Quantum leaps in biofuel crop efficiency – can sugarcane pave the way?

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Abstract

Triple challenge is confronting world plant production in a few forthcoming decades: population increase, worsening of growth conditions, and changeover from fossil-based to renewable energy and raw materials. The challenge cannot be met without utilizing the best modern biological techniques, genetic modification included. In the current era of rapid environmental changes, plant breeding should take even greater responsibility for food, feed, fiber and fuels than in the past. There are good prospects for remarkable improvements both in yield level and energy efficiency in plant production, as is exemplified with the cases in modern sugarcane breeding reviewed below. Such applications could be taken in use without delay, because any ecological risks connected with new plant varieties are generally much smaller than the ones caused by commonplace types of ecosystem manipulation such as the choice of crop plant species or the introduction of alien biocontrol organisms in the field.

Running title: Quantum leaps in biofuel crop efficiency

Introduction

The merits of the huge increases in agricultural production efficiency during the 10 000 latest years are attributed about fifty-fifty between the developments in crop husbandry, crop protection etc. *versus* plant breeding. Now that we live in rapidly changing and possibly hard times, the responsibility of plant breeding may surge. But then also the potentials of breeding are greater than ever before, thanks to the revolution in genetic knowledge and know-how in this millennium.

Current bioenergy crops are often criticized in the media for competing untenably with food, feed and fiber production in the fields. Such a new source of competition may tend to enhance future price speculations, and it may thus fuel the spiking of food prices in international markets. Indeed, due to the very low efficiency characteristic of the maize-based production of bioethanol in USA, a large proportion of maize production area has to be redirected from food and feed purposes to fuel uses even if the very first quantitative goals set down by legislators for biofuel production during forthcoming decades in USA are to be fulfilled.

International plant science organizations point out that great improvements are required in current bioenergy crops for achieving sustainable systems of biofuel production.[1] On the other hand, great prospects for such improvements exist, because relatively little breeding for such special traits has been done previously. Accordingly, genetic variation in certain “energy” traits may still be found in the breeding populations of the crop species. Further genetic diversity is available in the Nature. The 10,000 wild grass species in the world harbor riches of highly efficient solutions available for improving the productivity and ecological tolerance to environmental stresses of crop plants as soon as the genetic basis of the desirable traits is being unraveled by modern genome research.

The efficiency of the bioenergy crops, measured in savings in fossil inputs such as fertilizers and tractor fuels as well as in biofuel yields produced per hectare, depend much on the methods used in their production. Therefore, essential improvements in ecological and carbon efficiency can be reached, if bioenergy crops can be bred to manage with lower fossil inputs without compromising their high yield levels. When more efficient plant varieties become available, sustainable production of bioenergy and renewable products can be obtained without jeopardizing food security and wildlife.

Sugarcane is very efficient in assimilating solar energy into carbohydrates, and according to various evaluations tropical sugarcane production is sustainable both in terms of carbon efficiency and in ethanol yield per hectare. International Energy Agency states that ethanol from sugar cane produced in the tropical/sub-tropical regions such as Brazil, southern Africa and India, for example, has excellent characteristics in terms of economics, CO_2 reductions and low land use requirements.[2] Other studies also confirm that tropical sugarcane ethanol yields the highest savings achieved hitherto (85–98 %) in fossil energy use and greenhouse gas emissions.[3]

Regarding biodiesel production, oil palm (*Elaeis guineensis*) is far superior to any oil crops produced in Europe. It produces nine times more oil per ha than soybean and six times more than oilseed rape,[4] which means much less wastage of natural resources in agriculture.

Oil palm requires tropical climate. However, contrary to certain “activist” campaigns, palm oil need not be produced in rainforests but certified oil palm plantations can be founded on set-aside and waste lands. Furthermore, though all kinds of animal or plant fats can be used for biodiesel in the manufacturing process by the Finnish company Neste Oil, such food waste materials are only available in minor quantities that could provide for no more than a few percentages of the biodiesel volumes to be required. Accordingly, the decision by the company of using palm oil in its biodiesel process is environmentally justified.

