorrow's table: Organic farming, genetics and the future of food* - speakers from a wide range of actors attended. Several of the talks are contained in this year's first issue. Eelegantly moderated by Annika Åhnberg, the seminar discussed gene technology, or its limitations. Ronald and Adamchek, in their professional roles representing both sides, held the view that there is no contradiction between sustainable and resource-efficient crop production, and use of modern technologies. Rather, the perspectives are complementary to each other, they argued.

The discussion of modern biotechnology has many times come to regard it as a revolutionary tool with the ability to once and for all solve global food supply. This is of course not the case. Same arguments were voiced when mutation research made its appearance, or when the hybrid breeding entered the scene. Frankly speaking, biotechnology - embracing a variety of different and mutually varying techniques - is just another tool in the line of all the ones we will need to keep up with global needs. The UN Food and Agriculture Organization estimate that by 2050, we must increase our agricultural production by 70%, which is a somewhat formidable challenge. This should make it clear that we cannot afford to dispose of all the opportunities available to us.

But is not modern biotechnology just an issue for the developed world? After all, it is here that the financial resources available. It is probably true that this was the case early in the evolution of technology. But over a period of many years, researchers and students from developing countries have been able to assimilate more and close the gap to the developed world. Meanwhile, the methods have become increasingly available and costs have fallen. FAO nowadays strongly supports the use of resource-efficient biotechnological methods and argue that an infected GMO debate has overshadowed the value of these new techniques. The extensive work of the International Assessment of Agricultural Knowledge, Science and Technology for Development (IAASTD) also stressed the importance of applying modern technology to overcome many of the problems in agriculture of developing countries. This said, IAASTD also identified the risks and perhaps primarily in terms of the concentration of various forms of intellectual property protection with fewer, and larger, global companies.

Janne Bengtsson's critical reflections, on the other hand, serve as an intellectually stimulating counterweight to the arguments maintained by those already redeemed by biotechnology. He argues that where evolution has already tried, and failed, we will likely do so as well. Moreover, there are much simpler ways to increase agricultural production in developing countries. The solution includes, among other things, better resource management and that we give the concept of sustainability a new meaning.

Finally, you are invited on a fascinating journey in the wonderful world of the barley genome. Happy reading!

Jens Weibull
jens.weibull@telia.com

GMO inom jordbruk och forskning – en kort introduktion

GMOs in agriculture and in research – a short introduction

Jens Sundström

Agricultural practises are currently divided into conventional and organic farming practises. In short, conventional farming allows the use of synthetic inputs such as mineral fertilisers and chemical pesticides while such inputs are not allowed in organic farming, as stipulated by the organic producers and their certification organisations.

The rules that are being implemented in Europe surrounding the cultivation and import of genetically modified (GM)-crops prohibit the integration of GM-crops, not only in organic farming systems but also in conventional farming systems. So called ”co-existence rules" that demand labelling, traceability and separation of GM-crops from conventionally bred varieties hinders the integration of GM-crops in existing crop-rotation schemes and we are, in practise, well under way to create three separate agricultural systems: one conventional-, one organic- and one GM-agricultural system, see *e.g.* (Fagerström et al., 2012).

One might argue that the ambition for agricultural science should be to develop an environmentally friendly agriculture that has the possibility to sustain an increasing demand for food and agricultural products, using the most efficient technologies available. However, the legal division between different agricultural practises that currently are being implemented is a major and sometimes unnecessary obstacle to this ambition.

Within the EU a GMO is defined as: "…an organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination" (EU Directive 2001/18).med omkring 35-45 nya sorter per år. I höstvete, vårkorn, havre, höstraps och vårraps har 5-10 nya sorter årligen anmälts och i höstråg, rågvete, vårvete och ärter ca 1-5 nya sorter per år. Antalet sorter av åkerböna, potatis, vallväxter är betydligt mindre och det anmäls i medeltal mindre än en ny sort per art och år. Av de sorter som anmäls till provning är det i regel endast en liten del som godkänns och tas in på den svenska sortlistan och sedan släpps ut på marknaden.

In practise, what is considered a GM-crop is decided by the breeding technique used and not the final properties of the crop. Hence, two crops with identical traits, one being bred using transformation methods and one being bred using conventional methods such as mutagenesis, chromosome doubling and embryo rescue are treated as inherently differently when it comes to rules for risk-assessment and co-existence. The former being a GMO has to go through an extensive and very costly risk-assessment before any import or cultivation can be approved, while such rules do not apply to the latter. Despite the fact that many conventional breeding methods cause far more large-scale and unknown changes in the genome of a plant than the directed changes associated with transformation methods.

Modern biotechnologies such as large scale sequencing of entire genomes, functional studies of individual genes and marker-assisted breeding have also demonstrated that the domestication process in itself is associated with large and often unpredictable genomic changes. As also noted by Werner Arber in 2002, " …naturally occurring molecular evolution, i.e. the spontaneous generation of genetic variants has been seen to follow exactly the same three strategies as those used in genetic engineering. These three strategies are:
(a) small local changes in the nucleotide sequences,
(b) internal reshuffling of genomic DNA segments, and
(c) acquisition of usually rather small segments of DNA from another type of organism by horizontal gene transfer."(Arber, 2002).

Despite this, the misconception still prevails that GM-crops have more unintended effects than conventional crops (Ammann 2012).

One specific aspect of having a legislation that is technology-based rather than based on agricultural properties of the resulting crop is that the legislation becomes irrelevant as technology development proceeds. In Europe, we now have several breeding techniques under development that are not covered by the current legislation. Those techniques include, but are not limited to: Oligo-Directed Mutagenesis (ODM), Reverse breeding, Zink-finger nucleases and Agro-infiltration. Common for many of the new techniques are that they involve recombinant DNA in one step of the breeding process but the products that reach the market or crop that is cultivated do not harbour any recombinant DNA.

If legislators should decide that crops bred using the new breeding technologies indeed are GMOs this will pose a direct problem for risk-assessors, since many of the crops will be impossible to distinguish from existing conventionally bred varieties. In addition, depending on how legislators decide, further technology development of the new breeding techniques are likely going to be moved out of Europe causing loss of scientific know-how and future enterprises in Europe.

To conclude, the current GMO-legislation in Europe is not science-based and puts up unnecessary road-tolls on a specific technique that, if integrated with other improved agricultural practises, could help to develop a future sustainable agriculture. In fact, the current legislation hinders such integration and development. To amend this situation, European legislators need to reform the current bio-safety legislation and develop a legislation that is technology neutral and instead focuses on the properties of the developed products.

Sammanfattning

Den nuvarande GMO-lagstiftningen i Europa inte är vetenskapligt grundad och ställer upp onödiga hinder för tekniker som, om de hade integrerats med andra förbättrade jordbruksmetoder, skulle kunna bidra till att utveckla ett framtida hållbart jordbruk. I själva verket hindrar den nuvarande lagstiftningen sådan integration och utveckling. För att ändra på denna situation så behöver EU reformera den nuvarande biosäkerhetslagstiftningen. I stället bör man utveckla en lagstiftning som dels är teknikneutral och dels fokuserar på egenskaperna hos de utvecklade produkterna.

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Jens Sundström är universitetslektor vid SLU, Inst. för växtbiologi och skogsgenetik.
jens.sundstrom@slu.se

Jordbrukets bioteknologi – behovet av större vidsynhet

Agricultural biotechnology – the need for less myopic perspectives

Sven-Ove Hansson

It is now twenty years since the first genetically modified plant was commercialized (James and Krattiger 1996, p. 24). In the two decades since 1992, agricultural biotechnology has developed at a remarkably fast pace, and currently about 11 % of the land used for agriculture carries genetically modified crops (James 2011). Already a decade ago, it was estimated that 60-70 % of the food items available in North American grocery stores contained genetically modified organisms (MacDonald and Whellams 2007). Therefore, in a global perspective, genetically modified (GM) crops are something that we have considerable experience of, and we are now in a position to base our judgment on that experience.

It should be quite clear by now that genetic modification is a breeding tool that can be used to develop agriculture in quite different directions. The technology can be used to introduce traits that increase yields at the price of worsened environmental impact. But genetic modification can also be used to introduce traits that reduce the negative environmental impacts of agriculture (Hansson and Joelsson 2012). With the accumulated knowledge and experience of GMO it becomes increasingly untenable to judge all uses and potential uses of this technology in the same way. No one should close their eyes to the fact that this technology can be used to introduce undesirable traits, such as traits damaging the environment that cannot be introduced with conventional breeding alone. And neither should anyone close their eyes to the fact that the same technology can be used to introduce desirable traits, such as environmentally beneficial traits, that cannot otherwise be obtained.

Decisions are being made all the time about the future development of GM crops, about what traits to develop and about their integration into crop varieties. In my view, environmental issues and the requirements of a sustainable agriculture should have a much stronger influence on these decisions. For this to happen, corporate decision makers have to pay more attention to the environment and listen more to those who voice environmental concerns about their products. And friends of the environment will have to distinguish between different uses of biotechnology and even be willing to investigate under what conditions such technologies can be used to solve environmental problems.

In this contribution I will focus on three questions, or perhaps rather problem areas, that will require careful study and consideration if we want to get a grip on the environmental aspects of biotechnology. Being the programme manager of a recently started research programme, Mistra Biotech, that investigates the potential for using biotechnology as a tool in sustainable Swedish agriculture, I have benefited a lot from discussing these issues with other researchers in the programme.

A heavily criticized system

There has been a lot of criticism both against the legislative–regulatory system and against the major companies that are involved within that system in the development of agricultural biotechnology.

The legislative system has been criticized for putting a too heavy economic burden on the introduction of genetically modified varieties. Small companies have virtually no chance of introducing a product into the market, which has therefore arguably developed into an oligopoly. The high regulatory costs have also lead to a concentration of GM-supported breeding efforts to a few economically highly important crops at the expense of other crops that may nevertheless be important for farmers and consumers.

The legislative system has also been criticized for being technologically asymmetric. We have very strict regulations on the introduction of a new crop variety if it has been obtained with GM technology, but no such restrictions apply if the variety has been obtained by traditional means.

The large companies that develop new GM varieties have been criticized on several grounds. They have been claimed not to give sufficient priority to environmental and health-related breeding goals, to introduce undue dependence on environmentally damaging herbicides and to construct the crop system in ways that make farmers dependant on continuously buying their products.

The latter criticism should be understood against the background that the breeding industry is in the same situation as the film, music, and computer game industries: they all make products that can be copied at low cost. In order to conduct viable business, they need to find means to charge those who use their products. The legal system leaves certain ways for them to do so, including patents (for GM crops), plant breeder's rights (for both GM and non-GM crops), and (also for both GM and non-GM crops) various traits that make seed saving uneconomical for the farmer. We cannot take for granted that the current system is optimized in the sense of tailoring the incentives of business companies so that their self-interest is reasonably consonant with the furthering of socially desirable outcomes.

The need for a broader perspective

Much of the discussion has had a rather one-sided focus on criticism either of the regulatory system or the business corporations. In my view we need to see the issue in a broader perspective and ask the question: *What are the combined effects of the legislative-regulatory system and market-driven corporate decisions, and how does the outcome compare to our goals for a socially desirable development of the structure of food production and distribution?*

In answering this question we should consider the effects for instance on the choice of crops for breeding and on the selection of specific traits as breeding goals – for instance whether traits important for sustainability and human health are given sufficient priority. Furthermore, effects on the social and economic structure of the food production and distribution system need to be taken into account. Obviously, these are questions than can, and should, be asked with respect to plant breeding in general, irrespective of the technologies it makes use of.

A better understanding of the total public-private system involved in agricultural biotechnology may give us reasons to change it. Possible candidates for change include the regulations referring to GMO and to breeding in general, the property regime as it affects plant varieties, the division of research labour between university and business corporations, and research priorities in general.

It is a common misconception that large and entrenched social systems are unchangeable. History shows that even global systems can be changed, although it takes time, judiciousness and considerable knowledge to achieve such changes.

European exceptionalism

Europe differs from the rest of the world in having a legislation that has in practice all but excluded genetically modified products from farming and from the food and feed production system. However, the experience from the last two decades shows that the use of genetic technologies leads to faster progress in the development of improved plant varieties. It can therefore be expected that as time passes, the price of non-GMO products will be increasingly higher than that of equivalent GMO products, at the same time as some products can be obtained with but not without this technology. This gives rise to a second, very simple question: *How long is the European exceptionalism in the GMO area tenable, and what can happen if and when it is given up?*

Specifically, if the current European exceptionalism in the (non)use of agricultural biotechnology comes to an end, what will that end look like and what will come after it? What type of influence will European citizens have over what happens, through the political process and/or as individual consumers when buying their daily food? Will GM food be introduced without specific labelling as in other parts of the world, or will it be accepted by individual consumers in Europe because of some or other properties that they have?

It is important to realize that we European consumers are not all alike. There are different segments of the population. For instance there is a segment of consumers whose worldview is difficult to reconcile with the consumption of GMO foods, but there is also another segment of consumers that are much concerned with what food does to their health and who basically rely on medical science. For the latter group of consumers, foodstuff based on plants with a more healthy composition may well be interesting irrespective of the breeding technology. There is also, unfortunately, a large segment of the population for whom the price of food is of necessity the major determining factor.

The prerogative of direct advantages

When do contested new technologies become accepted? Judging by the experience we have, direct individual advantages are often a crucial factor in the processes leading to acceptance. One example of this is the almost universal acceptance of mobile phones, in spite of a considerable flow of assertions that they are dangerous to the health of those who use them. As far as I can see, the major reason for this is that this technology has a personal advantage that is direct, undoubted, and not achievable by alternative means. There is no other means than the mobile phone by which I can talk to a friend in another town while walking in the street.

Nuclear energy is one of the best examples of the opposite situation. There is considerable public opposition to nuclear energy, and there was indeed much such opposition already before Three Mile Island, Chernobyl, and Fukushima. As the nuclear industry often points out, consumers benefit from nuclear energy whenever they turn on the light or the TV, but from the viewpoint of the consumer it is not undoubted that the same advantage cannot be obtained without that technology. This should not be dismissed as a sign of irrationality on the side of the public; it is not irrational to be less moved by an uncertain than by a certain benefit.

Most of the traits that have been achieved up to now with agricultural biotechnology provide improvements in food production, rather than improvements in food quality. They may have direct advantages for the farmer, but they do not have the very direct advantages to the consumer as exemplified by mobile phones. It is an open question what the public reactions would be to genetically modified products with important health advantages. This leads to my third question, more immediately aimed at plant breeders than the previous ones: *What direct advantages to consumers can be achieved by plant breeding?*

All these questions and many others will be addressed in various ways within the Mistra Biotech programme. However, these are large issues that we cannot solve alone. We need to co-operate and communicate with others, both researchers and practitioners, in order to gain a better understanding of the complex relationships between biotechnology and environmental sustainability.

Sammanfattning

Artikeln är ett försök att se på och analysera de nya bioteknologiska metoderna utifrån ett samhällsvetenskapligt perspektiv. Författaren resonerar bland annat utifrån följande frågor: Vilka är de sammanlagda effekterna av de lagstiftande regelsystemen och de marknadsstyrda företagens beslut, och hur ser det resultatet ut jämfört med våra mål för en socialt önskvärd utveckling av strukturen för livsmedelsproduktion och -distribution? Hur länge är den europeiska exceptionalismen inom GMO-området hållbar, och vad kan hända om och när den överges? Vilka direkta fördelar kan man genom växtförädling uppnå för konsumenterna? Frågor som dessa kommer att belysas i det nya programmet Mistra Biotech. Det är en utmaning som kommer att kräva samarbete och samtal med andra, både forskare och praktiker, i syfte att få en bättre förståelse för de komplexa sambanden mellan bioteknik och miljömässig hållbarhet.

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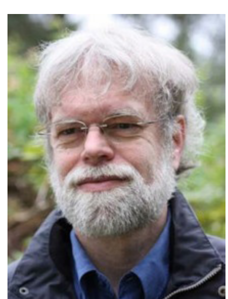

Sven-Ove Hansson är programchef för Mistra Biotech[1] och för närvarande knuten till SLU och Institutionen för växtproduktionsekologi.
soh@kth.se

[1] *http://www.slu.se/centrumbildningar-och-projekt/mistra-biotech/*

Vart är Europeisk växtbioteknisk forskning på väg?

Where is European research on biotech going?

Sten Stymne

Växtbioteknisk forskning inkluderar både forskning som använder bioteknik för att besvara frågor av grundläggande natur rörande livsprocesserna i växterna och tillämpad växtbioteknik. Den tillämpade växtbiotekniken har som syfte att styra växtens egenskaper på ett för människan optimalt sätt. Jag kommer här att enbart behandla situationen för den tillämpade växtbiotekniska forskningen som rör utvecklingen av växter med hjälp av genöverföring, dvs. GM-växter. Den tillämpade forskningens volym och resurser är beroende av det stöd som den får från de som tillgodogör sig dess resultat, dvs. näringsliv och samhälle. Därför är det förväntade framtida nyttiggörandet av växtbioteknikens resultat helt avgörande för investeringarna i denna forskning.

Det är i år (2013) precis 30 år sedan den första genmodifierade växten skapades. Vilket genomslag har då gentekniken på växter haft under dessa 30 år? Man kan nog säga att det varit en succéhistoria. Bara nio år efter den första GM-växten skapats kom den första kommersiella genmodifierade växten. Idag är ca 11 % (2011) av jordens åkerareal planterad med GM växter. Sjuttiofem procent av all soja, 82 % av all bomull, 32 % av all majs och 36 % av all raps som odlas är genmodifierad. Inom EU är odlingen av GM-växter dock nära obefintlig. Endast två GM-växter (s.k. event) är godkända för odling: Monsantos majs 'Mon810' med insektsresistens (Bt) och BASF:s potatissort 'Amflora' med hög halt av amylopektin. Av dessa två odlas bara majsen och det på en total yta av bara ca 100 000 ha (0.06 % av EUs jordbruksareal). Trots att dessa två GM-grödor är godkända inom EU så har regeringar i flera länder, i strid med EU-lagstiftningen, förbjudit odlingen. Man har då åberopat risker för hälsa och miljö som kan vara förenat med dess odling, ofta hänvisande till larmrapporter som, efter granskning av EU:s organ för livsmedelssäkerhet (EFSA), visat sig vara helt ogrundade.

EU:s GMO-lagstiftning föreskriver att odling av grödor för livsmedel som är gentekniskt förädlade skall särhållas från grödor som är förädlade med äldre, s.k. traditionella, metoder. För GM-grödor godkända för odling tillåts dock en viss oavsiktlig inblandning av GM-material, uppgående till maximalt 0.9% av just den aktuella livsmedelskomponenten i ett livsmedel. Överstiger inblandningen detta värde, eller är inblandningen avsiktlig, skall livsmedlet märkas med att det innehåller produkter från genmodifierade växter. För icke godkända GM-grödor, eller produkter som inte är godkända för livsmedel men kommer från för odling godkända GM-grödor, tillåts ingen inblandning alls. För Monstantos godkända majs 'Mon810' hade man inte sökt tillstånd för att använda pollenet i livsmedel, vilket uppmärksammades av tyska anti-GMO-aktivister som påpekade att honung innehållande sådant pollen inte får försäljas som livsmedel. Detta har orsakat stora problem för biodlare att få sälja sin honung i de få områden där man odlat 'Mon810'. Monsanto har nu sökt tillstånd att använda också pollen från 'Mon810' i livsmedel och tillstånd förväntas erhållas senare i år. Ett större bekymmer är att fältförsök med icke godkända GM-växter för att utröna dessas agronomiska potential, och eventuella miljöpåverkan, har blivit mycket svåra att genomföra. Skall lagstiftningen tillämpas strikt så får inte ett enda pollenkorn hamna i honung, vilket naturligtvis inte går att garantera.

Under säsongen 2012 gjorde vi fältförsök i Skåne med genmodifierad oljekål med oljekvaliteter som är skräddarsydda för industriella tillämpningar. För att minimera spridningen av pollen till honung blev vi ålagda av Jordbruksverket att dra ett tätt insektsnät över odlingarna, vilket ledde till att växterna inte utvecklades på det sätt de skulle ha gjort utan nät. Skörden blev ungefär hälften jämfört med odlingar utan nät, och både växter och frön blev svårt angripna av svamp, vilket gjorde försöket helt värdelöst ur vetenskaplig synpunkt. Om inte en pragmatisk överenskommelse om avstånd till närmaste bikupa för fältförsök med GM-grödor kan komma till stånd så kommer det i praktiken att stoppa all vidare utveckling av GM- grödor inom EU. Eftersom många anti-GMO-organisationer har ett stoppande av teknikens användning på sin agenda så är det inte troligt att sådan överenskommelse kan uppnås. De har ju GM-lagstiftningens nolltolerans att stödja sig på.

Det häftiga motståndet mot odling av GM-grödor och utformningen av EU:s GMO-lagstiftning har lett till att de bioteknikföretag som sysslar med utveckling av GM-grödor inte längre utvecklar några sådana grödor för odling i Europa. Det har också flyttat merparten av sin forskning utanför Europa. Sist i raden av dessa företag var BASF som förra året deklarerade att de stoppar all växtbioteknisk verksamhet i EU och flyttar merparten av sin forskning till USA. Detta trots att BASF hade GM-potatisen ’Amflora’ godkänd för odling och en bladmögelresistent GM-potatis långt framskriden i godkännandeprocessen.

Att EU avsagt sig odling och utveckling av GM-grödor betyder inte att EU inte använder GM-produkter. Soja är en huvudingrediens i djurfoder och EU importerar 98 % av all soja som konsumeras (40 miljoner ton). Av denna soja är 95 % GM. Produkter från trettiosex olika GM-växter är godkända för import till EU för användning i livsmedel och foder. Sjuttiotre ytterligare GM-växter är under regulatorisk prövning för import. I och med att antalet GM-växter med olika genmodifieringar växer lavinartat runt om i världen förväntas ytterligare många hundra sådana ansökningar under de närmaste åren. Anledningen till att EU å ena sidan förhindrar odling av GM-växter och å andra sidan importerar väldiga mängder sådana kan tyckas svårförståeligt. Det kan delvis förklaras med att frihandelsavtalen sätter gränser för vilken import som kan förhindras samt den merkostnad jordbruket skulle behöva belastas med om det avhände sig användandet av GM-produkter i foder.

De kommersiellt odlade GM-grödor vi har idag har i stort sett bara två egenskaper införda, insektsresistens genom produktion av Bt-protein och tålighet mot bredspektriga ogräsmedel. Genom den snabba utvecklingen av växtforskningen öppnas möjligheterna att med genteknik introducera mycket mera sofistikerade egenskaper med genteknik. Vad vi kan åstadkomma med tekniken är svindlande. Med en kraftig satsning på den gentekniska forskningen skulle vi inom en relativt kort tid få fram perenna grödor som kan binda väldiga mängder kol i marken. Om större delen av vår jordbruksareal skulle planteras med sådana grödor skulle inom några decennier koldioxidnivåerna i atmosfären vara nere på förindustriella värden. Jag och mina kollegor bedriver forskning som med hjälp av genteknik skulle kunna tredubbla produktionen av vegetabiliska oljor utan att behöva öka den odlade arealen och ändå få tillräckligt med växtolja för både livsmedel och för att ersätta hälften av all fossil olja i den kemiska industrin.

Att EU har avsagt sig tillämpningen av gentekniken på växter och att industrin har flytt återspeglas nu i en krympande budget av allmänna medel till denna forskning. EU har inte längre har en enda utlysning av forskningsprojekt syftande till att ta fram grödor förbättrade med genteknik. I Sverige gav Formas, det forskningsråd som ansvarar för jordbruksforskning, inte ett enda anslag till tillämpad växtbioteknisk forskning i 2012 års utlysning, trots Sveriges framstående position inom området. På sikt kommer detta också att drabba den grundläggande växtforskningen. Redan ser vi att växtvetenskaperna får allt färre studenter i många länder i Europa. Om det inte finns några utsikter till en näring som vill utnyttja denna forskning så är framtidutsikterna också dystra för de studenter som utbildar sig inom området.

Naturligtvis kommer växtbiotekniken, förr eller senare, att spela en avgörande roll också i EU för ett miljövänligt och högavkastande jordbruk. Frågan är vilket pris EU måste betala innan detta sker: miljömässigt, ekonomiskt och forskningsmässigt.

Abstract

Thirty years ago the first genetically modified plant was developed and nine years later the first GM crop was released. Today, 11 % of the world’s total acreage is planted with GM crops. EU imports 40 million tons of soybean annually, 95 % of which is GM and used mostly for production of animal feed. In addition, products from 36 other GM crops are free to import for use in food and feed. Nevertheless, the legislative framework in Europe maintains very strict regulations on biotech and GM research, so strict that large enterprises like BASF now move their related activities to USA. Obviously plant biotechnology, sooner or later,

will play a crucial role also in the EU for developing an environmentally friendly and high-yield agriculture. The question is what price Europe must pay before this happens: environmentally, economically and in terms of research.

Sten Stymne är professor i växtförädling vid SLU och Institutionen för växtförädling i Alnarp.
sten.stymne@slu.se

På vilka sätt skulle modern bioteknologi kunna vara en del av hållbart jordbruk?

In which ways could modern biotechnology be part of sustainable agriculture?

Jan Bengtsson

Abstract

I propose that biotechnology is not a major means to sustainable and sufficient food production in the future. There are several arguments for this: Firstly, a future with scarcity of natural resources implies that crop improvements based on biotechnology may be difficult to realise, because these actually usually rely on using more rather than less resources, despite arguments of the contrary among proponents of biotechnology. Secondly, for most traits discussed by proponents of biotechnology, evolution has already been exploring the simple trade-off-free improvements that can be made. Hence many of the promises of biotechnology may not be possible. Thirdly, other means to solve the problem of sustainably feeding the world, like developing resource-efficient and sustainable and multifunctional productive farming systems, are more likely to be successful, and more efficient. This entails a radical rethinking of politics and of social, economic and ecological organisation. However, within the constraints of sustainable farming systems, some biotechnological methods may be useful tools in breeding, but this requires a more thorough discussion about the meaning and implications of sustainability.

Key words:

Biotechnology, food production, natural resource scarcity, evolution, trade-off, sustainability

Introduction

The usefulness of biotechnology in future agriculture has been debated for years. Some proponents argue that biotechnology, and especially using transgenic methodology to produce genetically modified organisms (GMOs), is crucial to increase agricultural production, enhance food security and contribute to sustainability (Conway 1998; Fedoroff et al. 2010; Fagerström et al. 2012; see also Ronald & Adamchak 2008 and Denison 2012 for references to more examples). Critics of biotechnology, on the other hand, argue from different perspectives. Some are of the opinion that many aspects of biotechnology, especially GMOs, are inherently risky and therefore should be treated with utmost caution (e.g., Greenpeace[1]). There are also those whose main argument is based on viewing such genetical modification as ”against nature” and unethical[2].

During the last few decades, these debates have been caught in highly polarized positions unable to come forward with solutions to the challenges of sustainable and sufficient food production. In this article, I will provide a third perspective and discuss why biotechnology, whether risky or not, is unlikely to solve the problem of sustainably feeding the world. Instead, it is necessary to start from the notion of sustainable farming systems. This will call attention to issues related to social change, the distribution of resources and knowledge, and to changes in diets and other consumer patterns.

My aim is to add some considerations that have received less attention in the past. The first is related to the idea of the looming scarcity that is central in most assessments of future agriculture, food production and food security. A future with scarcity of natural resources challenges the arguments that biotechnology will be central to solving the problems facing future agriculture.

The second consideration is a corollary to the first, and is based on the fact that for most – but not all – traits discussed by proponents of biotechnology, evolution has already been exploring the simple trade-off-free improvements that are most likely. Hence many of the promises

[1] *http://www.greenpeace.org.uk/global*

[2] *http://en.wikipedia.org/wiki/Genetically_modified_food_controversies*

of biotechnology may not be possible, or at least much more difficult to realise than asserted (Denison 2012).

Finally, I will argue that if we really are interested in sustainably feeding the whole world, other measures and techniques to solve this dilemma are much more likely to be successful, and also more efficient. A focus on developing sustainable farming and production systems requires a radical rethinking of politics and of social, economic and ecological organisation. However, when designing sustainable farming systems, it may nonetheless be useful to seriously consider using aspects of biotechnology, perhaps also including GMOs, as argued by, for example, Ronald & Adamchak (2008). However, this should be done within the constraints of sustainable farming systems and by a thorough discussion on the meaning and operationalisation of sustainablity, rather than the other way around (i.e., technology defining which farming systems should be used, as in most modern intensive agricultural systems).

My key proposition is that the largest risk with biotechnology is that it is unlikely to be able to deliver what it promises. Thus excessive focus on this technology in research and development withdraws limited resources from other areas that are more likely to contribute to solving the challenges of future food production and food security (Vanloqueren & Baret 2009; Denison 2012).

Before proceeding it is important to emphasise that biotechnology is much more diverse than GMOs, encompassing a large set of modern techniques used in breeding, genetics, molecular biology, evolutionary biology, medicine and industrial processes. While some applications of biotechnology obviously suffer less from the critical arguments I discuss, the issues are more general than pertaining to GMOs only. There is also often a large difference between employing a technique in the laboratory and large-scale use in a non-contained environment.

Biotechnology and the predicted scarcity of natural resources

Most analyses of the challenges for future food production and land use have in common that natural resources and land for food production will be more scarce in the future, resulting in rising prices and more competition for land and water (SCAR 2011; The UK Foresight, The Royal Society 2009, Pretty et al. 2010; The Swedish Future Agriculture, Öborn et al. 2011). For example, the European Commission's Standing Committee for Agricultural Research (SCAR) concludes that " *… increasing scarcity of natural resources and destabilisation of environmental systems represents a real threat … to future food supplies*" and that "*many of today's food production systems compromise the capacity to produce food in the future*". A drastic transition towards efficiency and resilience that cannot follow the "*common narrative of increasing productivity*" is needed. Finally, the group argues that a "*radical change in food consumption and production in Europe is unavoidable to meet the challenges of scarcities … and uncertainty*". I have quoted this document at some length, because this is not stated by some obscure green doomsday prophets, but by an established body within the EU system.

Similar calls for considering a coming scarcity, and the ensuing price increases, have been issued for many natural resources necessary for agricultural production. Concerning energy, peak oil has been discussed for quite some time (Aleklett et al. 2010; Aleklett 2012) and according to some estimates it may already have happened during the first decade of the 2000-ies. Similarly, Heinberg & Fridley (2010) announced that the age of cheap coal is at its end, although the world will probably not run out of oil and coal, at least during this century. It is highly unlikely that bioenergy will be able to compensate for this, given concerns about water scarcity, biodiversity, land degradation and competition for land for food production (Foley et al. 2011; Offermann et al. 2011). Cordell et al. (2009) discuss the likelihood of peak phosphorus which they predict by 2030, and it is possible that some micro-nutrients may peak even earlier[3]. At the 2012 World Water Week in Stockholm it was reported that global water demand is likely to be larger than global supply by 2030. By then potential yields may have decreased by up to 30% in large areas of Africa and the US as a consquence of the drier and warmer climate following from

[3] Pernilla Tidåker & Ingrid Öborn, Dept. Crop Production Ecology, SLU. Personal communication.

climate change[4].

Competition for arable land will also become more intense in the future. Many of the world's largest cities are expropriating fertile agricultural land, turning it to deserts of asphalt, concrete and shopping malls that are difficult to revert to cropland. Production of bioenergy and other biomaterials are also likely to compete with food production on the approximately 10% of the global land area that can be used for crop production (Foley et al. 2011).

A problem less often recognised, but fairly well known in economics[5], is the fact that new technologies historically have resulted in increased rather than lower total use of resources, primarily energy. This presents a problem for sustainability (Wackernagel & Rees 1997). If technological development tends to increase total resource use, but the world's resources are becoming ever scarcer, the question arises if biotechnology can be an exception to this strong historical trend. And if so, why would biotechnology be able to perform such magic? Present use of biotechnology in agriculture does not support this the view that biotechnology will reduced total resource use, even though efficiency per unit input energy (or other resources) may increase. In fact, nitrogen use efficiency has rather decreased during the last 50 years (Tilman et al. 2001)

Is biotechnology able to address these concerns? My tentative answer is ”No”, if this technology is regarded as a spearhead toolkit for the world's future food production, as some proponents seem to argue (e.g., Wambugo, 1999; Fedoroff et al. 2010; Fagerström et al. 2012). I find it illuminating that many recent analyses of future food production do not highlight biotechnology as the main solution to feeding the world by 2050. Pretty et al. (2010), based on the UK Foresight program[6], has ”Crop genetic improvement” as one of thirteen central areas, and only 3 of 100 ”top questions” directly concern biotechnology. A larger number of important questions relate to natural resources, ecosystem services, agronomic practices and agroecology, and social and economic issues. Likewise, Foley et al. (2011) mention ”genetic improvements” of crops as one approach among several. Godfray et al. (2010) have a more optimistic view of biotechnology, in particular they consider GM crops to be potentially valuable, but they also state that public acceptance and trust is needed before ”*it can be considered as one among a set of technologies that may contribute to improved global food security*”. Still, Godfray et al. argue that a broad range of options need to be pursued simultaneously, and they also highlight the difficulties in navigating the ”*complex landscape of production, environmental, and social justice outcomes*”.

The IAASTD report “Agriculture at a crossroads” (2009) and the French Agrimonde (Paillard et al. 2011), on the other hand, highlight the importance of ecological processes and design of multifunctional farming systems for future food security, with biotechnologies playing a subordinate role. In particular, in addition to *ecological intensification*, Agrimonde emphasises questions about social issues, equity, consumption behaviour, trade patterns and governance. In the Swedish Future Agriculture research program (Bengtsson et al. 2010), based on global and European scenarios (Öborn et al. 2011), biotechnology, and risks associated with using or not using it, is mentioned, but the technology as such is given low priority compared to issues related to climate change, social values, land use conflict resolution and rural development.

None of these studies dismiss biotechnology, but it is not emphasised as a major tool for addressing future food production and security. While technological improvements are regarded as necessary in all analyses, the needs for technology development are rather directed towards farming practices and resource conservation than genetical improvements. In addition, social issues like equity and rural development are usually given much more emphasis than biotechnology.

[4] Colin Chartes, IWMI. Plenary talk on ”The food and Water paradox” at the 2012 World Water Week.

[5] http://en.wikipedia.org/wiki/Jevons_paradox. Here it is, for example, argued that ”To ensure that efficiency enhancing technological improvements reduce fuel use, efficiency gains must be paired with government intervention that reduces demand”.

[6]http://www.bis.gov.uk/assets/foresight/docs/food-and-farming/11-546-future-of-food-and-farming-report.pdf

Hence, one could question if biotechnology really should be regarded as an important tool for solving the future challenges concerning global food production. This becomes even more questionable if we turn to the next perspective – is it really likely that biotechnology can deliver what it promises?

Biotechnology in the light of evolution

It is a basic tenet of evolutionary biology that there are fundamental trade-offs among organism traits of importance for fitness[7]. Trade-offs occur when improvements in one trait or process has negative effects on other traits (processes). Trade-offs are ubiquitous in nature, which is clear from an assortment of important concepts in evolutionary ecology, for example, the r-K-selection theory (MacArthur & Wilson 1967), the competition-colonisation trade-off in metapopulation dynamics (Hanski & Ranta 1983), life history theory (Stearns 1992), and trade-offs between competitive ability and the ability to avoid predation (Brooks & Dodson 1965). Trade-offs are often related to resources and physiological constraints; it is a physiological fact that organisms cannot at the same time invest limited resources in, e.g., growth, defenses to predators or herbivores, dispersal and tolerance to environmental stress. This creates inescapable limits to what real organisms can do in real ecosystems. Trade-offs can also be genetic and caused by genetic correlations. Many trade-offs can be masked by increased resource inputs, as in modern agriculture, but this does not obliterate the trade-offs (Figure 1). They are still real and constraining what, for example, breeding can accomplish, as is evident from the fact that breeding for high milk production has resulted in breeds with shorter lifespans, health problems and a dependency on high quality food.

It has always seemed to me that the grand plans of biotechnology have disregarded such trade-offs when discussing its possible contribution to sustainability and food production, When exploring the writings of Ford Denison and collegues I realised that this concern about ”trade-off blind biotech-

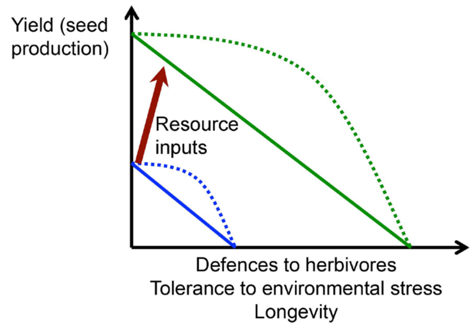

Figure 1. Illustration of the concept of trade-offs in evolutionary biology. Increases in yield will have negative effects on investment in defences against herbivores or tolerance to stressful environmental conditions. These negative relationships can take different forms (for example, as shown by the solid or hatched lines) and can be masked by increases in inputs that increase yield (arrow). For example, enhancing defense traits like Bt will – all else being equal – result in lower yield, the magnitude depending on the form of the curves.

nology” was actually shared by others (Denison et al. 2003; Denison 2012). To be fair, evolutionary considerations have for a long time been proposed as important for biotechnology (e.g. Gould 1988), but apparently with little understanding of its implications, except in cases concerning insect resistance to Bt[8] crops (see below).

If we believe in the power of evolution to have created the vast diversity of well-adapted organisms on earth, an inevitable consequence is that over millions of years, traits and physiological processes with high survival or reproductive value for individuals, i.e. fitness traits, have been improved almost to perfection. Examples of such traits are physiological pathways like photosynthesis, and efficiency of water and nutrient use. The continuous testing of organisms over long periods of time means that *simple* trade-off free improvements of fitness traits are highly unlikely to have been missed by evolution. Proponents of biotechnology seem to have disregarded this fact, for example when arguing that that genetically engineered crops will show higher yields because they will make more efficient use of sunlight, water and nutrients (Conway 1998; cited by Denison 2012). Increased tolerance to, e.g., drought has also been suggested,

[7]Fitness traits are traits or characteristics of organisms that are affect their relative reproductive success, i.e. survival and reproduction. See e.g. http://en.wikipedia.org/wiki/Natural_selection or …/Life_history_theory

[8]Bt = Bacillus thuringensis, Bt crops have received genes from these bacteria to protect crops to specific insect pests. See: http://en.wikipedia.org/wiki/Bacillus_thuringiensis

in this case by more balanced authors (e.g., Godfray et al. 2010). Of course, it is possible that some of these traits may be possible to improve in crops. It may be that evolution has not managed to find the paths to the peaks in the adaptive landscape (Wright 1932), or that evolution is not always producing organisms optimally adapted to their environment (Gould & Lewontin 1979). But to believe that such fundamental improvements can be made without negative consequences for other traits is naive – for example, increased water-use efficiency and having the physiological machinery conferring higher drought tolerance are likely to result in lower growth when there is no water stress (Denison 2012).

In fact, the evolutionary understanding by some proponents of biotechnology seems rudimentary. Consider, for example, the following quote from the project plan of a distinguished group in the Netherlands studying how (if) photosynthesis can be improved, by genetic engineering and synthetic biology[9]: ”*Plants are evolutionary adapted to their environment, but not more than necessary for their survival (sic!). Therefore, sub-optimal systems can occur. One known example … is the Rubisco enzyme. This enzyme is … only efficient at the high* CO_2 *concentrations which occurred in the early days of our planet (and) is much less efficient at the relatively low* CO_2 *concentrations of the last million years. Nature responded to this low efficiency by developing C4 plants (sic!). These plants possess extra features that lead … to better photosynthesis while using less water. The C4-system is not very common … knowledge of the C4-system … enables us to improve photosynthesis by the transfer of sets of genes to important C3 crops, enhancing the photosynthetic efficiency of these crops and in that way enhance their potential yield.”* No discussion of trade-offs, for example, that the C4-system performs poorly in colder climates (Denison 2012). No mention that conversion of C3 to C4-plants may actually result in *lower* photosynthesic efficiency (Long et al. 2006, cited in Denison 2012). No discussion of ”sub-optimal” with respect to what – in this case with respect to the scientists' own perception of what nature is and who it exists for, which is clearly a philosophical and social issue, not something than can be left to biotech scientists to prescribe. And on top of this a basic misunderstanding (or misinterpretation) of natural selection and evolutionary biology.

Disregarding such misunderstandings, I think it is safe to assume that attempts of making plants with ”new", more efficient, photosynthesis will be tremendously difficult, terribly expensive, and – if successful – highly risky. Risky because plants with much more efficient photosynthesis without strong trade-offs with other aspects of growth or survival will have been given a fitness advantage that allows them to spread in natural environments. This argument also holds for the other fitness traits discussed by biotechnology – drought tolerance, water-use and nutrient-use efficiency.