In this paper, however, sugarcane and ethanol were selected for the case of a detailed analysis, because a) there are many scientifically interesting developments going on in sugarcane, and b) our most important crop plants are cereals, i.e. grass species, and not palm plants.

The structure, water use, fertilizer intake, sucrose content, and the very nature of sugar production in sugarcane are likely to undergo major changes with the modern tools of genetic modification. Scientists predict that the ethanol yield of sugarcane per ha can be doubled in practical cultivations within the next 15 years.[5] Additionally, prospects for remarkable enhancements in resource use efficiency also exist in sugarcane, at least regarding water and nitrogen.

Locally well-adapted and highly productive biomass grasses are under development in temperate and cool climates, e.g. switchgrass (*Panicum virgatum*) in North America[6] and reed canarygrass (*Phalaris arundinacea*) in Finland.[7] What lessons could possibly be learned for their breeding from the experiences in sugarcane?

Current sugarcane production

Sugarcane (*Saccharum officinarum*) is cultivated in 22 million ha, and its average cane yield is 70,9 tn/ha. World production is 1,560 million tons of cane, which yields 68 million tons of sugar annually. World sugarcane production has increased by a quarter from the turn of the century onwards. The greatest cane producers are Brazil and India, with 33 % and 22.3 % share of world sugarcane production, respectively. Other great producers are China, Thailand, Pakistan, Mexico, Colombia and Australia, which in combination share 22.6 % of world sugarcane production.[8]

The bulk of sugarcane is produced in a zone surrounding equator: between 35 °S and 35 °N. Depending on varieties and growth conditions, yield is harvested in 9–24 month intervals by cutting the cane stalks. Sugarcane is a perennial crop, and it is economically viable to take 3–8 crops from the same cane roots in recurrent years. Commercial sugarcane is propagated vegetatively, and new cultivations are established by burying segments of stalks in furrows in the field. Furrow interval is 1.1–1.4 m, and one hectare of sugarcane cultivation contains 21,000–35,000 cane stalks.

Sugarcane is an efficient assimilator and may produce more than 200 tons of biomass per ha. An average cane yield is 50–150 tn/ha – in wet tropics good rainfed cane yield is 70–100 tn/ha, whereas in dry tropics and subtropics cane yield using irrigation is usually 110–150 tn/ha.

Sugarcane processing products and byproducts

Sugarcane stalks are pressed to produce syrups (molasses), which are then processed further in a few purification steps to yield purified cane sugar. Remaining molasses fractions still contain some sugars and can be utilized for alcoholic fermentation. Brazil produces the bulk of its ethanol from sugarcane molasses. Additional uses for the molasses fractions are feed additives and fertilizers in sugarcane cultivation.

Bagasse is the highly fibrous residue remaining after cane is pressed to remove sucrose. Bagasse is high in ligno-cellulose, and it is being burnt for energy in sugar mills or used for paper production. Regarding feed uses the disadvantage of bagasse is its low digestibility (25 %) because of the presence of lignin which protects carbohydrates from being digested by the rumen microbes. Consequently, chemical, biological or thermo-mechanical treatments are required to improve the digestibility to approximately 65 %.[9]

Following harvest quite a lot of harvest residues (e.g. leaves) are left in cane fields. Their quantity roughly resembles that of bagasse remaining after cane pressing. According to certain estimations up to 80 % of the harvest residues could be utilized for raw materials without compromising sustainable sugarcane production.[10]

Bagasse and harvest residues would be suitable raw materials for the future production of cellulosic ethanol. In sugarcane-cultivating countries the quantity of biomasses available from sugar production may vastly exceed that any other potential biomass sources combined together, municipal wastes included. For example in Australia, four times more biomass is available from sugar industry wastes than all other sources in combination.[10]