Trade-offs are indeed evident in present use of genetically modified crops. For example, the fact that there is a trade-off between yield and investing in plant defences is illustrated by the findings of several authors that differences in maize (corn) yield between Bt-maize and non-Bt-maize varieties are insignificant at low infestation levels of pests, or when the crop is treated with insecticides, and only at higher pest levels the advantages of Bt-crops (or pesticides) become evident (e.g., Catangui & Berg 2002; Ma & Subedi 2005; Ma et al. 2009). A similar result has been obtained for wheat, in which transgenic resistance to wheat mildew has incurred ecological costs (Kalinina et al. 2011). The lack of higher yield when pests are controlled by other means is also emphasised by the Failure to Yield report (Gurian-Sherman 2009), although this report from the Union of Concerned Scientists may be regarded as biased – just like many reports and studies performed by biotechnology proponents are biased[10]. From an ecological perspective, this point has been made by, e.g., Strauss et al. (2002) who stated that few studies had directly addressed this issue. An implication of these results is that if pests could be better controlled by other means, such as modifying the farming system, the genetically modified varieties would not be superior.

[9] *http://www.wur.nl/NR/rdonlyres/553F9FF7-78F0-43DE-8471-586D565EBB0F/87523/TBSCProjectplanShort010709.pdf. Project plan accessed 2012-08-26.*

[10] *For example, Monsanto (http://www.monsanto.com/ newsviews/pages/do-gm-crops-increase-yield.aspx. Dated 2009-09-21, accessed 2012-11-19) and the Agricultural Biotechnology Council (http://www.abcinformation.org; accessed 2012-11-19), both with vested interests in promoting biotechnology.*

Another important trade-off with respect to biotechnology is that between reproduction and longevity, in agriculture manifested by the fundamental difference between annual plants (surviving harsh periods by producing many seeds) and perennial ones, which have adaptations like roots, tubers, bulbs or deciduousness that allow winter or drought survival. Developing perennial crops such as perennial wheat has been suggested by several proponents of biotechnology as solutions to a number of sustainability problems (e.g., Fagerström & Sylvan 2010; Ortiz 2011). While such a development could be desirable, the difficulties seem to me overwhelming. Denison (2012) discusses this at length, pointing out that life-history theory predicts trade-offs that makes it impossible to maximise both early reproduction (seed production) and longevity (perenniality). Also, trade-offs relating to the conservation of matter precludes simultaneous investment in seeds and overwintering structures. It is highly unlikely that perennial seed crops will ever come even close to the yields of annuals. On the other hand, perennial forage crops can be very productive in terms of biomass, already now. But in that case biotechnology has not been needed. And many such forage plants require an intermediate herbivore to provide food for humans.

So the question is if biotechnology will be able to break out of such constraints in the future. Can it produce the super crops that its more optimistic proponents suggest are waiting behind the corner? To me these prospects seem bleak, indeed.

A third issue concerning biotechnology and evolution is the fact that all modifications of crops, and the changes in farming systems these entail, will result in evolutionary responses by natural selection among the other organisms that experience these modifications. The stronger, more persistent and large-scale these selective pressures are, the more rapid the responses are likely to be. Just like large-scale intensive pesticide and herbicide use has resulted in the evolution of resistance among insects, fungi and weeds, such responses have started to occur in response to the large-scale use of genetically engineered organisms. The intensive farming systems involving herbicide resistant crops have not managed to avoid the evolution of herbicide resistant weeds, which have increased dramatically in recent years (Neuman & Pollack 2010). Resistance to Bt sprays was observed several decades ago (Tabashnik 1994), and has recently been observed also in Bt-crops (Gassman et al. 2011; Zhang et al. 2012). The likelihood of this occurring has been known for a long time, and has been the basis for elaborate strategies to reduce such risks (e.g. Tabashnik et al. 2008). The larger the area of Bt crops, and the longer time they are used, the more such events will be observed.

My interpretation of this is that biotechnological changes of traits that are of importance for fitness and species interactions are likely to be problematic. Thus large-scale growing of, for example, Bt maize (corn), soy bean and cotton, and herbicide resistant crops such as maize, soy bean and rice, is unlikely to be sustained without large inputs of resources in the technological arms race against pests and weeds, as well as inputs of energy and other natural resources to production systems. Such resources may well be put to better use by investing them in developing better farming systems for a world with scarcer resources. And, to add to this, recent evidence suggests that not even the purported environmental benefits of these GM techniques have occurred. According to Benbrook (2012) the large-scale use of herbicide tolerant and Bt crops in the US seems to have increased the use of pesticides, especially herbicides, as a result of the development of Round-up resistant weeds, rather than the opposite.

Hence, from an evolutionary perspective, it seems wise to be sceptical about many of the claims of biotechnology proponents that this technology has the ability to increase crop production in a sustainable way to the extent that it will feed the world in the future.

Can biotechnology feed the world?

The challenge to feed more than 9 billion people by 2050 has been highlighted by many recent authors and scenarios for future food production (e.g., Conway 1998; IAASTD 2009; Fedoroff et al. 2010; Godfray et al. 2010; Bengtsson et al. 2010; Foley et al. 2011; Paillard et al. 2011; Magnusson et al. 2012). In my view, there is a clear division between those who seem to believe that biotechnology will be the key component solving this challenge (e.g., Conway 1998; Wambugo 1999; Fedoroff et al. 2010) and those that do not consider it as the main ingredient in how to sustainably feed the planet (e.g., IAASTD, 2009; SCAR 2010; Bengtsson et al. 2010; Foley et al.

2011[11]; Denison 2012). In between are more balanced authors, like Godfray et al. (2010), who suggest that biotechnology will provide humanity with wider production options, e.g., by improved salinity tolerance, disease resistance, and water and nutrient use efficiency. Here I will outline some reasons for my own (not Bengtsson et al's) scepticism to biotechnology being able to solve the problem of feeding the world in the future.

It seems to be widely accepted that presently, with 7 billion people on earth, enough food is actually being produced to feed all people. The fact that hunger still is common is a problem of distribution, an economic and political issue, rather than related to limitations in productive capacity. The more than 1 billion people being chronically hungry or living in extreme poverty (according to recent UN/FAO figures) simply do not have money to buy food or inputs to increase agricultural production that could alleviate their situation. Furthermore, the policies to improve the situation for these people often seem to be absent, in fact sometimes counteracted by international organisations. For example, fertiliser subsidies in Malawi combined with other measures almost doubled productivity on small-holder farms (Sanchez 2010), but this policy was actively opposed by, e.g., the IMF (Bello 2008[12]). Many small farms in South Saharan Africa are so deficient in soil nutrients and soil organic matter that, lacking the resources to buy basic inputs, many farmers simply cannot increase production on their farms – no matter the farming system they use[13]. For these farmers, who are crucial for food security in large parts of Africa, the question of using biotechnology or not, or whether to farm organically or conventionally, is simply irrelevant. They need to improve production by simpler means by enhancing soil organic matter and fertility, as the Malawi example shows.

Hence, to a large extent, the means to efficiently solve the problem of feeding the hungry 1-2 billion today is not to improve the already high production in the Western world and its associated areas with intensive production, by means of biotechnology. The task is rather to increase food production and provide infrastructure in the parts of the world where the yield gap is large, like Africa, Latin America and Eastern Europe (Foley et al. 2011). Here yield gaps can be partly closed by much simpler methods, such as improved soil fertility by local organic resources, cover crops, biological nitrogen fixation and synthetic fertilisers (especially phosphorus). In fact, each kilogram of fertiliser would do much more for food security if it were distributed among smallholder farmers in Africa or Latin America than when used on, for example, European or North American soils. That this is not done is a political issue, and does not need biotechnology to be solved.

However, a more challenging scenario is presented by the projected population increase until 2050 to approximately 9-10 billion people, and the assumed trend towards more meat consumtion in large parts of the world, especially

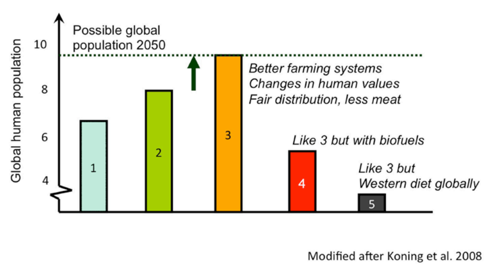

Figure 2. Simplified interpretation of the arguments in Koning et al. (2008) that it should be possible to feed the world by 2050 if policies are in place to improve farming systems, change diets and global distribution of food.
1 = Food production today.
2 = Food production with better farming systems, fair distribution of resources and more or better use of agricultural land.
3 = Changes in human values (ethics, equality, policies) and less meat consumption in addition to 2.
4 = Large bioenergy production on agricultural land in addition to 3.
5 = Like 3 but with Western diets globally.

[11]Although Foley et al. (2011) do not seem to dismiss biotechnology, they do not mention it or GMOs in their paper. It is interesting that their claim that the yield gap needs to be closed is interpreted as support for biotechnology, for example when Foley's talk on Greenbiz forum 2012 is introduced on the Council for Biotechnology Information web page (http://www.whybiotech.com/?p=3364; accessed 2012-10-21). In his talk, Foley himself is silent on this issue.

[12] http://www.worldhunger.org/articles/08/editorials/ bello_afag.htm. Accessed 2012-10-21.

[13] Ken Giller, personal communication at seminar at SLU, Uppsala, 2011.

the high- and middle-income countries. Present production possibilities and consumption patterns are incompatible with this development (e.g., Koning et al. 2008; Godfray et al. 2010). But does this mean that it will be impossible to produce enough food for all by 2050?

Koning et al (2008) argued that better and more productive farming systems globally would easily feed about 8 billion people, and if better farming systems are combined with changes in human values and policies concerning food production, i.e., diets, ethics and equality, resulting in a more fair distribution and less meat consumption, then feeding 9-10 billion is within reach (Figure 2). However, it does require that large parts of agricultural land is not used for bioenergy purposes to replace fossil fuels, and also that climate change and greenhouse gas emissions are not allowed to follow current trends. Similarly, both Agrimonde scenarios (Paillard et al. 2011) indicate that it should be possible to produce food for all by 2050.

My interpretation is that although producing food for all by 2050 will be difficult, there is no need for despair, nor do we have to put our faith in the unknown promises of biotechnological advances. And we do not all have to become vegetarians as suggested by Falkenmark (2012)[14], although the proportion of animal food would need to decrease in many parts of the richer world. Large parts of the crop production that could be used by humans directly are presently consumed by animals for meat production. This is especially so for grain-fed and soy-bean fed animal production (Foley et al. 2011). Improvements in *farming systems* under increasing scarcity – not biotechnology – is most probably the key to solving this challenge, together with strong policies to mitigate climate change (World Bank 2012)[15].

The role of biotechnology in farming systems

If (and of course there is an "if" when we are discussing the future) biotechnology is unlikely to be the key to solving the challenges facing future agriculture and food production, it follows that possible solutions lie elsewhere. I have emphasised the combination of diet changes and improved farming systems. The diet changes needed in the Western world are quite large, and depend on how much meat can be produced without competing with plant food production for direct human consumption. In addition, improvements in recycling of nutrients, integration of animal and plant production, and use of other land than arable land, are important (Bengtsson et al. 2010). Presently approximately 20 % of the global land surface can be used for animal production without competing with arable crops, compared to ≈10 % for crop production[16]. Many animals, for example, cattle, sheep, goats and wild game, utilise plant parts and habitats that are not useful for producing plant food for humans. Thus, although the areas that are unsuitable for crop production often are less productive, calls for most humans becoming nearly vegetarians are probably drastically exaggerated. Nevertheless, large changes in lifestyle and diet will probably be required if sustainability and global equity is to be achieved (Bengtsson et al. 2010; Foley et al. 2011; Paillard et al. 2011).

The question is, however, if the needed improvements in farming systems can be fully realised *without* improvements in crops and use of modern biotechnology. Biotechnology is in fact much more than the hotly debated GM technology. For example, molecular tools have revolutionised animal and plant breeding, have been fundamentally important for identification and monitoring the dynamics of microbial communities, and for evolutionary biology. Present understanding of gene-environment interactions, development, evolution and ecological interactions would not have been possible without modern biotechnology. Being sceptical to what biotechnology can achieve is not the same as being dismissive.

Once it is accepted that it is the *farming systems* that must be sustainable, it follows that farming systems should be the main focus of agricultural research and development. Biotechnology may have its role given that the methods are used for developing sustainable farming systems. The key

[14] Also reported in the Guardian August 26, 2012, by John Vidal. http://www.guardian.co.uk/global-development/2012/aug/26/food-shortages-world-vegetarianism.

[15] www.worldbank.org. Accessed 2012-11-19.

[16] http://en.wikipedia.org/wiki/Agricultural_land. According to this source ≈10 % of the terrestrial surface can be used as arable land, and an additional ≈20 % can be used for animal production.

question is not if biotechnology should be used at all, but whether biotechnology proponents and the biotechnology industry should be allowed to drive the development of farming systems, rather than farming systems driving biotechnology.

Such a focus on farming systems would require a complete volte-face that would drastically affect how society should view agriculture, agricultural policy and funding for education and research (cf. Denison 2012). It would put farming systems at the core of society's efforts to improve and make sustainable global food production and security, and force technologies to be subordinate to this goal. The focus of research under such a constraint on technology would emphasise understanding cropping and livestock systems, recycling of nutrients, energy efficiency at the systems level, and maintaining ecosystem services and soil fertility. The last decades of biotechnological development, for example, herbicide resistant and Bt crops, have not exactly contributed to these aims, but rather taken off in the direction of large-scale industrial farming, separation of crop and livestock systems, monocultures that may not even be the most productive systems (Bennet et al. 2012), and increasing environmental problems.

Modern biotechnology may indeed contribute to sustainable farming systens (Ronald & Adamchak 2008). One example may be systems that simultaneously use a combination of methods to control pests, through appropriate crop rotations, breeding for crop resistance to pests and biological control measures associated with natural or semi-natural habitats. Modern biotechnological methods could also be useful in providing crops with traits that clearly aren't fitness-related, such as starch composition in potatoes or oil composition in oil crops (Dyer et al. 2008), providing essential vitamins if farming systems fail, as has been suggested in the case of the golden rice (e.g., Enserink 2008), and when managing diseases, where possible benefits for humans are large and may outweigh potential risks and negative effects (Urquhart 2012)[17]. In addition, given the risk of climate change exceeding +4°C by 2100 (World Bank 2012), it may be risky to entirely dismiss the possibility to use biotechnology to produce crops that can tolerate higher temperature, flooding and drought, despite the fact that these traits will trade-off with high yield.

The view that improved farming systems, rather than biotechnological innovation, should be main target for increased food production and sustainable (ecological) intensification is supported by the observation that in long-term agricultural research trials, yield differences due to crop management are huge relative to gains from biotechnology (Denison 2012). If this is true, the major risk with a belief in modern biotechnology solving these problems is not primarily the risks to the natural environment or GM varieties spreading into natural ecosystems. Rather, the main risk is that a strong belief in biotechnology will result in large-scale investments in biotech and less interest in improving real, input-limited farming systems (cf. Vanloqueren & Baret 2009). This will result in less resources for the research that is most needed – farming systems, resource conservation and recycling technology, social science and ethics – and place all our bets on biotechnology. How are we to handle the possible situation when this technology has been oversold and that despite massive investments the promised benefits of biotech ”improvements” have not materialised (Denison 2012)? Then agricultural research will have been locked up in a biotech corner from which it will be difficult to take off in a different (and less high-tech and fancy) direction.

A corollary of this is that future agricultural research must hedge its bets in a planned way. A more pluralistic approach is needed, in which research in all kinds of disciplines relating to agriculture has a place – in my view mainly on farming systems. Several aspects of biotechnology may be needed in the quest for sustainable food production (see also Denison 2012). This means that many of the polarised controversies and debates that has plagued agricultural research in recent years – organic vs. conventional farming, GM crops or no GM crops, large-scale vs. small-scale agriculture – need to be replaced by acknowledging and respecting that there may be different forward-looking perspectives on sustainable production systems.

Proponents of biotechnology have not been especially good at understanding the needs for plurality in agriculture. This is an obstacle for resolving some of the controversial issues.

[17] *Also: http://www.guardian.co.uk/environment/2012/jul/15/gm-mosquitoes-dengue-fever-feature*

Consider, for example, the following quote from Fagerström et al. (2012): ”*… sustainable and productive agriculture is not by maintaining expensive, parallel production systems, using different sets of crop varieties, and relying on expensive regulations for their coexistence. Instead, agricultural systems should use the best available technology at all stages …*” To me, this implies a remarkable lack of understanding of the importance of plurality and diversity in research and practical farming. It also reveals an amazing certainty about the needs in terms of technology in an uncertain future. And a discomforting reluctance to reflect on the fact that the meaning of terms like ”sustainable” and ”best available” technology is not at all clear-cut and should not be left solely to natural (or, even worse, biotech) scientists to decide. In the long run, we need different ideas to select from, rather than trusting one single way of doing agriculture (Denison 2012). Pluralism has always been more useful in solving scientific and societal problems and will also be a more resilient way of addressing a future that is essentially unknown.

Costanza et al. (2000) highlighted the importance of society hedging its bets when ”managing our environmental portfolio”. There is no room for large mistakes globally, if the global aim is persisting on the planet and progressing towards sustainability. This includes hedging the investments (in agricultural research, in the present case) and, most importantly, strategies that avoid the worst possible scenarios. My personal interpretation of this is to maintain a sceptical view of what biotechnology can do. This view implies investing more in understanding farming systems and policies that decrease the overall environmental impact of agriculture, including how to bring about diet changes in richer parts of the world. In my view, the worst possible scenario would be a neglect of understanding the ecology and biology of farming systems combined with a failure of biotechnology to deliver in the longer term. However, I may be wrong, and others may perceive a resource-poor low-tech world filled with a diversity of poorly producing farming systems as the worst possible scenario. That is why we need to ground the discussions on future agriculture in a pluralistic view on science and a respect for different perspectives.

Concluding remarks

Debates on technology and its role in the future are common within all fields of society, so the debates on biotechnology are hardly something special. Many of the general issues in the present essay have been discussed - but not solved - earlier (e.g., Andersson 2012). What is special is the present setting of this debate, which takes place against a background of an increasing scientific discussion, in fact often consensus, about the effects of human activities and technology on the global state of natural resources and the environment. Global climate and land use changes, decreases in biodiversity and ecosystem services, the possibilities of planetary boundaries and several crucial resources becoming more limited in the future, financial crises, global social and economic equity, all these and many other factors profoundly affect the possibilities to produce enough food for future generations and even the future of humanity as such. Science and society must take these challenges seriously, and understand that the strong linkages between these factors make an interdisciplinary systems approach necessary. I am simply not convinced that major investments in biotechnology will be able solve the above challenges, although it may contribute some pieces when laying the puzzle of the global future.

At the heart of these debates lies the notion and meaning of ”sustainability”. Ever since the term was coined in the 1980-ies, it has been a problematic concept (e.g. Smit & Smithers 1993, Owens 2003; Hediger & Knickel 2009, FAO 2012), and it is used and made operational from many different perspectives. For example, already in the 1990-ies, it was clear that sustainability in agriculture can be judged by different sets of criteria – food sufficiency, environmental stewardship, and economic and social concerns (Smit & Smithers 1993). Recent discussions in FAO emphasise at least four different perspectives – good governance, environmental integrity, economic resilience and social well-being (FAO 2012). The same document also emphasises that ”*despite the valuable efforts for making sustainability assessments in the food and agriculture sector accurate and easy to manage, no internationally accepted benchmark unambiguously defines what sustainable food production entails. There also is no widely accepted definition of the minimum requirements that would allow a company to qualify as sustainable.*”

Clearly, defining what is a sustainable agricultural system is not a simple matter, neither is defining terms such as ”planetary boundaries”, ”sustainable intensification”, ”ecological intensification” and the idea of ”producing more with less” that are becoming prominent in the recent literature (e.g. Rockström et al. 2009; Godfray et al. 2010; Paillard et al. 2011).

Most definitions of sustainability, however, do require some kind of systems perspective (Smit & Smithers 1993; UN 2007; USDA 2009[18]). The Agrimonde scenarios make it clear that agriculture according to sustainability criteria will be very different from a trend-based Business-As-Usual scenario (Paillard et al. 2011). Thus single measures in isolation cannot be counted as sustainable. If biotechnology is used to sustain an otherwise unsustainable production system, like large-scale monocultures or producing soy bean feed for the global meat production, then arguments that biotech (GMOs) contribute to sustainability just because it, for example, decreases herbicide use, ring hollow. It may also be untrue (Benwick 2012). In addition, if long-term soil fertility is of concern, as it should be, then using Bt to be able to maintain a monoculture maize-soy bean cropping system, for example, is not likely to contribute to sustainability, even if it decreases the unsustainable use of pesticides. It is the farming and production systems that define sustainable agriculture, not piecewise tinkering without understanding how to make the agricultural *system* sustainable.

Proponents of biotechnology should more seriously discuss the meaning and operationalisation of sustainability in the context of farming systems, before arguing that this or that biotechnology is contributing to sustainability. The same goes for the opponents of biotechnology – biotech is not intrinsically un-sustainable – it depends on what it does and in which context or farming system it is used, and on its long term consequences.