Alternatively, part of the wastes could be burnt in special furnaces into coal to be used for agriculture. Namely, such coal degrades extremely slowly in the soil, and it could therefore be applied for improving soil structure and organic matter content in cultivation.[11]

Growth requirements

Water

Water is often the limiting factor in sugarcane production.[12] During their growth stage sugarcane varieties need much water (in total 1500–2500 mm, evenly distributed in the period) as well as warmth (Table 1). Cane yield is directly proportional to the amount of water used by sugarcane in each climatic conditions. About 37–330 kg of water is used for producing one kg of cane and 1000–2000 kg of water for producing one kg of sucrose, respectively.[13–16]

Sugarcane is being cultivated both rainfed and applying irrigation. Irrigation has been traditionally based on furrows, but recent trends favor sprinklers and drip irrigation (especially in Hawaii). Much water and work is saved using drip irrigation. Therefore, its use is economically sustainable, even if the drip hoses damaged by the burning treatments of the plantations must be replaced after harvest.[17]

Temperature

On the contrary when harvesting period is approaching sugarcane needs dry, sunny and cool conditions in order to ripen to harvest state and boost its sugar content to 10–12 per cent. Rooting and sprouting of the planted stem pieces occurs at its best in 32–38 °C, and stalk growth reaches its optimum in 22–30 °C, but ripening of stems and their sugar enhancement proceeds most successfully in 10–20 °C.[9]

Soil

Sugarcane has no requirements for a special soil type. Optimum soil pH for sugarcane is 6.5 but the plant can be grown in soils with pH 5–8.5. Sugarcane grows best in more than one meter deep layer of soil, and parts of its root system may extend into the depth of five meters. However, the bulk of its roots (85 %) typically harbor the uppermost 60 cm layer of soil, especially if the plant is irrigated often and with small doses of water at a time.[17]

Deeper root systems could be generated by irrigating the plants less frequently and with greater doses at a time. Deeper-rooting varieties could presumably be developed with plant breeding and at least with genetic modification. Deeper root systems would diminish the susceptibility of the canes to damages caused by occasional drought periods in certain areas.[13]

Nutrient requirements

In order to be productive sugarcane needs quite a lot of nitrogen (100–200 kg/ha, referring to yield level 100 tn/ha) as well as potassium (125–160 kg/ha), but rather little amount of phosphorus (20–90 kg/ha) is sufficient. Though, in the ripening period nitrogen content in the soil should be as low as possible in order to reach high sucrose content in the stems (especially in hot and wet conditions).

For reducing the amount of harvest residues sugarcane stalks or plantations are often being burned before harvest or after having cut the stalks down in the field. However, at least the Australian sugar industry is trying to get rid of such a traditional procedure, because burning pollutes air with particles harmful to human health.[18]

Leaving harvest residues on the plantation as green mulch and for decomposition might beat burning also as regards soil nutrients. However, not much is known about the effects of such cultivation method on the nitrogen or carbon balance of the soil. It may apparently not have much effect on improving nitrogen availability of the next cane vegetation or rising permanent carbon content in the soil.

In studies in wet tropical Australia less than 6 % of the nitrogen in the harvest residue was utilized, i.e. found its way to the next harvested cane yield. The bulk of the carbon in the harvest residue was burnt to CO_2 due to microbial activities and lost in the air. Though, in wet tropical areas only about

6 % of fertilizer nitrogen is utilized by the cane plant as well, whereas in temperate regions 20–40 % of fertilizer nitrogen is being utilized by sugarcane for yield production.[19–21]

Classical cane breeding

Sugarcane originated in Asia. Sugarcane varieties in cultivation are species hybrids between the primitive cultivated sugarcane *Saccharum officinarum* (2n=80) and a wild cane species *S. spontaneum* (2n=40–128). Sugarcane varieties are highly polyploid plants i.e. they contain each of the cane basic chromosomes in 5 to 14 copies in their cells. Many varieties are even aneuploid, which means that different basic chromosomes may occur in different numbers. Therefore sugarcane varieties are often quite sterile.

Actually even *S. officinarum* itself is of complex species-hybrid origin and may have received whole chromosomes intact from as far as other plant genera (*Erianthus* and *Mischantus*).

High sugar content came from *S. officinarum*. Unfortunately, the species also harbors many poor traits unsuitable for cultivation: it is very susceptible for diseases, devoid of ecological adaptability and lacks sprouting ability necessary for the perennial cropping system. Thus, *S. officinarum* cannot usually manage without human help, and its few ephemeral occurrences outside cane plantations cannot spread further in Nature.

Vigour, disease resistance, tolerance to poor cultivation conditions, and great biomass production have been introduced into sugarcane varieties from the wild cane, *S. spontaneum*. As a trade-off, the sugar content of the wild species is negligible. Much genetic variation occurs in its populations and the species is a troublesome weed in certain areas of the world. Though, its weedy characteristics have not been carried along to cultivated sugarcane varieties.[9]

In order to retain the sugar content high enough in sugarcane cultivation, primary species hybrids have been crossed back to *S. officinarum* for several generations. Consequently 80–90 % of the genes in currently cultivated sugarcane varieties originate from that high-sugar but primitive ancient cane species.

Sugarcane breeding takes decades

For genetic reasons considered above, the bulk of sugarcane varieties are more or less sterile. Furthermore, sterility is favored, because flower formation decreases sugar content in the stalks. When viable seed is rare, breeding via crosses becomes more difficult. In addition, seeds are tiny and their growth to adult canes may take years which retards the progress in selection.

High level of polyploidy remarkably complicates traditional plant breeding. Because each allele may occur in 5–14 copies in the genome, replacing poor alleles with desirable ones may often prove much more unreliable and take a lot more of time than in a diploid plant species such as rice. Namely, simple Mendelian heritability rules may not apply but these are usually replaced with much more complicated statistics of segregation typical of polyploid plants.

If a recessive allele is being introduced in sugarcane using crossing, the trait it encodes does not express itself in plant phenotype until every single original allele has been replaced with the introduced one in plant's genome. The probability of finding such a fortunate genetic recombination among cross progeny may be practically nil.

For example, it is impossible to breed aromatic wheat using conventional methods.[22] Wheat is a hexaploid species so that the harmful cereal gene for scentless grains occurs in altogether six copies. It is statistically impossible to switch all these copies off simultaneously (or even sequentially) with traditional, non-targeted means such as mutagenic treatments using radiation and chemicals. Whereas all the six copies can easily be silenced concurrently e.g. using RNA interference, a genetic modification method winning Nobel prize in Medicine in 2006.[23]

Accordingly, very high numbers of progeny are often screened through, in the hope for finding a lucky hit in the stochastic lottery of traditional plant breeding. In clonally regenerated crops such as sugarcane, apple, pear, grape, potato, strawberry etc. it is enough to find one superior genotype which is thereafter being vegetatively multiplied into millions of genetically identical shoots for cultivation as a new variety.

In traditional sugarcane breeding programs, progress is slower than with most staple crops, as rationalized above. Typically, a cross is made and its progeny are scrutinized for valuable genetic recombinants combining the best traits of both parents. Selection work usually starts with 100,000 progeny seedlings and proceeds in 4–6 stages (Table 2). Finally a single one new sugarcane variety may be released for cultivation, typically in 12–15 years' time after the cross was made.[24–25]

In the first two stages seedlings are picked for further selection stages according to their visual scoring in vigor and disease resistance. During the later selection stages individual seedlings are being multiplied into clones to be used for measuring their cane yield in consequent harvests during 2–3 years (primary cane crop and 1–2 re-growth or 'ratoon' crops in the subsequent years). Since then productivity is also taken into account in selecting the rather limited number of progeny genotypes to be kept for the final field test stages. The final production tests are performed in several regions of cultivation, because results only based on one district cannot usually be generalized to the whole area of sugarcane cultivation.[26]

The multi-phased and arduous process of selection is the most important and expensive stage in traditional breeding programs.[27] Whereas 1,000 times fewer plant individuals are started with when an established sugarcane variety is being improved with one desirable new trait applying genetic modification. Consequently, the modern plant breeder may proceed directly to the penultimate or last stage of field tests, saving much costs and time.