Debates on future food production and what it needs are often based on the erection of straw men that are either good or evil. This goes for biotechnology, but also debates on organic farming, monocultures and the role of technology in general. In the present context, I have begun to wonder what biotechnology really is, and what different people mean when they discuss it. Is biotech a philosophy or a toolbox? Do we understand our own prejudices and perceptions about biotech? How much do different personal world-views (some would call it ideology) about the future and what it should look like influence our views on biotechnology? These are very difficult questions to handle, especially for many natural scientists whose training usually have not made them well qualified to discuss such value-laden issues. But once we accept that most of the important issues for future agriculture require less certainty and more discourse, more weighing of perspectives, evaluation of arguments, and value judgements, we can make some progress. It is not a matter of saying a categorical ”Yes” or ”No” to technologies like biotech, or farming systems, but understanding what they can do given different possible futures, what their consequences may be, how they may contribute to sustainability, and by which criteria such progress actually can be assessed.

[18] *http://www.nifa.usda.gov/nea/ag_systems/in_focus/sustain_ag_if_legal.html*

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Jan Bengtsson
SLU, Department of Ecology,
Box 7044, SE-75007 Uppsala,
and Future Agriculture, SLU.
jan.bengtsson@slu.se

Hållbart jordbruk – behöver det modern bioteknologi?

Sustainable agriculture – does it need modern biotech?

Maria Larsson

Genetic engineering could be used to improve organic farming, say UC Davis geneticist Pamela Ronald, Professor of Plant Pathology, and her husband Raoul Adamchak, a former organic farmer and now the manager of the certified organic part of the Student Farm at UC Davis. Currently, this is controversial in the US and more or less impossible in Europe, where even conventional agriculture bans GM crops.

Pamela Ronald and Raoul Adamchak were main speakers in a seminar on *Sustainable agriculture – does it need modern biotech*, organized by the KSLA on the 30th of August. Together, they have written *Tomorrow's Table, Organic Farming, Genetics, and the Future of Food*. In the book they share their vision of an ecologically based system of agriculture. In their vision, the interaction of agricultural biotechnology and agroecological practices is the key to increase the production in an ecological balanced manner.

Increase in sustainable food production…

The global challenge is to provide a growing population with good and healthy food produced in a sustainable way. Today, although we produce enough food in the world, one billion peo-ple are hungry. Furthermore, food production will have to increase by estimated 70 % by 2050 to a planet with approximately nine billion people. That is the big challenge.

There are threats against a positive development: multiple interacting driving forces push ecosystems towards tipping points, like natural fire regimes, biomass burning, palm-oil expansion and El Niño going from a regenerative to a destructive force. Climate Change, land and freshwater use, nitrogen and phosphorus overload are other factors crucial for food production. Stability and democracy might be threatened by severe droughts.

Prof. Johan Rockström, SEI, presented three scenarios for the development. With 3000 kcal per person and day, whereof 20 % is coming from animal food, there will not be enough water for agriculture in 2050. Reducing the share of animal food to 5 % will mean that 1.5 billion people live in dry, low income areas, 4.1 billion people in water deficit areas and not more than 3.3 billion people in areas with water surplus. Reducing calorie intake to 2200 kcal per person and day (still with 5 % coming from animal food) will resemble the ambition of the green revolution. But still many will live in areas where they cannot produce enough food. The number of people living in dry, low income areas will be about the same as in the second scenario. But fewer people (3.6 billion) will live with water deficit and more (3.7 billion) will live in areas with water surplus.

…on current cropland

Johan Rockström demanded a new "global specification" for world food production:

1. Stay within 350 ppm CO_2, an agricultural system that goes from being a source to a global sink.
2. Essentially a green revolution on current cropland (with a small expansion from 12 to 15 % of land area).
3. Keep global consumption of 'blue water' less than 4000 km^3 per year. We are at 2,600 km^3 per year today and rushing fast towards 4000 km^3 per year.
4. Reduce the extraction of N from the atmosphere to 25 % of the current level.
5. Do not increase P inflow to oceans.
6. Reduce loss of biodiversity to less than 10 E/MSY (rate of diversity loss, extinctions of species per year) from current 100–1000 E/MSY.

Social, economic and environmental sustainability

Using the term sustainable agriculture you tend to see it from the farmer's standpoint, but Raoul Adamchak stressed that farmers must be able to make a living and consumers must be able to afford the food. So the definition must be twofold. He also set criteria for a more sustainable agriculture in three different areas: social, economic and environmental.

Social sustainability includes local food security and abundant, safe and nutritious food. Viable farm and rural communities together with affordable food make up the economic sustainability. Environmental sustainability consists of reducing harmful inputs, energy and erosion, improving soil fertility, minimizing use of land and water, and enhancing biodiversity.

Back to "organic" farming – but better

A little more than hundred years ago all agriculture could be regarded as organic, with no chemical fertilizers or pesticides, no biotech and hardly any medicine for farm animals. Indeed very "natural". But was it sustainable? No. Although harvests were poor, leakage of nitrogen had already started, in Sweden due to draining of wetlands. The soils lacked phosphorous.

Research and development made the conventional agriculture flourish with higher and higher yields. The fixation of nitrogen from the atmosphere was an early milestone. However, over the years conventional farming has shown its disadvantages with e.g. leakage of nutrients and pesticides to groundwater, monocultures, high energy use and dependence on fossil fuels. A demand for a more natural agriculture, but high yielding and based on modern methods, has developed. Modern organic farming relies on crop rotation, support and enhancement of beneficial organisms for pest control, manure, compost and cover crops.

– Pam has said that in Sweden you can drink water from the rivers, but in Davies you can't even drink it from the well, Raoul Adamchak said, as a comment to the reduction of nitrogen leaching by 50–80 % from organic farming compared to conventional.

He emphasized that yields in organic farming can be comparable to conventional farms depending on the crop and location, but for some key crops, such as rice, yields are often lower. On the whole, yields vary from 45 to 100 % of yields in conventional systems. A recent report, published in Nature[1] 2012, finds that overall yields are 25 % lower in organic systems, analysing 316 different comparisons in 34 crops.

So far, organic farming comprises only a small amount of all agriculture, in the US 3.5 %. In Sweden, 12.6 % of the arable land was used for organic farming in 2011, 4.5 % of the cereal acreage and 22 % of the forage production acreage was certified as organic.

Modern biotech can help

To feed the world 2050 without additional yield increases, it will be necessary to almost double the world's cropland area. But there is not much more land available; in fact cropland is continuously lost to urbanisation, infrastructure, etc. So what can we do in a situation where 30–60 % of the yield is lost to pests, diseases and environmental stress?

– Modern genetic approaches can contribute to sustainable agriculture, Pamela Ronald continued her husband's speech. She gave several examples, starting with how the papaya ringspot virus could be defeated by genetic engineering in the late 1990s. Thirty years before, the virus hade decimated the total papaya production on the Island of Oahu and forced farmers to abandon the island. Later it was spread to Hawaii, where a research team engineered papaya for resistance. Pamela Ronald compared the immunization with human vaccinations against polio or small pox. Today most of the papaya eaten in California is genetically modified. This papaya is the oldest GM crop in the world and was developed completely without engagement from the large commercial biotech companies.

– I have done research on rice. An old rice variety, highly tolerant to submergence, was found in India, but it was low producing. My team isolated a gene called Sub1A-1 and found that it was sufficient to confer submergence tolerance in nearly

[1]*Seufert, V., N. Ramankutty & J. A. Foley (2012). Comparing the yields of organic and conventional agriculture. Nature Volume:*

485, Pages: 229–232.

all highly intolerant, highly producing and good tasting varieties. It was so exciting to see that it worked, Pamela Ronald exclaimed.

But again, genetic engineering doesn't solve all problems. Pamela Ronald told the story of how the introduction of Bt-cotton in China reduced the use of insecticides strikingly and how the number of insecticide-related illnesses among farmers fell to 25 % of the previous level. But after seven years, populations of other insects increased so much that farmers resumed spraying other pesticides. To prolong the efficiency of Bt-cotton the farmers need agroecological approaches like increased crop diversity and crop rotation. For instance they can grow refuges, patches of traditional cotton, intermingled with fields of Bt-cotton.

– The refuges ensure that the few pink bollworm moths that are resistant to Bt are most likely to mate with Bt-susceptible pink bollworm moths that grew up in refuges. The offspring from such matings die when they eat Bt-cotton, Pamela Ronald explained.

She and her husband were asked what comes first, agroecological practices or biotech?

– Both are very important and it really depends on what the problem is. A potato disease could be fought by a rotational system, but a resistant potato variety is better, said Raoul Adamchak and Pamela Ronald added:

– Each problem has to be addressed on its own. The Golden Rice has saved thousands of children from vitamin A deficiency, while efforts of changing their diet have failed.

GM success – but not in Europe

Thirty years of genetic engineering on plants has taken us from the first genetic engineered plant reported in 1983 to 11 per cent of agricultural land cultivated with GM crops in 2011. 82 % of all cotton, 75 % of all soybean, 32 % of all maize and 26 % of all rapeseed were GM in 2011. However, the development in Europe has been quite different. Worldwide, the first GM plant was commercialised in 1992. Six years later the first GM plant – Monsanto's maize Mon 810 (Bt) was approved for cultivation in the EU. 1999-2004 a moratorium on approval of GM plants stopped the cultivation. In 2010 the Amflora high amylopectin potato from BASF was approved for cultivation in the EU. However, the cultivation of these two approved GM plants are negligible, only 0.1 million hectares in 2011, which is 0.06 % of the global cultivated area.

– There has been a political resistance against GMO in Europe. The situation for plant biotech is catastrophic, with no biotech company willing to invest in developing GM plants in Europe, said Prof. Sten Stymne, SLU. Partly, he blamed media for focusing on problems, even when the main story is positive. But the lack of cultivation of GM plants in the EU doesn't mean that we don't use GM products. Sten Stymne gave an example:

– EU imports 98 % of its soybean consumption – or 40 million tons – and 95 % of this is GM soybean.

Maria Larsson
Liv Journalistik
maria.larsson@livjournalistik.se

Hållbart jordbruk – behöver det modern bioteknologi?

Sustainable agriculture – does it need modern biotech?

Lennart Wikström

A hot topic

The greatest challenge to agriculture and our global food supply system is how to make productivity and sustainability meet. Future production systems must increase productivity in order to meet the increasing demands of food, feed and renewable energy and at the same time reduce its environmental footprint and sustain and eventually increase biodiversity. In order to achieve this, we have to explore every possible technological tool and evaluate it against the long term challenge. This includes plant biotechnology, which has been much discussed and to a large – maybe too large – extent discarded, expecially by environmentalists and the protagonists of organic farming.

But, as the authors Pamela Ronald and Raoul Adamchak point out in their book ”Tomorrow's table”, it is not a question of either or, but of both ecology and technology.

At the seminar ”Sustainable agriculture – does it need modern biotech?” arranged by the Royal Swedish Academy of Agricultural Sciences (KSLA) and the Royal Swedish Academy of Engineering Sciences (IVA) i Stockholm on August 30th , 2012, this issue was well covered with a number of initiated presentations and with the authors present.

The presentations, represented in previous articles, were followed by a series of discussions on specific questions regarding the main topic and what was presented.

Research – risks and benefits

The moderator, Annika Åhnberg, KSLA, first asked how the assessments are done by EU authorities.

– Assessing food safety is one of the European Food Safety Authority EFSA´s main tasks, answered Dr Dr Ilona Kryspin Sørensen, senior scientist and research director at the Danish Technical University, and a member of the GMO panel at EFSA. This is done by a panel with qualified representatives from membership countries.

According to Dr Kryspin Sørensen, applications are done in the groups environment, food and feed and molecular scientific assessments. Aspects of GMO are specifically allergies and statistics.

– Qusetions are focused on the minute areas, and there is not any subject area that has been so well scrutinized as the GM-applications, said Dr Kryspin Sørensen. The membership states in turn are doing their own assessments with support from EFSA.

– The EU law has been completed with the EFSA regulations. The membership states have very much saying in this, and are obliged to answer to other membership states concerns. The work in the panel has been performed with great transparency and high level of science. Some issues in EFSA have been given special interest, such as marker genes for antibiotic resistance and their persistence, and that has now been technically reported.

– But many of the national decisions in turn are not based on scientific assessment and the extensive work of EFSA.

Dr Jürgen Logemann, Vice President Technology Management at BASF PlantScience, represents one of the companies that have invested large sums on developing GM crops.

– We have spent an enormous amount of work the last ten years, he said. The farmers ask for yield, yield and yield, and we want to provide solutions. We work on protecting yield and on developing intrinsic yield. One interesting program is our resistance to potato late blight. By adding two genes from a distant relative to the cultivated potato, we achieved almost 100 % resistance.

In the US, BASF PlantScience works with nitrogen uptake and drought tolerance.

– With potato in the EU we have been fighting

Talare och paneldeltagare/Speakers and panelists (clockwise, starting upper left): Inge Gerremo, Torbjörn Tännsjö, Jenny Jevert och Ingemar Kroon, Jürgen Logemann, Carl-Eric Ehrenkrona, Bengt Persson.

to be able to perform field trials. In the end, the difficulties in the EU have more or less forced us to move our plant biotech activities overseas to the US.

Dr Logemann meant that forcing the companies developing GM crops to leave Europe leads to brain drain and loss of investment in knowledge, so either the scientists have to move to the US or do something else. He saw no future for GMO on his company´s part in the EU.

– If the consumers in the EU are prepared to pay for the cost of not using GMO I can accept it, but it also makes it of no interest for us in staying on the European market.

– We will not be able to stop GMOs in the world, the question for the EU is more how long we can resist, he concluded. I have no problems with integrating organic, conventional and biotechnical components, but what I have problems with is when organic farmers impose their values upon others. Mutual respect would help to find integrated solutions.

– Increasing food prices has made developing countries more interested in GMO. Saying no to GMO is a luxury problem, said Prof. Rodomiro Ortiz, Department of Plant Breeding at the Swedish University of Agricultural Sciences (SLU) in Alnarp. Whatever you do you will be criticized. In southern Europe we will a see a situation similar to that of the developing countries.

– I do my scientific work on oil crops, specifically host plant resistance, and I will work with any technology that can be efficient both in plant production and in reducing pollution. Weed control is essential in a reduced input system, and that includes herbicide tolerance, either with or without GM technology.

The moderator Annika Åhnberg asked the panel if the loss of knowledge will be a problem for Europe.

– We need to increase the input in plant breeding in order to handle future challenges, and that includes the need for advanced plant biotechnology. Northern Europe is a specific area with specific needs, and we can not just take a solution from another part of the world and expect it to work just as good here, said Prof. Ortiz.

Organic farmers reaching out

The question of modern plant biotechnology coming into use involves at least four agents. Beside scientists, authorities and commercial companies developing varieties, there has to be farmers willing to grow the crops.

– Under special circumstances it could be possible for organic farmers to accept GM crops, said Carl-Eric Ehrenkrona, chairman of the Swedish Association of Organic Farmers. The Swedish organic farmers are very much against genetic engineering. I have tried to understand why our members are negative and found three main objections. The first argument is about consumer concern, and that the use of GM crops in organic farming would erode the added value of organic products. Also, we want to produce in harmony with nature, and many regard modern plant biotechnology as being in conflict with that. The third objection is against the patent system, where farmers risk ending up in court by unintentionally having plants with GM genes in their crops.

– But if these problems can be solved, my belief is that also organic farmers would consider GM as an acceptable means. Organic farming also needs research and development, and new technologies could be accepted as long as they are in harmony with nature and do not include the use of pesticides and fertilizer. But you have to show us a good example.

The Federation of Swedish Farmer (LRF) organises the majority of Swedish farmers, and is consequently very heterogenous.

– LRF is divided, where many farmers are very pro, and others are negative to GM plants. Our policy has changed recently, where we want focus more on traits than on the technique used. Our main problem is that our agriculture is declining from lack of competitiveness, said Bengt Persson, member of the board of LRF and chairman of Swedish Farmer´s Foundation for Agricultural Research.

Since it is extremely difficult to completely avoid occurence of GM genes in crops even where no such crops are grown, there are suggestions on putting forward specific threshold values.

– We must be aware of what the consumers say and therefore try to avoid GM plant contamination as much as possible. The discussion is very infected, and we have to spread knowledge, said Mr Ehrenkrona. In order to meet the challenges of producing more food, we believe that existing non-GM technology will suffice. With the current knowledge, we do not regard GM plants a sustainable solution.

– As farmers, we are very much dependent on what both the industry and the consumers say, and right now the dairy industry says no to GM feed, and that limits the possibilities. For the future, we have to focus on the traits and on more knowledge. But still the issue is handled more with emotional and personal arguments than rational, said Mr Persson.

Consumer issues

GM field trials at Rothamsted were threatened to be destroyd by activists, an attack which also would risk harming the long term production system trials, which have been on the same site for more than 150 years. In an attempt to persuade the activists not to attack the trials, a group of scientists published a plea on YouTube[1].

This turned out to be an excemption from a large number of failed attempts from scientists to explain to the public about the positive potential of GM technology.

– The YouTube video is an example of how scientists can act, where you reach through and create trust, explained Jenny Jevert, a popular science journalist, specialised in biotechnology. The threatened scientists adressed the activists in an unusually emotional tone. The outcome didn´t stop the attack, but the news reports were very balanced, and the comments and editorials were all on the scientists' side. Compared with the 1990s, this time headlines were quite different, and we can see how media has changed the way they report on this subject.

Jenny Jevert meant that the media are very important in communicating science, but the media debate is running on repeat, and that is why new methods are welcome – and efficient.

– The GM debate is very much based on value-based arguments, and must therefore be met by value-based arguments, Ms Jevert concluded.

– What if the starting point had been something else than herbicide tolerance, asked the moderator Annika Åhnberg.

– The question of plant nutrition is much more relevant than the debate on GM technology, and a small amount of fertilizer would do wonder in the agriculture of most third world countries, said Dr Inge Gerremo from the Office of Global Affairs at the Swedish University of Agricultural Sciences (SLU), and with a long time experience of academic cooperation with developing countries. The Golden Rice is a good example, which should have been launched earlier, and I believe it would have changed the general opinion to be more positive towards GM plants.

– We need the modern biotechnologies on our agenda in order to be able to assist developing countries in their legitimate struggle for increased food availability, said Dr Gerremo.

– There were a lot of scepticism already in the 1970s when there were no products, said Ingemar Kroon, chief communications officer at Axfood, one of the major Swedish food retail chains. But the medical industry went the right way, targeting on products where there were clear setbacks with the current use, insulin and growth hormone for example. The acceptance was also helped by the general perception of medicine not being ”natural”, and that all medicine have setbacks. But food must be safe, and that is why the public has a greater difficulty to accept GM crops.

– Normally when people make up their minds they look for the good guys, and in this case they have chosen Greenpeace and the Swedish Society for Nature Conservation, SNF. If GM crops were to be accepted by the organic farmers, that would be a breakthrough.

– It is not a question of lack of transparency or knowledge, but it is all about values and attitudes, and in order to influence the public you need to tell good stories. The story so far has been a story of the small good guys against and the big bad guys in favour of GMO. But for us as a food retailer it would be commercial suicide to be the first to introduce a GM product and promoting it. What we can do is to avoid doing or saying anything stupid, or thrive on and encourage lack of knowledge.

– Farmers can do a lot. In Boulder farmers stopped a ban on GMO sugar beet by protesting, said Dr Pamela Ronald, coauthor of the book ”Tomorrow's table”.

Ethical considerations

Modern plant biotech and genetical modification of plants has been given much more attention in the public debate than in practical production. In most parts of the world it is not a controversy, and is used in agricultural production. The biggest attention is given to the issue in Europe, where there

[1] *http://www.youtube.com/watch?v=I9scGtf5E3I*

are virtually no GM crops grown.

– Is it more important to discuss ethics related to this technology, than ralted to other parts of agriculture, asked the moderator Annika Åhnberg.

– It is a kind of enigma, GM is a technique people have strong feelings about, said Prof. Torbjörn Tännsjö, professor in practical philosophy at Stockholm University. Applied ethics has for a long time been interested in the environment, and to begin with the philosphers could be very radical. They could, as the famous Norwegian philosopher Arne Naess, aim to reduce the world's population – Man was not as valuable as nature itself. The sentiment of ecological conservatism was to preserve, not to eradicate, existing species and not to create new ones. Do not meddle with the perfection of evolution.

– How can we meet this kind of discourse? Man is able to manipulate nature and that is why we can accept our manipulative capabalities. Why should we stay satisfied with the current situation, and things as they are right now? With new technologies, we can add species where nature and evolution has failed.

Today there is less interest in basic ecology, and instead we have achieved a more speciocentric view and discuss more of other issues such as Climate Change. Man is important, not nature. It is the people around us that matter today. We strive for sustainability instead of preserving what is here today.

– What if humanity goes extinct, Prof. Tännsjö asked. One answer to this could be that it is not a problem, since there will be no one around to complain. But this view I resent. It matters whether there are people around or not, and we should do what we can to stay around. It is extremely difficult to defend this position, but it is what I try to do.

– One consequence of this moral view is that we have to care for those who will be around after we are gone. If we were to restrain our own possibility of growth, we would have to face the decision of who should be sustained and how. Increased population can in itself be regarded as a sign of our world getting better. A greater number of human beings can be sustained, and GM technology can be a part of the solution to this sustenance, Prof. Tännsjö concluded.

– Man playing God is very central in this discussion, said Prof. Nils Uddenberg, KSLA. In a study ten years ago we asked people on how they looked upon nature. One observation was that very many Swedes look upon nature as good when it is stable, like a good mother – a religious thought. In Sweden nature has taken the place of God.

On the question of humanity´s existence, Prof. Uddenberg noted that it is a good thing that we exist, and if Man were to go extinct, it would not be very probable that all would disappear at the same time.

Prof. Uddenberg is a member of the Swedish Board on Gene Technology and as such expected to take part in the ethical evaluation of the new scientific tools.

– When you do an ethical evaluation, you can do it in two ways. One is an ontological way where it is always wrong to take genetic material from one organism and put it into another organism. This is a problematic position when there are other ways of obtaining the same goals, and those tools are not assessed in the same way. There is a risk that we would get a lot of genetically engineered crops that have not been scrutinized.

– The other way of evaluation is through consequential ethics, which I regard as a more constructive way that could also include the scrutiny of all gene technologies. The more remote consequences you discuss, the more difficult it becomes. Health and a good environment are important, but when the uncertainty in far away consequences is large, it becomes meaningless to consider them. The tractability of a mobile phone and its advantages makes us more accepting towards this technology than towards the abstraction of gene technology.

– The two opposite trenches of pro's and con's is a bad way to solve the problem of sustaining nine billion people in 2050. We will have to be prepared to use other new, even more questionable technologies, it does not stop with GMOs.

Prof. Uddenberg pointed out that food is very personal, it is very intimate and something we put inside us. Therefore our reactions to food are, and should be, very emotional.

– Food is not only about gene technology. I would like to put forward an idea, that may seem a bit mad, but then again, that is my privilege. What if we could manipulate Man to improve intelligence, should we do that instead of manipulating plants? Then, maybe, we would be able to solve this discussion once and for all. But when it comes to enhancing Man's performance, we accept it as long as it does not involve going into the genes.

With these last ethical reflections, the moderator Annika Åhnberg closed the day, and made a final specific address, where she thanked the authors Prof. Ronald and Dr Adamchak. Both the presentations and the following discussions showed that on the issue of genetically modified crops and plant biotechnology, much would be gained from focussing more on results and effects, than on the tools themselves. But we still have a long way to go before the technology is generally accepted, which also was underlined by the low attendanceduring the day of representatives from two important stakeholders, namely consumers and policy makers.

Lennart Wikström
Cultimedia Information AB
lennart.wikstrom@cultimedia.se

Hållbart jordbruk - behöver det modern bioteknologi?

Sustainable agriculture – does it need modern biotech?

Annika Åhnberg and Anders Nilsson

TIn the future, sustainable agricultural production systems must increase productivity, reduce its ecological footprint and its use of resources, while meeting demands on food supply, nutrient intake, biodiversity and industrial/bio-energy feedstocks. Science is offering plant biotechnology as one of the tools in the toolbox providing solutions to these seemingly contradicting requirements.

But modern biotech is sometimes seen as opposite to sustainable agriculture. In Europe there is a strong resistance towards cultivation of genetically modified crops and there is a very complicated system of regulations and restrictions concerning them. Rules for organic farming include a sharp borderline to the use of genetically modified crops.

Pamela C. Ronald and Raoul W. Adamchak are the authors of 'Tomorrow's Table', a book that has attracted much attention over the last years. Pamela Ronald is Professor of Plant Pathology and a geneticist; Raoul Adamchak is the manager of the certified Organic Market Garden at the Student Farm, both at UC Davis. The two of them are also happily married to each other. In their book, the authors discuss how research and development on plant biotech and organic farming can profit from each other. They also present examples that show how modern science in the biotech field can be intertwined with high ambitions for environmentally friendly and resource lean production systems that also meet with ethical considerations.

The Royal Swedish Academy of Agriculture and Forestry (KSLA), The Royal Swedish Academy of Engineering Sciences (IVA), and the Swedish Seed Association (SUF) invited to an open seminar on August 30th, 2012, where "Tomorrow's Table" was presented by the authors and commented by Swedish stakeholders and policy makers in the fields of plant biotech, agriculture and environment. Close to 100 persons attended the seminar in person and it could also be followed on the web. Besides presentations by and discussion with Pamela C. Ronald and Raoul W. Adamchak, the program of the seminar contained five more presentations and four panel discussions on different themes:

- research
- risk and benefits
- production issues
- consumer issues
- ethical considerations

The five presentations had the following titles:

- Biotechnology for Sustainable and Compet itive Agriculture and Food System – the Mis tra Biotech project/ Sven Ove Hansson, KTH
- In which ways could modern biotech be part of sustainable agriculture/ Jan Bengtsson, SLU
- GMO:s in agriculture and in research/ Jens Sundström, SLU
- Where is biotech research in Europe head ing?/ Sten Stymne, SLU
- Challenges for sustainable agriculture/ Johan Rockström, Stockholm Resilience Centre

The entire seminar was moderated by Annika Åhnberg. The presentations and discussions at the seminar are summarized and commented from different perspectives in this issue of the Journal of the Swedish Seed Association.

One of the objectives of the seminar was to challenge the conventional European view of plant biotech as incompatible with a sustainable development of agricultural production systems and address these issues from an opposite angle. The discussions at the seminar clearly showed that the European attitude can be questioned and that indeed sustainable production systems in agriculture can be combined with modern biotech.

Another objective of the seminar was to continue an initiative taken by KSLA in 2007 on establishing a dialogue between proponents and opponents to the use of plant biotech. This had the form of a small dialogue group that had met for some 8-10 sessions on different themes. This activity had been part of KSLA's project "A knowledge-based biosociety" – "Det gröna Kunskapssamhället", and had been finalized with a seminar in 2009 where the Standing Committee on Environment and Agriculture in the Swedish Parliament participated. The set-up of this dialogue group aimed at developing a better understanding of the arguments used by the respective side and the rationale behind the positions taken.

Annika Åhnberg
Tankeföda AB
annika.ahnberg@ystad.nu

Anders Nilsson
SLU, Alnarp
anders.nilsson@slu.se

Framtiden för växtbioteknik i Europa

Future of Plant Biotechnology in Europe

Anna Lehrman, Erik Alexandersson

This seminar was organised on 7 November 2012 by Plant Link, Partnerskap Alnarp and Mistra Biotech, and gathered over one hundred participants, but still more would have joined if they could. We have therefore made an attempt to summarise what was presented and discussed during the day. The power point presentations can be found at www.slu.se/mistrabiotech (under News).

Experience with GMOs in Spain, the first country in Europe cultivating Bt-maize

Prof. Pere Puigdomènech

Pere Puigdomènech is the Director of the Centre of Research in Agricultural Genomics (CRAG) in Barcelona and has been a member of the EU advisory group on Biotechnology and the European Science Foundation's expert group on Biology and Society. At CRAG model plants as well as cereals, fruit trees, horticultural crops, and farm animals are studied. They also provide services in molecular methods such as genotyping, genomics, proteomics, and metabolomics.

Puigdomènech focused his talk on the cultivation of Bt-maize (maize containing a gene from *Bacillus thuringiensis*) in Spain, which has been successful in comparison to cropping of genetically modified (GM)-crops in other European countries. He also explained the structure of the Spain's National Commission for Biosafety, which is composed by members of different ministries and scientific experts. This commission regulates and provide public reports on field trials.

There are several reasons why Bt-maize has found acceptance in Spain. To start with, Spanish farmers face stiff competition from other maize produces and there is a considerable and steadily increasing import from other European countries and the Americas. Bt-maize was first introduced on the market in 1998 and is today mainly grown in two Spanish regions where the European corn borer (*Ostrinia nubilalis*) causes problems. Today it accounts for about 30% of the total maize production and is used for feed and industrial uses.

It is well known that the public perception towards GM-crops varies between different EU member states, with Greece and Austria having a very negative perception. However, in spite of the large use of Bt-maize in Spain, the public is not that sceptical. Puigdomènech lifted that one reason for this could be that the general public in Spain are more confident in scientists than in other EU countries, and rank the trustworthiness of scientists higher than e.g. NGOs. Furthermore, the ruling party in Spain, Partido Popular, has taken a firm stand in favour of the technology. At the same time, the principle of co-existence of conventionally and ecologically grown crops, together with GM-crops is well-established. These factors together with the stiff competition farmers meet have led to that the cultivation of GM-maize is fairly uncontroversial in today's Spain.

A question was raised regarding the development of resistance and requirements for refuges with non-GM maize? In Spain it is recommended to sow refuges but because no region has more than 50% Bt-maize selection pressure is very low.

Risk assessment of GM crops within EFSA and experience of GM field trials at Rothamsted Research

Prof. Huw Jones

Huw Jones is the research leader in the Plant Biology and Crop Science Department at Rothamsted Research and a member of the Panel on Genetically Modified Organisms (GMO) within the European Food Security Authority (EFSA) based in Parma. At his Institute a genetically engineered wheat has been developed that emits (E)-beta-farnesene, which is a volatile compound aphids use as an alarm pheromone when attacked. The gene, originating from peppermint, has been transformed into wheat plants. This pheromone deters aphids from the wheat crop and, in addition, it attracts predators of aphids such as ladybird, lacewing and parasitic wasps. The idea is that the plant can defend itself, resulting in less need for insecticides. Because wheat is self-pollinated and not compatible with wild relatives it is highly unlikely to spread the genes to other plants.

The wheat has been tested in lab and in fact repelled the aphids more strongly compared to synthetically produced (E)-beta-farnesene. Last summer a first field trial was conducted at Rothamsted outside of London. Before the field trial a 60 day public consolation period was held, this time resulting in many letters of objection from individuals and anti-GMO organisations. However, Huw Jones thinks that a greater understanding and acceptance regarding GM-crops has emerged during the last year, maybe due to extensive and mostly positive media interest in the Rothamsted trials. In addition public acceptance has been greater probably because it is the first GM-wheat tested and that it has not been produced by one of the big multinational breeding companies. During the field trials an open letter was published and some Rothamsted scientists made a short information film to explain the research and ask the protestors not to destroy the trial which had a positive effect on the public's and media's attitude. The only incident at the site was a person that managed to breach the security and get into the field in order to spread organic wheat seed. But by that time the test crop was already well-established and the contaminating seeds could be distinguished and removed. The researchers at Rothamsted will repeat the trial in 2013 and publish their results of both trial years in a peer-reviewed journal.

The European Food Safety Authority (EFSA) works on a €77 million budget and use 167 scientific experts on 10 panels. EFSA is an independent scientific agency of the EC that carries out a risk evaluation of GMOs and publishes its opinion. This outcome requires approval by EU member states, often after protracted political discussion. The usual voting habits of the EU member states are that South and East EU tend to vote NO more often than other parts. So far 53 applications have been adopted by EFSA, and a further 60 are in process – of which about 50 are stacked events i.e. crops with combinations of different GM-traits. Most of the previous applications have been various herbicide tolerance or insect resistance traits but recently some new traits such as altered starch and fungus resistance (the Amflora and Fortuna potato varieties), thermo-stable maize for ethanol production, and soybean with reduced poly-saturated fatty acids are in the pipeline. The cost for regulation and risk assessment in bringing a new GM-crop to the market is somewhere between 7-15 million USD. This is a staggering amount which effectively restricts activity in this area to big multi-national biotechnology companies.

The Amiga project: Assessing and monitoring the impacts of genetically modified plants on agro-eco systems

Dr. Tina d'Hertefeldt

Tina d'Hertefeldt is a researcher at the Department of Plant Ecology and Systematics at Lund University and part of the Seventh Framework Program (FP7) financed program AMIGA; Assessing and monitoring the Impacts of Genetically modified Plants on Agroecosystems. She is a member of the board of the Swedish Ecological Society, Oikos, and the Swedish Board for Gene Technology.

Tina d'Hertefeldt focused her talk on practical GM-tests and communication. In the AMIGA program EU has been into regions to answer the question; can genetically modified crops have different effects in different parts of EU due to local environment? A lot has already been done for risk assessment and management. What are the EFSA decisions based on? AMIGA strived to simplify this document. In AMIGA, researchers will do practical test of ecological studies, look into post market environmental monitoring, and evaluate the economic impact with Argentina as an example country. The goal is to increase the confidence in guidance document and develop a robust risk assessment and effective post-market monitoring. The Bt-maize MON810 is used as a model crop with field trials outside Lund along with similar trials in Spain and Slovakia. Denmark and Rumania will also host maize trials in 2013. Amiga also includes studies on non-target organisms in potato, and a GM-potato resistant to late blight is grown in Ireland.

It is difficult to assess on how much effort is needed to sample insects enough to get good measures of non-target effects: How many samples over one season? When in season to sample? To which taxonomic level should insects be identified? In Sweden, there is a specific interest to investigate the presence of honey bees in the maize field, which is linked to the EU pollen verdict that states that honey may not contain any GM pollen. It has been important to inform beekeepers about when the maize would flower and a pollen trial on honey bees and bumble bees is also performed in the project.

Initially there was no interest to grow Bt-maize in Sweden because maize was such a small crop with no problems with the target pest, the European corn borer. However, increased maize cropping and climate change have brought the pest, not only closer to Sweden but signs of attacks in fields have been found on Öland and also outside Lund, and such a field is planned to be included in the next field season. The experience from the first field season shows that there is a strong need to customize the sampling protocol of non-target organisms so that it is possible to evaluate possible effects. The open communication about the trial is in line with the aim of the Amiga project and has been important in order to communicate why we planted the field trial. Findings of the first season will also be communicated back to the public.

Emerging techniques

Dr. Marie Nyman

Marie Nyman is Head of Division at the Swedish Gene Technology Advisory Board in Sweden and she talked about new techniques for plant breeding and GMO-legislation. She pointed out the fact that the biotech techniques regarded to result in a GMO are listed in the annex of the EU directive on genetically modified organisms (201/18/EC). These include recombinant nucleic acid techniques as well as heritable material prepared outside the organism and introduced in a host organism. Another annex to the directives lists exempted techniques, such as mutagenesis and cell fusions of plant cells from organisms which can exchange genetic material though traditional breeding methods. These techniques are exempted on the condition that recombinant nucleic molecules have not been used. Since some of these techniques create site-specific mutations using nucleic acid molecules, one of the key issues is whether a e.g. synthetic oligonucleotide is a recombinant molecule or not. Parts of the EU regulation on those techniques dates back to 1983, which creates problems since molecular biological techniques are rapidly developing, and there are today several examples of techniques for which it is unclear if the end-result should be regarded as GMO or not. Consequently it is uncertain whether these should be regulated or not. Examples of techniques which are currently discussed are oligonucleotide-directed mutagenesis (ODM), site-directed nuclease (e.g. ZFN and TALEN), cisgenesis/intragenesis, grafting of non-GM scion on GM-rootstock and vice versa, reverse breeding and synthetic genomics. A working group to evaluate these new techniques with members from the EU states was set up after a proposal from the Netherlands 2007. Their task was to evaluate a list of techniques, provided by the commission, in the light of the definition of GMO/GMM, the techniques listed in the annexes and the most recently available scientific data. The working groups report was distributed to the member states competent authorities in the beginning of 2012. According to a report from DG Joint Research Center the end-product of many of these techniques cannot be detected and a method of detection is a requirement in the legislation.

The largest uncertainty around new breeding techniques is of course whether they are going to be classified as GM or not by the EU, a decision which will have large consequences on the costs associated to risk assessment and registration. In general, Marie Nyman noted, science moves forward faster than the EU regulation. The crop closest to the market based on such a technique is Cibus' herbicide tolerant rapeseed. This crop is not considered as being genetically modified in the US, and interestingly not in the UK or Sweden either.

Risk assessment and regulation of new breeding techniques

Dr. Frank Hartung

Frank Hartung is a researcher at the Julius Kühn-Institut (JKI) Quedlinburg, Germany and member of the EPSO-Working Group on Agricultural Technologies and member of the

EFSA working group on the risk assessment of plants developed through new techniques.

Frank Hartung continued to talk about risk assessment and new breeding techniques with a deeper focus on the actual molecular methods used. He started off with reminding the audience that today 160 million hectares GM-crops are grown worldwide by no less than 16.9 million farmers. As a member of the EFSA working group on risk assessment of plants developed by new techniques he has examined these in the context of the European GM legislation. A final report was presented in February 2012. Frank Hartung especially stressed different techniques for site directed nuclease (SDN). Organisms as an outcome of some of these are, suggested by the working group, not to be considered as genetically modified; among these are oligonucleotide-directed mutagenesis (ODM) and certain zink-finger nuclease methods if recombinant DNA is not used (for more details, see presentations at www.slu.se/mistra-biotech). In comparison to the introduction of recombinant DNA or classic mutagenesis these new breeding techniques facilitate more precise mutations, transfer and integration of DNA, and have less side-effects. It is also possible to avoid the usage of selection markers. In fact, transgenes and non-transgenes cannot be distinguished after the events, which is a pre-requisite for monitoring and regulating GMOs. In addition many of the new breeding techniques are more cost-efficient also at the initial developmental stages compared to other breeding techniques.

Marie Nyman's and Frank Hartung's presentations led to a discussion in the audience on the currently very technique-based regulation within the EU and whether it would not be more appropriate to regulate and risk assess the end-product irrespective of technique used.

Policy of Federation of Swedish Farmers on GM plants

Jan Eksvärd

Jan Eksvärd is environmental manager at the Federation of Swedish Farmers (LRF), and he began by pointing out that a sustainable agriculture both needs to deliver more food to the plate and reduce environment impact. Social, ecological and economic factors have to be weighed in. Some of the future challenges are that 50-30% of all food is lost on its way to the plate, how to use the available land for food, feed, fuel or fibres and adaption to climate change with 5 month longer growing period.

Jan Eksvärd expressed that the EU approval system on GMOs is too expensive, reducing development and competition among breeding organisations and that knowledge and companies in the sector are leaving the EU. Besides new cropping management practices, agriculture needs crops and varieties with new appropriate properties for the Northern climate to meet our challenges. Focus should be on how to develop a more sustainable agriculture. All old and new techniques are probably needed. Farmers want new varieties to be safe so all new properties should be tested in an approval system that gives a safe enough result, is fast, cheap and encourages competition among breeding companies and institutions. Jan Eksvärd also believed that the current debate which focuses on for or against GMOs need to be held in the broader context of sustainable development and new properties independent of breeding method.

A review system should be developed that focuses on sustainability and health issues on a system level, independent of breeding technique. Investigations could differentiate depending on type of new properties, earlier experience, gene mapping and used technique. This could initially work in parallel with the GM review system.

LRF has a general policy for genetically modified products stating that they e.g. should contribute to a sustainable development, be evaluated on the basis of precaution, follow the values of farmers and consumers, be labelled, and allow the coexistence with non-GM cultivars. The debate around GM-crops should be conducted in an open manner.

After the presentation the question who should take the lead in taking the debate to the next level arose. To this Jan Eksvärd pointed out that farmers and scientists to a large extent have similar views on what is needed, not to forget that consumer trust is a key for success. The question should be broadened to new properties for sustainable development and that both old and new techniques will be needed. With an approving system that focuses on new properties, not only the breeding industry can develop but also the debate can be held in a broader context.

Public acceptance of different biotech and GM-technologies

Prof. Sven Ove Hansson

Sven Ove Hansson is Head of the Division of Philosophy, Royal Institute of Technology, and Program director at Mistra Biotech, at SLU. He is also President of the Society for Philosophy and Technology.

From one point of view GM crops have been very successful with an incredible increase during the last 20 years. There is no other example of an agricultural practice adopted so fast. Of the food sold in North American grocery stores, 70% contains at least some GMOs. At the same time, the technology has encountered strong resistance in many European countries, but from a global perspective this stand against GM-crops can be seen as European "exceptionalism". But there are also other techniques that encounter public resistance, even if few quite as much as GM.