Classic breeding is a Sisyfos task

When a clonal plant variety with a highly heterozygous genetic constitution is being crossed further, its fortunate gene combination inevitably disintegrates due to sexual reproduction. Once lost, the unique genetic combination cannot be reassembled in the progeny generations in practice.

Thus, traditional sugarcane breeding is a Sisyfos task: previous achievements are lost to a major degree each time new improvements are being pursued.

No wonder that e.g. sucrose content in sugarcane has not increased in several decades, even though studies show that genetic variation for the trait occurs in its breeding populations.[28] On the contrary, sucrose content even slightly decreased during 1970–1990 in Australia, though 50 new sugarcane varieties were released for cultivation in the period.[9] Main focus was on biomass production and disease resistance.

When major progress is tried for, new genes or alleles must be retrieved from other cane species. E.g. genes for higher biomass production exist in S. *robustum* or S. *spontaneum*. However, for winning back the bulk of the desirable traits achieved hitherto in cultivated sugarcane, each species cross should be complemented with consequent backcrosses (usually with *S. officinarum*). Accordingly, the time required for breeding would be multiplied in proportion.

Even if such completing crosses and progeny selection would be made during 10–20 generations, which is possible in grain crops with shorter generation intervals, hundreds of undesired arrival genes might still remain in the progeny plants. E.g. five hundred alien genes still remained in maize progeny after 14 generations of backcrosses and selection following the original cross of maize with *Tripsacum* (gamagrass).[29]

In traditional plant breeding such compromises are a commonplace, however, and a new (though impure) variety is being released so long as it looks better than old ones.

Better focusing available with genetic modification

A major advantage of genetic modification is its high degree of focusing. Not thousands of unknown genes but one desired gene without any hitchhiking ones is introduced from a wild plant species. The transferred gene is added to the genome of the recipient plant variety in its vegetative phase of life cycle, and consequently its superior genotype is retained and not disrupted by meiosis.

That is why the Sisyfos task can be avoided and the achievements of prior breeders be conserved and developed further. Furthermore, there is no need for subsequent crosses for purging the variety of unwanted alien alleles.

Consequently, using genetic modification 1,000 times fewer plant individuals have to be scrutinized than in traditional breeding. Therefore, much time and costs can be saved.

Though, making one improved plant individual is usually not enough in genetic modification, either, but some degree of selection is carried out. In practice the desired gene is often transferred to 50–200 individual plant lines. After comparisons in the laboratory, the few best-functioning plant lines are then being selected for final field trials.

Namely, the site of fixation of the gene in plant's genome may also have influence on how well the gene functions in the plant cell. In most techniques of genetic modification the site of fixation cannot yet be determined in advance (but in any case it is always specified afterwards). On the other hand there are thousands of locations in the chromosomes where the transferred gene is able of functioning well. It is therefore sensible to screen through a modest number of individual transformation events in order to optimize the modification results.[30]

Doubling of sugar content in one step of genetic modification

There are several obstacles in raising the sucrose content in sugarcane. One basic reason is that a great number of genes are involved in sugar content, each with a fairly modest effect.

Alleles for high sucrose content originate from *S. officinarum*. In polyploid hybrids it is a demanding task to enrich such "sugary" alleles in one genotype, because there may exist up to 14 copies of the gene in the cell. Furthermore, if the bulk of efforts are concentrated on improving one trait, other traits may often deteriorate as a trade-off.

Other obstacles to rising sugar content in the plant with traditional breeding methods are its homeostasis and sugar sink systems. Sugar is stored in stalk cells in the amounts that may prove beneficial for the plant's further development. If that level is exceeded, the homeostasis systems of the plant may start using the sugar more for other than storage purposes. Therefore, major improvements in sucrose content may call for finding such homeostasis genes and optimizing their functioning according to human purposes.

So it may prove easier to breed sugarcane cells for producing in addition to sucrose some kind of sugar that the plant is not able of utilizing itself. Such production would likely not be governed by the built-in regulation mechanisms of the plant.

Accordingly, the sugar content of sugarcane was doubled in one step of genetic modification by introducing a gene for sucrose isomerase enzyme in the plant.[31–32] The modified cane produces normal amounts of sucrose in its cells but on top of that also similar amounts of isomaltulose, which is an isomeric form of sucrose. Because sugarcane is not able of utilizing that type of sugar itself, isomaltulose is readily accumulated in its storage tissues. It was channeled by the breeder to find its way to the vacuoles, i.e. the "waste sacks" of plant cells.

Isomaltulose is a slowly-degrading sugar produced in growing amounts for functional food using microbial cultivations. The present production via microbial fermentation is quite costly, however. Isomaltulose can also be used as an acariogenic sweetener, because mouth bacteria cannot usually break it down. Regarding biofuels, isomaltulose can be exploited for a raw material in alcoholic fermentation just as sucrose.

Field trials proceed in Australia

Altogether 120 different lines of isomaltulose sugarcane are being tested in field trials in Australia in 2005–2010.[33–34] Experimental area is 3.25 ha in total, and its products are not used for food or feed for the time being.

Diverse regulatory elements (promoters) obtained from sugarcane or maize are being tested for controlling the functioning of the sucrose isomerase enzyme in sugarcane. In the plant lines, the enzyme is being produced in different amounts and it has been channeled to different parts of the plant. Different combinations of regulatory elements are being compared with each other in their ability of accumulating isomaltulose in sugarcane without harming plant growth in customary growing conditions.

After field test stage clearance for commercial cultivation as sugarcane varieties may be applied for the most promising experimental lines. Varieties may be available for cultivation at the earliest in five years' time.[35] From the biological point of view the novel sugarcanes could be taken in use fairly rapidly after the field tests. Nonetheless, forecasts for the start of isomaltulose cane cultivation vary from three to seven years depending on how obstructive the permission bureaucracy may prove to be in practice.

Cellulosic ethanol from self-degrading cane varieties

Sugarcane produces biomass up to 200 tn/ha, but on average less than 100 tn of cane is being harvested per ha annually. The bulk of the biomass is water, but about 10 % of it is cellulose which remains as bagasse after the pressing process. Similar amounts of cellulose also remain on the fields in harvesting residues, 80 % of which could be utilized as raw materials without compromising the sustainability of sugarcane cultivation.

Cellulose is a polysaccharide which could in principle become degraded into sugars to be fermented into alcohols. If the cellulose in sugarcane bagasse could also be utilized for ethanol, current ethanol yield per ha of sugarcane would be approximately doubled.

At present degrading cellulose into sugars is by far too expensive to be economically viable. Plant cell walls need expensive pretreatments in hard process conditions in order to loosen the structure of the walls so that the degrading cellulase enzyme could have sufficient access to cellulose molecules in the walls later on. Fairly large amounts of the enzyme are needed and its purchasing from the markets would be very costly.[36]

Therefore, sugarcane is now being modified genetically to produce the necessary cellulase enzyme itself, free of charge, in its cells. When produced from inside the cells the enzyme is also more efficient, having better access to the cell walls, and there is less need for expensive pretreatments as well.

Based on an inducible promoter cellulase production in sugarcane cells is being started with a special treatment not earlier than 2–3 days before harvest. That is why plant growth is not affected.[10]

Also the lignin in sugarcane cell walls is being modified genetically in Brazil (Allelyx SA) to a better-degrading type consisting almost exclusively of syringyl instead of the more recalcitrant guaiacyl lignin. Thereafter cell-wall lignin can be loosened more easily, providing cellulases with better access to the cellulose fibers.