What are the characteristics for such technologies? If there are direct personal advantages, a technology will be accepted even if 'dangerous'. An obvious example is the internet (which e.g. enables pedophilia, terrorism). Another example is mobile networks where the public is usually more concerned about the radiation from base stations than from mobile phones, which are perceived to be very useful in everyday life, in spite of the fact that the base stations give rise to lower doses of (non-ionizing) radiation than the mobile phones. The same is true in biotechnology, where there is little public resistance against recombinant DNA if used for therapeutic purposes. Frank Hartung continued to talk about risk assessment and new breeding techniques with a deeper focus on the actual molecular methods used. He started off with reminding the audience that today 160 million hectares GM-crops are grown worldwide by no less than 16.9 million farmers. As a member of the EFSA working group on risk assessment of plants developed by new techniques he has examined these in the context of the European GM legislation. A final report was presented in February 2012. Frank Hartung especially stressed different techniques for site directed nuclease (SDN). Organisms as an outcome of some of these are, suggested by the working group, not to be considered as genetically modified; among these are oligonucleotide-directed mutagenesis (ODM) and certain zink-finger nuclease methods if recombinant DNA is not used (for more details, see presentations at www.slu.se/mistrabiotech). In comparison to the introduction of recombinant DNA or classic mutagenesis these new breeding techniques facilitate more precise mutations, transfer and integration of DNA, and have less side-effects. It is also possible to avoid the usage of selection markers. In fact, transgenes and non-transgenes cannot be distinguished after the events, which is a pre-requisite for monitoring and regulating GMOs. In addition many of the new breeding techniques are more cost-efficient also at the initial developmental stages compared to other breeding techniques.

Members of the public - irrespective of political views - are generally suspicious against technology good for governments or big companies, and show low tolerance to techniques benefiting these parts of society. Other aspects that can raise concern in the general public is the notion that humans should not play God, which is an example of issues not linked to risk but rather to ethics. This and other issues linked to belief or religion can be classified as "world-view issues".

Human beings tend to treat new technologies as a matter of risk, which might be problematic. In the case of GM-crops it would be wise to take precautionary measures only for some types of genes. But it is hard to argue that this should be inferred in general for all genes and GM applications. Still, the technique as such is considered dangerous.

Risks are often perceived by the general public who then express their fear, but it is important to distinguish that concerns may have different origins. It is extra difficult when public concern is not linked to true risk introduced by a technique.

And sometimes even experts might be wrong! For example experts in nuclear science in the 70's (when also the concept of GMOs first evolved) calculated that one melt down would occur in every 1,000,000 reactor years. The result to date is 5 melt downs in 50,000 reactor years, which gives a frequency of 1/10,000 not 1/1,000,000. Most technologies are connected to some sort of risk. The best solutions are those that make accidents impossible (inherent safety), not only reduce their probabilities.

The goal should be "transparent safety", where it is easy for non-experts to ascertain safety measurements taken and make these readily understandable.

With respect to GM-crops the following should be considered:

1. We should distinguish between different GMO applications; all GMOs can't be treated alike

2. Personal advantages such as health benefits or price with GM-crops should be highlighted.

3. What is benefiting big companies and governments or farmers and consumers should be distinguished, highlighting farmer focused advantages.

4. Find better ways to address world-view issues

5. Specific examples where transparent safety can be applied should be identified.

After the presentation the question of the influence of price on GM choice among consumers was raised in the audience; generally people are not willing to trade off safety for price.

Panel discussion

Q: What will be the situation for GM in Europe in 20 years?

Jan: No difference, legislation has to be replaced. GM feed will still be imported.

Huw: No dramatic changes in legislation. For public confidence, risk assessment is needed for the specific products if GM crops are to exist. Importation of GMO will continue but cultivation of GMOs will be slower; but new attempts to introduce GM-crops on a member-state by member-state basis might occur. There is an EU proposal of non-cultivation clause for member states over-ruling scientific evaluation and recommendations.

Tina: Real needs should be put in the first place and be communicated well.

Marie: Slight optimism even without a new legislation and a new legislation will be initiated.

Frank: Better than today because of worldwide spreading of GM-food and feed.

Pere: New traits that benefit people might appear, like the golden rice. Traits that are viewed as positive could improve status. Economical down-turn in Europe might lead to focus on more pressing questions other than GMOs. Communication is still important.

Q: What measures could be taken to achieve a more positive future?

Jan: There should be a focus on properties of crops independent of breeding technique.

Tina: We should ask ourselves which ecosystems services we need rather than "how can this method solve the problem?" GM should be approached as a whole package with advantages and disadvantages.

Jan: I do not agree; focus on properties independent of breeding technique instead.

Q: Companies have now withdrawn from Europe; what will the funding be in the future?

Huw: No big biotech companies left in Europe. Only academic research today and this is very small since route to application/market is difficult and expensive. Many scientists in applied biotech have left this research field or left EU. Smaller breeding companies cannot afford the costs of R&D and regulation. Still Europe has become a consumer of GM crops.

Jan: There is not much time; present legislation need to be replaced.

Q: Surprises might occur and play a role, like the BSE crisis for example. We need to be humble also when we talk about properties.

Jan: Agree, but we should not get stuck in present situation.

Huw: Legislation based on processes has to continually chase new technologies all the time and will constantly lag behind; and will as a consequence become illogical. Better to evolve a regulatory framework based on trait/product.

Pere: Properties a possible way out. But environmental arguments are difficult since targets are complicated.

Tina: Money to protect biodiversity and function has no clear links. We need to evaluate all crops not only GMs, which crops advances ecosystems services and which do not.

Q: Third world will bring technology forward, and break the viscous circle in Europe. Such a change could influence NGOs and make them realize that they should focus on larger issues. GM is also a question of European protectionism. Sometime in the future even European sceptics can change target if progress in the developing world goes well.

SO: That would be an unusual technology-transfer from Africa to Europe, as usually it is the other way round!

Q: Who should drive the public discussion on plant biotech inventions forward?

Frank: Bring up something people are interested in. It is hard for scientists to reach the heart of people.

Jan: We should communicate together to create trustworthiness; farmers, scientists, environmentalists.

Huw: Scientists should share the platform together with farmers and NGOs complimenting each other. There is a lack of scientific understanding among European politicians.

Tina: Risk assessment; clear risks are identified and we need to communicate the unattractive areas linked to GM as well. And acknowledge also areas that are not acute risks.

Marie: Developing world might be the way forward. Many people are surprised when they hear about the state in EU.

Pere: Who is an expert? Experts can also come from industry. Being on different boards is time consuming and does not much for your career. Who should we choose? Scientists will have an important role there.

Comment: Legislation lags behind at the same time as development rushes. Now with even more powerful tools at hand, scientists should be even more pedagogical towards to the general public not to increase worries. There is a danger that new breeding technologies will be regarded only as a way for scientists and companies to bypass legislation. Biosafety legislation is needed, not a technique-based one.

About PlantLink and Mistra Biotech

Plant Link is an alliance between Lund University and the Swedish University of Agricultural Sciences in Alnarp (SLU Alnarp). Our mission is to stimulate and coordinate plant research and higher education in Southern Sweden. We strive to increase the interest and competence in molecular plant science and we want to create an environment that promotes research, innovation and a dynamic interaction between the universities, private companies and the general public.Plant Link has financial support from the Skåne Regional Council (Region Skåne). More information at www.plantlink.se

Mistra Biotech is a research programme that started in the beginning of 2012. The research is focused on different aspects of the use of biotechnology in agriculture and involves scientists from several disciplines. Most researchers work at SLU but researchers at KTH, Lund University, Aarhus University and Roskilde University are also involved. The goal for Mistra Biotech is to contribute to sustainable agricultural and food production, from an environmental, social and economic perspective. The programme is funded by The Swedish Foundation for Strategic Environmental Research (Mistra) and SLU. More information: www.slu.se/mistrabiotech

Sammanfattning

Artikeln är en sammanfattning av ett seminarium som arrangerades 7 november 2012 av Plant Link, Partnerskap Alnarp och Mistra Biotech. Seminariet samlade över hundra deltagare, men ännu fler skulle nog ha gått om de hade kunnat. Visade power point-presentationer finns att tillgå på www.slu.se/mistrabiotech (under Nyheter).

Plant Link är en allians mellan Lunds universitet och SLU i Alnarp. Uppdraget är att stimulera och samordna växtforskning och högre utbildning i södra Sverige. Vi strävar efter att öka intresset och kompetensen i den molekylära växtforskningen och vi vill skapa en miljö som främjar forskning, innovation och en dynamisk samverkan mellan universitet, privata företag och allmänheten. Plant Link har finansiellt stöd från Region Skåne (Region Skåne). Mer information finns på www.plantlink.se

Mistra Biotech är ett forskningsprogram som startade i början av 2012. Forskningen är fokuserad på olika aspekter av användningen av bioteknik inom jordbruket och involverar forskare från flera discipliner. De flesta forskare arbetar vid SLU, men forskare vid KTH, Lunds universitet, Aarhus Universitet och Roskilde Universitet är också engagerade. Målet för Mistra Biotech är att, från ett miljömässigt, socialt och ekonomiskt perspektiv, bidra till hållbarhet i jordbruk och livsmedelsproduktion. Programmet finansieras av Svensk Stiftelsen för miljöstrategisk forskning (Mistra) och SLU. Mer information finns på www.slu.se/mistrabiotech.

Erik Alexandersson är forskarassistent vid SLU/Växtskyddsbiologi i Alnarp.
erik.alexandersson@slu.se

Anna Lehrman är knuten till SLU/Institutionen för växtproduktionsekologi i Uppsala.
anna.lehrman@slu.se

Translokations- och duplikationslinjer hos korn, än en gång

Barley translocation and duplication lines revisited

Robert Hasterok, Justyna Majlinger, Lukasz Kubica, Kerstin Brismar and Waheeb K. Heneen

Abstract

The focus of this cytological study is on the two nucleolar chromosome pairs 6H and 7H of barley (Hordeum vulgare L., 2n=14). These chromosomes carry the major sites of rDNA that harbour repeats of the 18S-5.8S-26S ribosomal genes at the secondary constriction. These sites act as nucleolar organizer regions (NOR 6 and NOR 7) that form nucleoli, when the ribosomal genes are expressed. If NOR 6 and NOR 7 occur on the same chromosome due to reciprocal translocations or segment duplications, nucleolar dominance prevails, implying activity of the ribosomal genes in NOR 6 and suppression of those in NOR 7. Secondary constrictions, activity of NORs and nu-cleolar features were studied in the cultivar 'Bonus' and translocation lines T6-7ab and T6-7d, and duplication lines D2 and D24 from the late Professor Arne Hagberg´s collection of barley mutants. NOR 6 and NOR 7, either intact or partial, occurred on the same chromosome in all four translocation and duplication lines. Correlations were found between size of secondary constrictions, activity of NORs visualized by silver nitrate staining, maximum number and size of nucleoli in mitotic nuclei, and associations between nucleoli and rDNA sites of meiotic bivalents 6H and 7H. Com-plementary information was also gained by determining the relative amounts of intact rDNA regions, or portions of rDNA regions, appearing as fluorescent signals after using fluorescence in situ hybridization (FISH) with rDNA probes. NOR 6, whether intact or partial, showed nucleolar dominance over NOR 7 in the translocation and duplication lines. Of relevance is the finding in the cultivar 'Bonus' of relatively higher amounts of rDNA in 7H than in 6H, and a more active NOR 6 compared to NOR 7 which is reflected in the size of nucleoli in mitotic nuclei and in the nucleolar associations with rDNA sites of meiotic bivalents 6H and 7H. Nucleolar dominance in the translocation and duplication lines may be considered as an accentuation of the existing minor differences in activity between NOR 6 and NOR 7 in the standard bar-ley cultivar 'Bonus'. Expression and suppression of NORs are currently understood as being epigenetic phenomena. DNA methylation, histone modifications, chromatin modulation, and short interfering RNAs are of possible significance in this context. Meiotic aberrations in the translocation and duplication lines were also documented.

Keywords: Barley translocation and duplication lines, rDNA, FISH, nucleolar organ-izer regions, nucleoli, intra-chromosomal nucleolar dominance.

The late Professor Arne Hagberg (1919-2011).

Introduction

Barley (*Hordeum vulgare* L., 2n=14) is a model cereal crop for cytological and cytomolecular studies (Heneen, 2011). Many chromosome translocation and duplication lines have been developed in barley, largely by the Hagbergs and their co-workers (Hagberg, A., 1986, 1994 and references therein). Translocation lines were the material where the phenomenon of intra-chromosomal nucleolar dominance was discovered and studied (Nicoloff *et al.*, 1977a and b, 1979; Anastassova-Kristeva *et al.*, 1980; Rieger *et al.*, 1979; Schubert and Künzel, 1990; Kitanova and Georgiev, 2005; Ruffini Castiglione *et al.*, 2008; Dimitrova *et al.*, 2009). Intra-chromosomal nucleolar dominance is encountered when a translocation between two different nucleolar chromosomes leads to the occurrence of their nucleolar organizer regions (NORs) on the same chromosome. As a consequence, expression of one of the two NORs is suppressed. The phenomenon is described as intra-chromosomal since inter-chromosomal nucleolar dominance, initially referred to as "differential amphiplasty" (Navashin, 1934), has been already reported in intergeneric and interspecific hybrids carrying intact nucleolar chromosomes of two parental origins.

The NORs are the transcriptionally active regions of the tandem rDNA repeats that encode for 18S-5.8S-26S ribosomal RNAs (hereafter referred to as 45S rRNA, which is the primary transcript). These regions coincide with the secondary constriction in the short arm of chromosomes 6H and 7H. Expression of ribosomal genes can be visualised after staining with silver nitrate as silver bands (Ag-bands) of relic proteins at the NORs of condensed chromosomes during division, and as maximum numbers of darkly stained nucleoli at late telophase or early interphase, before their fusion takes place. Early studies on nucleolar dominance in barley focused on the interrelationships between different types of translocations and number and size of nucleoli (see review by Rieger *et al.*, 1979). It was shown that when the NORs of 6H and 7H occurred on the same chromosome, it was the NOR of 7H that was always suppressed, whether it was transposed or not (Anastassova-Kristeva *et al.*, 1980; Kitanova and Georgiev, 2005).

Possible explanations of inter- or intra-chromosomal nucleolar dominance, in plant and animal materials, have been proffered (Reeder, 1985, Pikaard, 2000; Santoro, 2005). Using different approaches, it was found that suppression of NORs was not due to loss of rDNA repeats (Subrahmanyam and Azad, 1978b; Schubert and Künzel, 1990; Kitanova and Georgiev, 2005). Nucleolar dominance/suppression, being an epigenetic phenomenon and reflecting chromatin modulation in terms of DNA methylation and histone modifications, has been implicated in the regulation of rRNA gene expression (Grummt and Pikaard, 2003; Pontes *et al.*, 2003; Neves *et al.*, 2005; Santoro, 2005; Preuss *et al.*, 2008; Ruffini Castiglione *et al.*, 2008).

In the present work on barley, two translocation lines and two duplication lines from the Hagberg collection, together with the cultivar 'Bonus', were chosen for further analysis. Interrelationships and correlations between relative amounts of 45S rDNA repeats, appearance of secondary constrictions, activity of NORs, maximum number and size of nucleoli in mitotic cells, and association of nucleoli with meiotic prophase chromosomes were determined. Application of both Ag-staining and FISH with rDNA probes on the same or different cells of all materials envisaged correlations between relative activity and amounts of rDNA. A new maximum number of six nucleoli were recorded in the translocation lines. This number correlated with the observed Ag-banding patterns of metaphase chromosomes. Nucleolar dominance of NOR 6 over NOR 7 in the four studied lines can be regarded as an accentuation of interrelationships between these NORs in the standard barley cultivar 'Bonus'. The findings are discussed in view of earlier works on these and similar lines and in view of current understanding of nucleolar dominance.

Material and methods

Plant material: Kernels of barley, *Hordeum vulgare* subsp. *vulgare* L. cultivar 'Bonus', and of barley lines homozygous for the translocations T6-7ab and T6-7d, and duplications D2 and D24 (Table 1) were kindly obtained from the gene bank NordGen (www.nordgen.org) in Alnarp, Sweden. In addition, kernels heterozygous for the translocation T6-7ab were made available by the late Professor Arne Hagberg. The duplication lines originated from crosses between two reciprocal translocation lines (T6-7w x T6-7ae) induced in the cultivar 'Bonus' in the case of D2, and between translocation lines induced in the cultivars 'Bonus' (T6-7ae) and 'Betzes' (T6-7q) in the case of D24 (Hagberg,

Accession number	Accession name	Description
NGB14657	'Bonus'	Standard karyotype
NGB131647	T6-7ab	Translocation
NGB131625	T6-7d	Translocation
NGB131690	D2	Duplication
NGB131686	D24	Duplication

Table 1. Accessions of barley (Hordeum vulgare subsp. vulgare L.) obtained from the gene bank NordGen (www.nordgen.org)

P. and Hagberg, A., 1978; Subrahmanyam *et al.*, 1994). All the above translocations were induced by gamma rays, except T6-7q which was spontaneous (Hagberg, A. *et al.*, 1978). The studied lines are among many other barley lines with structurally changed chromosomes, developed by the Hagbergs, and available at the gene bank NordGen.

The material for mitotic and meiotic chromosome squashes: Seeds were germinated at 22 ± 2 °C in dark in Petri dishes on filter paper moistened with tap water. Whole seedlings with a root length of 1.0 - 3.0 cm were immersed in ice-cold water and incubated for 24 - 26 h. Excised roots were fixed in 3:1 (v/v) methanol/glacial acetic acid at room temperature for 4h, and then stored at –20 °C until use. Immature inflorescences from potted plants were fixed in 3:1 (v/v) ethanol/glacial acetic acid at room temperature for 4 h, and then stored at –20 °C until use.

Feulgen staining: Fixed roots containing apical meristems were rinsed briefly with distilled water and then hydrolysed in 5M HCl at 20 °C for 40 - 60 min. Afterwards, the material was immediately transferred into Schiff's reagent (Sigma) for 2 h at room temperature or until the meristematic tissue stained *in toto* deep purple. After brief washes in distilled water apical root meristem tissue was dissected from the rest of the root and squashed on a microscope slide in drops of 45 % acetic acid and frozen. After freezing, cover-slips were removed and the preparations were air dried and mounted in synthetic resin (DPX).

Preparation of root tip meristem tissue for mitotic chromosome squashes: The preparations were made according to the protocol described in detail in Jenkins and Hasterok (2007). In brief, fixed roots were washed in 10 mM citric acid – sodium citrate buffer (citrate buffer, pH 4.8) for 15 - 30 min and subjected to enzymatic digestion in a mixture comprising 20 % (v/v) pectinase (Sigma) and 2 % (w/v) cellulase (Calbiochem) for 2.5 h at 37 °C. Meristems were dissected out from root tips, squashed in drops of 45 % acetic acid and the preparations were frozen at –60 °C. After freezing, cover-slips were removed and the preparations were air dried.

Preparation of anther cell tissue for meiotic chromosome squashes: The preparations were made as described in detail in Jenkins and Hasterok (2007) with minor modifications. Briefly, fixed immature inflorescences were washed in 10 mM citrate buffer (pH 4.8). Isolated anthers were enzymatically digested in solution containing 10 % (v/v) pectinase (Sigma), 0.65 % (w/v) cellulase Onozuka R-10 (Serva), 0.5 % cellulase (Calbiochem), 0.15 % (w/v) cytohelicase (Sigma) and 0.15 % (w/v) pectolyase (Sigma) in 10 mM citrate buffer. Further steps of the procedure were similar to the treatment of root material. After removing coverslips, the preparations were post-fixed in chilled 3:1 ethanol/glacial acetic acid, dehydrated in absolute ethanol and air dried.

Staining with silver nitrate and slide destaining: Silver staining followed the protocol of Hizume *et al.* (1980) with minor modifications. In brief, slides were immersed for 10 min in a borate buffer, pH 9.2 (Merck) and air-dried. 50 µl of freshly prepared 50 % aqueous solution of silver nitrate was applied to the preparation. Slides were covered with a nylon mesh and incubated in a humid chamber at 42 °C for 30 - 120 min, then washed several times in double distilled water, air dried and mounted in glycerol. Slide destaining prior to FISH follows the method described in detail in Idziak and Hasterok (2008).

Fluoresecence in situ hybridization (FISH): The following DNA probes were used in this study:

(1) 2.3-kb *Cla*I subclone of 25S rDNA derived from *A. thaliana* (Unfried and Gruendler, 1990). This probe was labelled by nick translation either with digoxigenin-11-dUTP (Roche) and visualised by immunodetection using fluorescence isothiocyanate (FITC)-conjugated anti-digoxigenin antibodies (Roche) or labelled and directly visualised

using tetramethyl-rhodamine-5-dUTP (Roche). This probe allowed detection of 45S rDNA loci containing the genes coding for 18S, 5.8S and 25S rRNA.

(2) pTa794 clone containing a 410-bp fragment of 5S rDNA unit of *Triticum aestivum* (Gerlach and Dyer, 1980) was labelled by PCR with the same labels as above. Both labelling and FISH were carried out using the protocols described in details in Hasterok *et al.* (2002) and Jenkins and Hasterok (2007).

Staining with Snow´s carmine: Pollen mother cells (PMCs) were studied after fixation in ethanol and acetic acid (3:1), storage in 70 % ethanol and staining in Snow´s carmine (Snow, 1963). Of special interest were the nucleolar associations with bivalents during pachytene and diakinesis.

Chromosome numbering: Numbering of the seven chromosome types of barley, 1H – 7H (henceforth used to refer to these chromosomes) is according to the internationally adopted system (Linde-Laursen *et al.*, 1997). The nucleolar chromosomes 6H and 7H carry the NOR 6 and NOR 7, respectively.

Results

Emphasis has been placed upon the two nucleolar chromosome pairs 6H and 7H of barley, since they are the chromosomes involved in the studied translocation and duplication lines. These chromosomes are identifiable morphologically in mitotic spreads by their secondary constrictions and satellites (Fig. 1). The secondary constriction and neighbouring proximal chromatin harbour the 45S rDNA, a fraction of which is the NOR.

Schematic idiograms of 6H and 7H in the studied barley material are presented in Fig. 2. In these the chromosomes are divided into segments as employed by Subrahmanyam *et al.* (1994) for defining sites, content and orientation of translocated and duplicated chromosome segments. Chromosome size relationships, sites of the centromere and secondary constrictions embodying the NORs, and the segments involved in the reciprocal translocations and the duplications are apparent (Fig. 2). The numbering of segments in the idiograms facilitates the mapping of break sites as well as the content and orientation of involved chromosome portions.

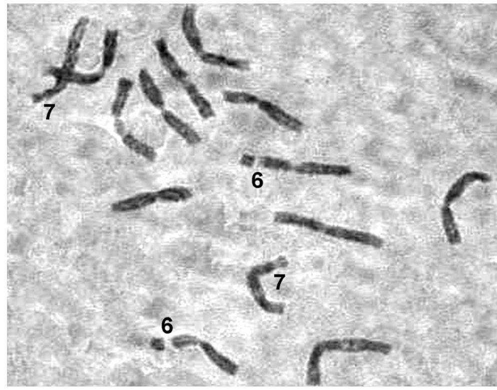

Figure 1. Feulgen stained mitotic metaphase chromosomes of barley cultivar ’Bonus’ with chromosomes 6H and 7H distinguishable by the positions of their centromeres and secondary constrictions. Scale, 10 µm.

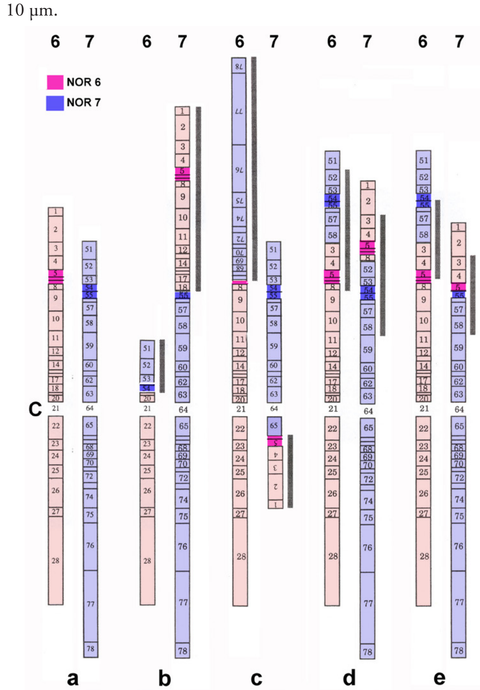

Figure 2. Idiograms of the nucleolar chromosomes 6H (pink) and 7H (blue) subdivided into numbered segments 1-28 and 51-78, respectively, with the centromeric region (C) uncoloured and the NORs densely coloured; a, standard chromosomes of Hordeum vulgare subsp. vulgare cultivar ’Bonus’; b and c, translocation lines T6-6ab and T6-7d, respectively, the translocated regions are indicated by adjacent grey rods; b, 6H with partial NOR 7, and 7H with intact NOR 6 and partial NOR 7; c, 6H with partial NOR 6 and 7H with intact NOR 7 and partial NOR 6; d and e, duplication lines D2 and D24, respectively, with duplicated regions indicated by adjacent grey rods; d, 6H with distal intact NOR 7 and proximal intact NOR 6 on the same arm, and the reverse positions of NORs in 7H; e, 6H with intact proximal NOR 6 and intact distal NOR 7 on the same arm, and 7H with one NOR composed of partial NOR 6 and partial NOR 7.

Barley cultivar 'Bonus'

Of relevance in the present context is the appearance of the satellite metaphase chromosomes 6H and 7H during mitotic metaphase in the control 'Bonus' material (Fig. 1 and 2a). Chromosome 6H has a more median centromere and a larger satellite than 7H. To be emphasized here is the difference in the size of the secondary constrictions between 6H and 7H (Fig. 1). In 6H, the constriction is more distinct and wider, a condition that can lead to separation of the satellite during slide preparation, which usually is not the case for 7H. Similarly at meiotic diakinesis, the secondary constriction in bivalent 6H is the one that is often visualized (Fig. 3e-g and j).

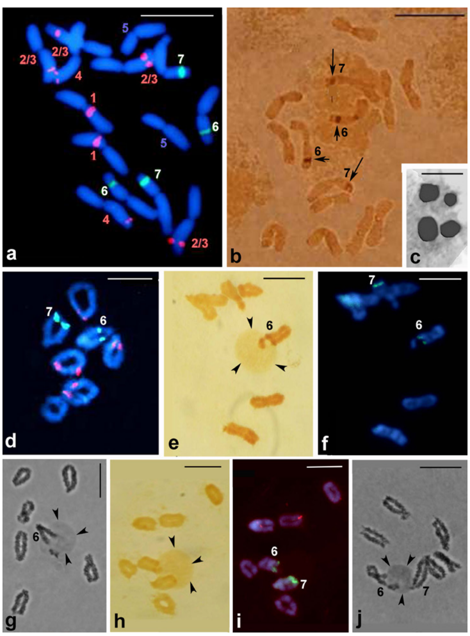

Figure 3. Chromosomes and nucleoli of barley Hordeum vulgare subsp. vulgare cultivar 'Bonus', subjected to FISH with 5S rDNA (red fluorescence) and 25S rDNA (green fluorescence) (a, d, i) or only 25S rDNA (f), or stained by silver nitrate (b, c, e, h), or Snow´s carmine (g, j), short and long arrows mark NORs 6 and 7, respectively, arrowheads mark contours of the nucleolus; a-c, mitotic cells; a, metaphase chromosomes, pairs 1H-4H contain 5S rDNA, pair 5H no signal, pairs 6H and 7H contain 45 S rDNA, a larger signal in pair 7H than in pair 6H; b, staining of NORs of 6H and 7H, more prominent bands on 6H than in 7H; c, four nucleoli in an interphase nucleus, two large and two smaller nucleoli organized by pairs 6H and 7H, respectively; d-j, diakinesis bivalents; d, larger signals on 7H than on 6H; e and f, same cell, one nucleolus associated with 6H; g, one nucleolus associated with 6H; h and i, same cell, one nucleolus associated with 6H and 7H; j, one nucleolus associated with 6H and 7H. Scale bars, 10 µm.

FISH with rDNA probes disclosed the localisation of 5S and 45S rDNA sites on the mitotic metaphase chromosomes (Fig. 3a). Sites and intensities of signals were characteristic for the different chromosomes. Pairs 1H-4H harboured 5S rDNA sites, chromosomes 5H did not carry any rDNA sites, while 6H and 7H exhibited their distinctive sites of 45S rDNA. Chromosome 6H had a less intense signal than the larger and more distal signal in 7H. Size differences of these signals reflected the larger amounts of rDNA in 7H than in 6H. The application of silver staining, which discloses the relative activity of the rDNA sites revealed the opposite situation, a more dense and larger Ag-band on 6H compared to 7H (Fig. 3b). The sites of the Ag-bands mark the NORs, and their size reflects the activity within the sites. Nucleolar formation starts at late telophase and early interphase by the appearance of four nucleoli, two of which being slightly larger than the other two (Fig. 3c), likely inferring their synthesis by 6H and 7H, respectively.

During meiosis in PMCs at diakinesis and after FISH with 25S rDNA, less distal signals on one bivalent were, as expected, smaller than the more distal signals on the other, apparently representing pairs 6H and 7H, respectively (Fig. 3d). Only one nucleolus, either associated with one or two bivalents, was most frequently observed in PMCs at diakinesis. When associated with one bivalent, this was bivalent 6H, defined by its smaller 25S rDNA FISH signal compared to that on bivalent 7H (Fig. 3e and f), or by its large often detached satellite (Fig. 3f and g). Association of one nucleolus with both bivalents 6H and 7H, defined by FISH (Fig. 3h and i), has also been documented after Snow´s carmine staining (Fig. 3j).

Thus, in the standard barley karyotype 6H has a lower amount of 45S rDNA, a more prominent secondary constriction, and a higher activity of its NOR, than is the case in 7H.

Translocation line T6-7ab

The break points of the reciprocal translocation T6-7ab are at a proximal site on the short arm of 6H and in the NOR of 7H (Fig. 2b). The result is a shorter than normal 6H with a partial NOR 7 together with the satellite region of 7H, and a longer than normal 7H with a partial NOR 7 and almost the entire short arm of 6H (Fig. 2b). The

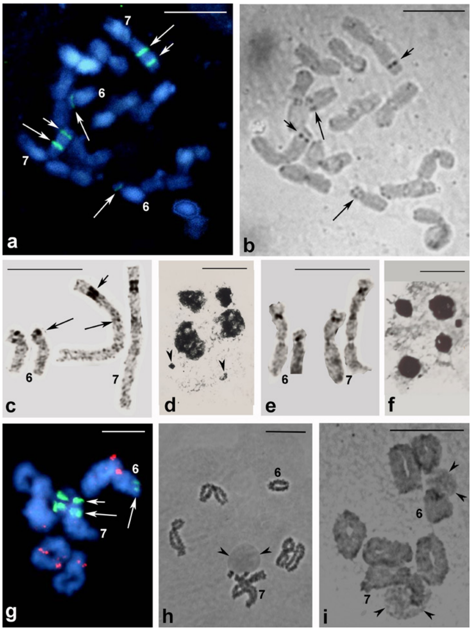

Figure 4. Chromosomes and nucleoli of translocation line T6-7ab in homozygotes (a-d, g-i) and in a heterozygote (e,f), hybridised with 25S rDNA (a) or both 5S rDNA (red fluorescence) and 25S rDNA (green fluorescence) probe (g), or stained with silver nitrate (b-f, i) or Snow´s carmine (h), short and long arrows mark NORs 6 and 7, respectively, arrowheads mark nucleoli; a-f, mitotic chromosomes and interphase nucleoli; a and b, the same metaphase spread; a, partial NOR 7 on 6H, and distal intact NOR 6 and proximal partial NOR 7 on 7H; b, distinct silver bands at the distally located NOR 6 on 7H and weak bands at the partial NOR 7 on 6H; c, 6H and 7H with stained NORs, two large regions at intact NOR 6 on pair 7H, two intermediate bands at partial NOR 7 on pair 6H, and two minor bands at partial NOR 7 on pair 7H; d, two large and two intermediate nucleoli and two minor nucleoli (arrowheads) in an interphase nucleus; e, stained NORs in two standard and two translocation chromosomes 6H and 7H, all five sites are silver stained (compare with c); f, two large and two intermediate nucleoli and one small nucleolus in an interphase nucleus; g-i, diakinesis bivalents; g, more intensive signal on partial NOR 7 than on intact NOR 6 of 7H; h, NOR 6 on 7H associated with one nucleolus; i, a small nucleolus associated with partial NOR7 on 6H and a large nucleolus associated with intact NOR 6 on 7H. Scale bars, 10 µm.

reconstructed chromosomes are distinguishable in mitotic metaphase complements of homozygous plants after FISH with 25S rDNA (Fig. 4a). The most prominent signal is that of the proximal partial NOR 7 on 7H followed by the distal signal of the intact NOR 6 on the same chromosome, and the signal with least intensity is that of the partial NOR 7 on 6H (Fig. 4a). This indicates that the amount of rDNA in the partial NOR 7 on 7H is larger than that of the intact NOR6 on the same chromosome, and that only a minor part of NOR 7 is translocated to 6H. The same chromosome spread stained with silver nitrate exhibits the Ag-bands at the NORs representing transcriptionally active rDNA sites (Fig. 4b). The opposite situation prevailed regarding the size of Ag-bands. The Ag-band of the distal NOR 6 on 7H was the largest, followed by that of the partial NOR 7 on 6H, while that of the partial NOR 7 on 7H was not detectable in Fig. 4b but distinguishable as a minor band on the less contracted metaphase chromosomes shown in Fig. 4c. By comparing Fig. 4a and c, it is apparent that in spite of the inferred presence of a higher number of rDNA repeats in the partial NOR7 on 7H than in the portion of this NOR translocated to 6H (Fig. 4a), the Ag-band on 7H was smaller than that on 6H (Fig. 4c), indicating suppression of the partial NOR 7 on 7H. The maximum number of nucleoli at interphase being two large, two intermediate and two small nucleoli (Fig. 4d) is thus likely organized by two intact NORs 6, two partial NORs 7 on two 6H, and two partial NOR 7 on two 7H, respectively.

In plants heterozygous for this translocation, pairs 6H and 7H comprise one standard chromosome and one reconstructed chromosome each, exhibiting the expected five sites of Ag-stained NORs (Fig. 4e, compare with Fig. 4c). The maximum number of nucleoli observed at interphase was similarly five comprising two large nucleoli, two intermediate nucleoli and one small nucleolus (Fig. 4f). Taking into consideration the size of the Ag-bands in Fig. 4c and e, the origin of the nucleoli can be deduced. The two large nucleoli are apparently organized by the intact NORs 6, one on the standard 6H and one on the reconstructed 7H. The two intermediate nucleoli are seemingly organized by the NOR 7 on standard 7H and the partial NOR 7 on 6H. The small nucleolus is organized by the partial NOR 7 on 7H.

The size relationships of rDNA FISH signals, observed in mitotic cells of homozygous plants (Fig. 4a) are similarly expressed in bivalents 6H and 7H of PMCs at diakinesis (Fig. 4g). One nucleolus may occur associated with NOR 6 on bivalent 7H (Fig. 4h). Alternatively, a large nucleolus occurs at this site and a small nucleolus appears associated with the partial NOR 7 on bivalent 6H (Fig. 4i).

Thus, in T6-7ab the amount of 45S rDNA of 7H type that remains on the reconstructed 7H is still higher than the total amount of 45S rDNA of 6H type translocated to this chromosome. The part of 45S rDNA of 7H type translocated to 6H is a minor part. The activity of the NORs was highest in the intact NOR6 sites, followed by the partial NOR 7 on 6H, and lowest at the partial NOR 7 on 7H, thus demonstrating intra-chromosomal nucleolar dominance of NOR 6 over NOR 7.

Translocation line T6-7d

The break points of the translocation T6-7d are in the NOR 6 and in the proximal region of the long arm of 7H. This yields a large 6H with a partial NOR 6 and the translocated almost entire long arm of 7H, and a short 7H made up of its original short arm and a shorter other arm composed mainly of a partial NOR 6 and the satellite of 6H (Fig. 2c). Pairs of such reconstructed 6H and 7H are found in plants homozygous for this reciprocal translocation. A mitotic metaphase spread subjected to FISH using 5S and 25S rDNA probes is shown in Fig. 5a. As expected, the intact rDNA site of 7H exhibits a more prominent signal in comparison with that of the partial rDNA translocated from 6H to 7H and that of the partial rDNA remaining in its original site on 6H (Fig. 5a). Silver staining of mitotic chromosomes results in Ag-bands at these three sites (Fig. 5b). At interphase up to six nucleoli have been recorded in two or three decreasing size categories, either four plus two or two plus two plus two (Fig. 5c). The smallest nucleoli are possibly synthesized by the partial NOR7 on pair 7H, which is inferred from the observations made on meiotic pachytene and diakinesis PMCs, presented below.

In the two pachytene PMCs shown in Fig. 5d and e, one large nucleolus is associated with a markedly short bivalent, apparently representing the reconstructed 7H. The association site seemingly coincides with the site of the partial NOR 6. An additional small nucleolus appears associated with a long bivalent at a site that could be the partial NOR 6 on bivalent 6H (Fig. 5e). The association of one large nucleolus with both sites of partial NOR 6 on bivalents 6H and 7H is apparent in the diakinesis cells shown in Fig. 5f and g. The analysis of diakinesis cells stained by silver nitrate and sequentially subjected to FISH with 25S rDNA probe (Fig. 5h and i) further ascertains that it is the partial NOR 6 and not NOR 7 on bivalent 7H that is associated with a nucleolus. Thus, nucleoli

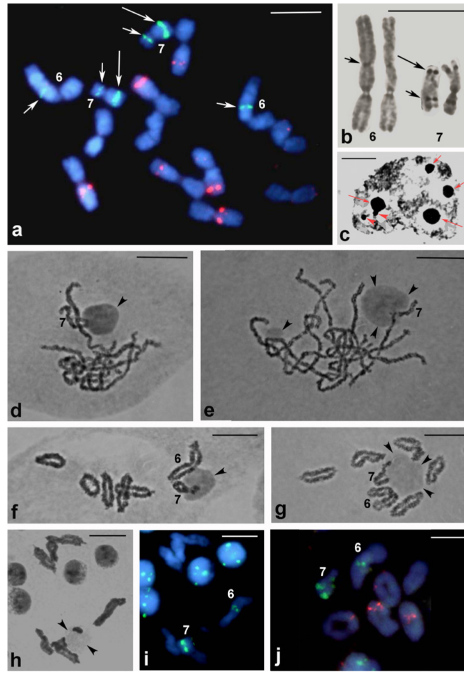

Figure 5. Mitotic and meiotic chromosomes and nucleoli of homozygous translocation line T6-7d hybridised with 5S rDNA (red fluorescence) and 25S rDNA (green fluorescence) (a, j) or only 25S rDNA probe (i), or stained by siver nitrate (b, c, h) or Snow´s carmine (d-g), short and long arrows mark NORs 6 and 7, respectively, arrowheads mark nucleoli; a and b, mitotic chromosomes; a, 6H with partial NOR 6, and 7H with partial NOR 6 on one arm and distinct intact NOR 7 on the other arm; b, nucleolar chromosomes, stained partial NOR 6 on 6H, and intact NOR 7 and partial NOR 6 on chromosome 7; c, mitotic interphase nucleus with six nucleoli in three size categories; d and e, pachytene bivalents; d, one nucleolus at NOR 6 site on 7H; e, large nucleolus at site of partial NOR 6 of 7H and a small nucleolus at the other site of partial NOR 6 on 6H; f-j, diakinesis bivalents; f and g, one nucleolus associated with NOR 6 sites on 6H and 7H; h and i, same cell, one nucleolus associated with 7H; j, 25S rDNA signals on 6H and 7H. Scale bars, 10 µm.

are associated mainly with the two partial NOR 6 sites, one on 6H and one on 7H. At late diakinesis and metaphase I, 45S rDNA sites specifically labelled with FISH can be defined on bivalents 6H and 7H (Fig. 5j).

Thus, in T6-7d the relative amounts of intact and partial 45S rDNA are distinguishable. The nucleolar patterns in mitotic cells and at meiotic pachytene and diakinesis are suggestive of nucleolar dominance of partial NOR 6 over intact NOR 7, when both are carried by 7H.

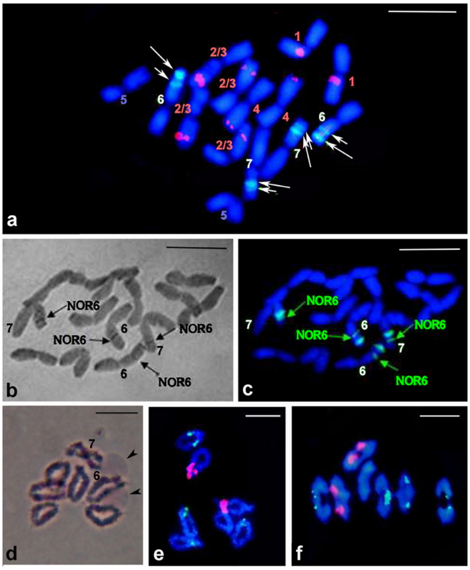

Figure 6. Mitotic and meiotic chromosomes of duplication line D2, short and long arrows mark NORs 6 and 7, respectively, arrowheads mark nucleoli; a-c, mitotic chromosomes subjected to FISH with 5S rDNA (red fluorescence) and 25S rDNA (green fluorescence) (a), or only 25S rDNA probe (c), or stained by silver nitrate (b); a, 45S rDNA seen as a large distal signal and smaller proximal signal on one arm of 6H, and as a small distal signal adjacent to a larger proximal signal on one arm of 7H; b and c, the same mitotic spread for comparison of FISH with 25S rDNA probe with the silver nitrate stained NORs restricted to NORs 6; d-f, meiotic diakinesis (d, e) and metaphase I (f) stained with Snow´s carmine (d) or hybridised with 5S rDNA (green fluorescence) and 25S rDNA (red fluorescence) probes (e, f); d, two nucleoli associated with bivalents 6H and 7H; e and f, labelling of four bivalents with 5S rDNA (green fluorescence) and two bivalents with 25S rDNA (red fluorescence). Scale bars, 10 µm.

Duplication line D2

As shown in the idiogram of 6H and 7H in this line (Fig. 2d), the duplicated segments in these two chromosomes comprise the NORs 6 and 7 with NOR 6 being located proximally in 6H and distally in 7H. Accordingly, after FISH of mitotic cells with 25S rDNA probe, the less intense signal is located proximally in 6H and distally in 7H (Fig. 6a). The fact that the rDNA sites are closer to each other in 7H than in 6H (Fig. 2d) may lead to their possible fusion, thus appearing as one large signal in condensed 7H metaphase chromosomes (Fig. 6a). When applying silver nitrate staining prior to rDNA FISH on the same mitotic spread, it can be seen that the visible Ag-band is located more proximally in 6H than in 7H, inferring the dominating activity of NOR 6 (Fig. 6b and c).

During meiosis, association of two nucleoli, one at a distal site of one bivalent, and one at a less distal site on another bivalent most likely reflects the positions of the main active sites of NOR 6 on bivalents 7H and 6H, respectively (Fig. 6d). Application of only rDNA FISH to diakinesis and metaphase I cells differentiated bivalents 6H and 7H from the rest of the complement (Fig. 6e and f). Due to the condensed state of diakinesis and metaphase I bivalents, the neighbouring rDNA sites were often fused, thus not permitting differentiation between bivalents 6H and 7H (Fig. 6e and f).

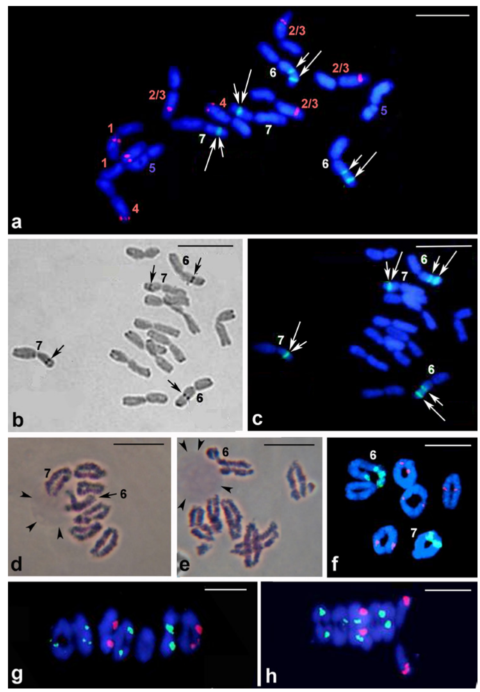

Figure 7. Mitotic and meiotic chromosomes of duplication line D24, short and long arrows mark NORs 6 and 7, respectively, arrowheads mark nucleoli; a-c, mitotic chromosomes hybridised with 5S rDNA (red fluorescence) and 25S rDNA (green fluorescence) (a), or only 25S rDNA probe (c) or stained with silver nitrate (b); a, two signals marking NOR 6 and NOR 7 on 6H, and one signal marking the composite site of partial NORs 6 and 7 on 7H; b and c, the same mitotic spread for comparison of 25S rDNA FISH signals with silver nitrate stained NORs mainly comprising NORs 6; d-h, meiotic diakinesis (d-f) and metaphase I (g, h); d and e, stained with Snow´s carmine; d, a nucleolus associated with bivalents 6H and 7H; e, a nucleolus associated with bivalent 6H; f, hybridised with 5S rDNA (red fluorescence) and 25S rDNA (green fluorescence) probes, two sites of 25S rDNA on bivalent 6H and one site on bivalent 7H; g and h, bivalents 6H and 7H distinguishable from the rest of the bivalents by their specific hybridisation pattern (red fluorescence); g, seven ring bivalents; h, six ring bivalents and one rod nucleolar bivalent. Scale bars, 10 µm

Thus, silver staining patterns of mitotic chromosomes, and nucleolar associations with bivalents at meiotic diakinesis, indicate the dominating activity of NOR 6 over NOR 7.

Duplication D24

The duplication in line "D24" pertains to a segment which in 6H is bordered at one end by a part of an intact distal NOR7 and at the other end by a part of an intact proximal NOR 6 (Fig. 2e). The corresponding segment in 7H contains the partial NORs 6 and 7 adjacent to each other forming one NOR (Fig. 2e). FISH of mitotic chromosomes exposes 45S rDNA at these sites as a prominent distal signal and a less prominent proximal signal on 6H, and as one prominent signal on 7H (Fig. 7a). Silver staining and FISH applied sequentially to the same metaphase spread portrays clear Ag-bands at sites of the intact NOR 6 on 6H and at the composite NOR on 7H containing portions of NOR 6 and NOR 7 (Fig. 7b and c).

In meiotic diakinesis cells, a nucleolus often appeared in association with two bivalents, presumably 6H and 7H (Fig. 7d). Association with the site of the intact NOR 6 in bivalent 6H is recognizable by the frequent increased distance between the paired chromosomes, or occurrence of a constriction, at this site (Fig. 7d and e). In Fig. 7e, 7H is not unambiguously identifiable. It was possible to differentiate between bivalents 6H and 7H in diakinesis configurations subjected to rDNA FISH by the appearance of double signals on bivalent 6H and a single signal on 7H (Fig. 7f). However, differentiation between bivalents 6H and 7H is not possible at metaphase I due to further chromatin condensation, which in effect causes fusion of signals in bivalent 6H (Fig. 7g and h).

Thus, silver staining patterns of mitotic chromosomes imply nucleolar dominance of NOR 6 over NOR 7.

Meiotic irregularities

Meiosis was generally normal in the barley cultivar 'Bonus', with seven ring bivalents as the most common configuration at M I, and with predominantly regular later stages. PMCs at M I with one or more rod bivalents, or two univalents, and aberrant later stages, were common in the translocation and duplication lines studied. The occurrence of rod bivalents and univalents in these lines is illustrated in Fig. 7h and Fig. 8b, g and h. Non-disjunction and lagging of bivalents, and occurrence of bridges, fragments and micro-nuclei at A I and T I are also documented in these lines (Fig. 8c-f and i). The chromosomes with translocations or duplications were largely involved in these aberrations. Similar irregularities during the second meiotic division, and occurrence of micronuclei in tetrads, were common in the translocation and duplication lines.

Discussion

Cytological aspects relating to the major sites of the 45S ribosomal RNA genes on the nucleolar chromosomes 6H and 7H of barley are the subject of this study. Other minor sites of these genes on four other chromosomes (Leitch and Heslop-Harrison, 1992; Pedersen and Linde-Laursen, 1994), not

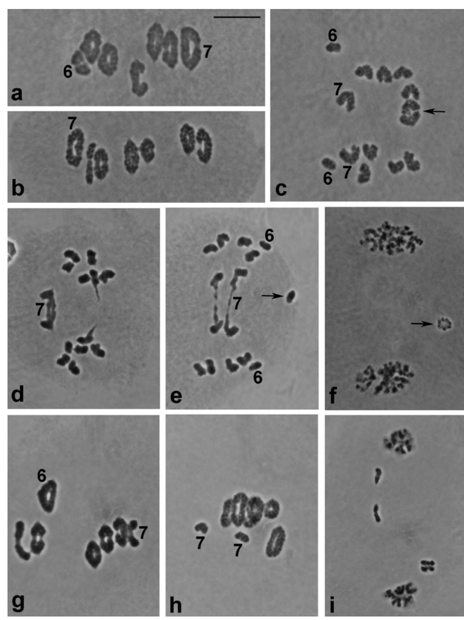

Figure 8. Meiotic metaphase I (a, b, g, h) and aberrant anaphase I (c-e) and telophase I (f, i) of translocation lines T6-7ab (a-f) and T6-7d (g-i); a, bivalents 6H and 7H as ring bivalents; b, one rod (possibly bivalent 6H) and six ring bivalents, the largest of which is bivalent 7H; c, normal separation of chromosomes of bivalents 6H and 7H and possible non-disjunction of chromosomes of one other bivalent (arrow); d, lagging of bivalent 7H; e, normal separation of chromosomes 6 and two bridges of lagging bivalents, one of which is bivalent 7H with a lagging fragment (arrow); f, a lagging micronucleus (arrow); g, five ring bivalents the largest of which is bivalent 6H, and two rod bivalents the smallest of which is bivalent 7H; h, six ring bivalents and two univalents likely representing chromosomes 7H; i, a lagging chromosome and lagging chromatids. Scale bar, 10 µm.

readily detectable in the present work, are not of concern in the current context. Focusing on 6H and 7H, emphasis has been placed upon disclosing the interrelationships between the size of the secondary constriction, patterns of 25S rDNA signals after FISH denoting the relative amounts of 45S rDNA repeats, Ag-staining patterns portraying expression of this kind of rDNA, and nucleolar features relating to these parameters during mitosis and meiosis. For a better understanding of these interrelationships in the studied translocation and duplication lines, it is important to regard these aspects in the barley cultivar 'Bonus', which is the main background material of the reconstructed karyotypes studied. As currently observed in the cultivar 'Bonus', it is well established that the secondary constriction of 6H at mitotic metaphase is generally more pronounced than that of 7H (e.g. Tsuchiya, 1960; Linde-Laursen, 1984). Consistent with this are the observations of a larger Ag-band at this site on 6H than at the corresponding site on 7H, and of a larger nucleolus synthesized by NOR 6 reflecting its higher expression activity in comparison with NOR 7, as earlier reported (Linde-Laursen, 1984; Gecheff *et al.*, 1994; Kitanova and Georgiev, 2005). This is further shown during meiotic pachytene and diakinesis stages by the more frequent occurrence of one nucleolus in association with only bivalent 6H than one or two nucleoli associated with bivalents 6H and 7H. Contrary to this is the observed minor signal on 6H compared to the major signal on 7H after FISH with 25S probes, indicating lower amounts of 45S rDNA in 6H. This is in agreement with the quantitative estimation of 1580 and 2690 repeats of the 45S ribosomal genes determined for 6H and 7H, respectively (Subrahmanyan and Azad, 1978a). Thus, less rDNA in 6H than in 7H, and more active NOR 6 than NOR 7 in the standard barley karyotype are fundamental features to be taken into consideration when interpreting findings in the translocation and duplication lines.

Of the currently studied barley translocation and duplication lines, only T6-7d was examined before regarding the maximum number and size of nucleoli in mitotic nuclei, determined as two large nucleoli and two micro-nucleoli and interpreted as synthesized by NOR 6 and NOR 7 on 7H, respectively (Anastassova-Kristeva *et al.*, 1980). In the current work on this line, the activity of the partial NOR 6 on 6H has also been recorded, and accordingly a maximum of six nucleoli in three size classes were found in mitotic nuclei. Six nucleoli, in three size classes, were also recorded in T6-7ab, in accordance with what is expected from the observations made after Ag-staining of mitotic chromosomes and after analysis of diakinesis cells. The maximum number of nucleoli in D2 would be expected to be similar to what has been recorded in a similar line D3 (Fig. 1c in Anastassova-Kristeva *et al.*, 1980), namely four large nucleoli and four micronucleoli. In all the materials studied, correlations were apparent between the magnitude/clarity of secondary constrictions in mitotic and diakinesis chromosomes, density of Ag-staining of mitotic and meiotic chromosomes, maximum number and size of mitotic nucleoli when determined, and nucleolar association with bivalents at pachytene and diakinesis. The FISH results gave hints as to the relative amounts of 45S rDNA at the different sites, which added to the validity of other identification criteria employed for determining the identity of these sites.

The activity/expression of 45S rDNA in mitotic and meiotic cells of the translocation and duplication lines was largely manifested by the intact or partial NOR 6 and to a lesser extent by intact or partial NOR 7 when present on another chromosome, and to a minimal extent by intact or partial NOR 7 when present on the same chromosome as a partial or intact NOR 6. When on the same chromosome, partial or intact NOR 6 and NOR 7 occurred either on the same arm or on different arms. In all cases this resulted in nucleolar dominance of intact or partial NOR 6 over intact or partial NOR 7. The dominance of NOR 6 over NOR 7, when present on the same chromosome, has been shown in T6-7d (Anastassova-Kristeva *et al.*, 1980) and in other similar translocation lines (Nicoloff *et al.*, 1977a and b, 1979; Schubert and Künzel, 1990; Kitanova and Georgiev, 2005). The dominance observed in the lines studied by Schubert and Künzel (1990) was manifested at meiotic diakinesis by the association of a large nucleolus with NOR 6 and a micro-nucleolus with NOR 7, at distal ends of the same bivalent. Apparently, the occurrence of two different types of NORs on the same chromosome leads to nucleolar dominance, in the present case the 9.9 kb repeats of NOR 6 and the 9.0 kb repeats of NOR 7 (Subrahmanyam *et al.*, 1994). This is substantiated by lack of suppression when two NOR 6 are present on an isochromosome (Schubert and Künzel, 1990) and when NOR 7 is split due to an inversion (Georgiev *et al.*, 2001). Relating to the findings in the barley cultivar 'Bonus', it is relevant to indicate that nucleolar dominance of NOR 6 over NOR 7, when on the same reconstructed chromosome, may be considered as an accentuation of the situation already prevailing when these NORs naturally occur on separate chromosomes in 'Bonus'.

The fact that nucleolar dominance can pertain to certain tissues but not to others has been encountered in *Brassica napus*, in which meristematic root tip cells, vegetative tissues and floral organs have been studied using different approaches (Chen and Pikaard, 1997a and b; Hasterok and Maluszynska, 2000). Mechanisms behind inter- and intra-chromosomal nucleolar dominance have been mooted with emphasis on their epigenetic nature that affects transcription of ribosomal genes (see reviews by Reeder, 1985; Pikaard, 2000; Santoro, 2005). Both cytosine methylation and histone acetylation are of relevance in this context, since their inhibition by 5-aza-2´ deoxycytidine and sodium butyrate or trichostatin A led to de-repression of NOR activity (Chen and Pikaard, 1997b; Grummt and Pikaard, 2003). The possible role of rDNA methylation in nucleolar dominance in reconstructed barley chromosomes was first considered of limited importance (Schubert and Künzel, 1990; Papazova *et al.*, 2001), but was later emphasized (Ruffini Castiglione *et al.*, 2008). The possible epigenetic pathway behind nucleolar dominance, implying rDNA methylation and histone modification, is apparently the same as the common pathway behind rRNA gene dosage control (Lawrence *et al.*, 2004). The significance of establishing and maintaining heterochromatic domains of ribosomal genes as to their differential expression has been stressed (Neves *et al.*, 2005). No differences were found between normal and reconstructed barley chromosome complements manifesting nucleolar dominance as to DNase I sensitivity of ribosomal intergenic spacer regions, thus excluding possible effects on the transcription initiation and termination factors (Dimitrova *et al.*, 2009). Whether the epigenetic chromatin modulation factors act directly on the ribosomal genes, or on other regulatory genes, needs to be elucidated. Control of nucleolar dominance by unlinked genes has been shown (Neves *et al.*, 1997). Possibly, genetic or epigenetic factors can be behind the natural variation of nucleolar dominance in a specific interspecies hybrid combination (Pontes *et al.*, 2003). Of relevance are also the other components of the epigenetic systems (Grant-Downton and Dickinson, 2005), like the role of short interfering RNAs in silencing of ribosomal genes (Preuss *et al.*, 2008).

The observed meiotic irregularities in the translocation and duplication lines studied are characteristic features of lines with restructured chromosomes, characterized by their reduced fertility. In spite of the general reduced plant vigour in duplication lines, some of these lines exhibited enhanced vigour and positive agronomic characters implying their possible use in barley breeding (Hagberg, A., 1886; Hagberg, A. and Hagberg, P., 1991; Hagberg, A., 1994).

In conclusion, correlations were apparent between size of secondary constrictions and Ag-bands in mitotic chromosomes, and maximum number and size of nucleoli in mitotic nuclei, and nucleolar associations with bivalents during meiosis, in standard and reconstructed chromosome complements of barley. FISH with 25S rDNA probes provided complementary data on the relative amounts of 45S rDNA at the NOR sites. Nucleolar dominance of NOR 6 over NOR 7 was consistent in the translocation and duplication lines studied. This feature was an accentuation of existing interrelationships between standard NOR 6 and NOR 7 in barley.

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In commemoration of an esteemed mentor and scientist, the late Professor Arne Hagberg, who gave us inspiration to undertake this work on his favourite material, barley translocation and duplication lines. The authors are also grateful to Dr Glyn Jenkins (Aberystwyth University, UK) for his helpful comments on the manuscript.

Sammanfattning

Denna cytologiska studie fokuserar på två kromosomtyper hos korn (*Hordeum vulgare* L., 2n=14). Det är nukleolkromosomparen 6H och 7H, vilka kännetecknas av sina sekundära insnörningar i den korta armen. Insnörningarnas lägen sammanfaller med lägena för ribosomalt DNA (rDNA), vilket består av repeterade kopior av 18S-5.8S-26S ribosomala gener. Dessa ställen kallas också för nukleol-organisatör-regioner (NOR 6 och NOR

7), eftersom början av nukleolbildningen sker där, när ribosomala gener kommer till uttryck. NOR 6 och NOR 7 kan hamna i samma kromosom som ett resultat av kromosomstrukturella förändringar, vilka leder till uppkomsten av reciproka translokationer eller segmentduplikationer. I dessa fall råder ”nukleoldominans”, vilket innebär att ribosomala gener i NOR 6 uttrycks medan motsvarande gener i NOR 7 undertrycks. För att utröna dessa förhållanden studerades kornsorten ’Bonus’ som kontrollmaterial samt translokationslinjerna T6-7ab and T6-7d och duplikationslinjerna D2 och D24 från professor Arne Hagbergs kollektion av kornmutanter. Undersökta parametrar var de sekundära insnörningarnas storlek, NOR-aktiviteten belyst genom färgning med silvernitrat, samt nukleolförhållandena hos mitotiska kärnor och vid meiosens pachyten- och diakinesstadier. Intakta eller delar av NOR 6 och NOR 7 fanns i samma kromosom hos alla fyra studerade translokations- och duplikationslinjer. Samband konstaterades mellan den sekundära insnörningens storlek, NOR aktivitet, maximalt antal nukleoler hos mitotiska kärnor, och nukleoler associerade med rDNA-lägen hos bivalenterna 6H och 7H under meiosens pachyten och diakines. Kompletterande uppgifter erhölls genom kartläggning av relativa mängder rDNA hos 6H och 7H, genom att synliggöra dessa regioner som band efter fluorescerande *in situ*-hybridisering (FISH) med rDNA-prober. Intakta eller delar av NOR 6 visade alltid nukleoldominans över NOR 7 hos translokations- och duplikationslinjerna. Det är viktigt att jämföra med förhållandena hos standardsorten ’Bonus’, där det finns en relativt högre mängd rDNA vid NOR-regionen i 7H än i 6H, och högre aktivitet (genuttryck) hos NOR 6, vilket även visar sig i nukleolstorleken hos mitotiska kärnor och i nukleolassociationer med rDNA lägen hos bivalenterna 6H och 7H under meios. Nukleoldominansen hos translokations- och duplikationslinjerna kan betraktas som en accentuering av de mindre betydande skillnaderna mellan NOR 6 och NOR 7 hos ’Bonus’. Uttryck och undertryck av NOR uppfattas nu som ett epigenetiskt fenomen. Således kan DNA-metylering, histonmodifikationer, kromatinmodulering och ”short interfering RNA” (siRNA) vara av betydelse i detta sammanhang. Vidare har meiotiska avvikelser hos translokations- och duplikationslinjerna dokumenterats.

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Robert Hasterok, Justyna Majlinger and Lukasz Kubica
Department of Plant Anatomy and Cytology
Facuty of Biology and Environmental Protection
University of Silesia
Jagiellonska 28
40-032 Katowice, Poland

Kerstin Brismar
Department of Plant Protection Biology
Swedish University of Agricultural Sciences
PO Box 102, 23053 Alnarp, Sweden

Waheeb K. Heneen
Department of Plant Breeding and Biotechnology
Swedish University of Agricultural Sciences
PO Box 101, 23053 Alnarp, Sweden

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