Self-degrading sugarcane for cellulosic ethanol production is being developed in a broad-based Australian–Brazilian research coalition. GM varieties already occur in field tests, and varieties may be released for cultivation in 2–5 to seven years' time depending on how slow the license bureaucracy is evaluated to be.[36]

Halving N-fertilization with NUE cane?

Sugarcanes need quite a lot of nitrogen fertilizers, which impairs their production economy and carbon efficiency and pollutes environment. Grain crops can usually utilize less than half of the nitrogen administered to them in fertilizers (the reminder finds its way to air, groundwater and waterways). In temperate regions, sugarcane may utilize 20–40 % of fertilizer nitrogen but in wet tropics only 6 %.[20]

Role of biologic nitrogen fixation

It is often told that sugarcane especially in Brazil may obtain a notable part of its nitrogen demand from nitrogen-fixing bacteria living in its root system. However, there is not much convincing evidence available, and most studies even lack systems of measurement reliable enough for the problem.[37]

Though, in reliable new studies small but positive (5–16 %) shares of biological nitrogen fixation have been recorded in sugarcane in Australia. However, securing favorable conditions in cane root system seems to be difficult in practice, and more research knowledge would be needed.[38]

Deficiencies may occur e.g. in the availability of efficient nitrogen-fixing bacteria for the plant species. Sugarcane roots cannot be inoculated with optimum nitrogen-fixing bacterial strains in advance, because plantations are founded from rootless pieces of sugarcane stalk.

In the long run breeders aim at developing grain crops capable of fixing their required nitrogen in their roots. That could be achieved most reliably in symbiosis with *Rhizobium* bacteria in plant root nodules. Several plant genes necessary for root nodule formation have been cloned, and early root nodule development can already be induced in legumes without the presence of rhizobia.[39] Though, many years may still be required for developing efficient nitrogen fixation in major grain crops.

Reducing nitrogen fertilization with NUE crops

Crop plants with much higher Nitrogen Use Efficiency (NUE) are being developed by genetic modification e.g. in maize, oilseed rape, wheat, rice, barley and sugarcane. In these applications, one single gene has usually been utilized, providing for more efficient intake of nitrogen from the soil in plant roots.

According to the field tests in maize, oilseed rape and African rice these first generation NUE crops are able of producing customary yield levels using 2–3 times less nitrogen inputs in cultivation. Consequently, less nitrogen is wasted in the water systems or in the air.[40–41]

That particular NUE gene is being bred also in sugarcane at least in India.[41] NUE sugarcane is under development also in Brazil, where a project has been started by Monsanto Inc. in collaboration with local breeding companies for improving the resource use efficiency of sugarcane.[42]

Field trials are going on in Australia in 2007–2010 with GM sugarcanes expressing enhanced nitrogen use in nitrogen-poor conditions. The gene being utilized originated from maize.[43–44]

The reductions in the necessary amounts of nitrogen fertilizer inputs improve both economical and carbon efficiency of field crops. Furthermore, with lower nitrogen levels in the soil, nitrogen-fixing bacteria thrive better. Thus NUE sugarcanes would create more favorable conditions for taking advantage of microbial nitrogen fixation in cane production as well.

In the above crop plants, NUE varieties are estimated to be released for cultivation in 8–10 years' time. Though, the yield evaluations in a perennial crop such as sugarcane may require a couple of more years than in annual crop species.

Improving drought tolerance

Provided climates warm up, water deficiencies are getting worse in large areas. Consequently, the necessity of irrigation also increases in cultivation. Though, in dry and hot regions traditional systems of irrigation result in soil salinization.[45] Such harms could be avoided by developing drought-tolerant plant varieties.

Drought-tolerant varieties would produce customary yields using less water. One important type of drought tolerance helps the plant to survive occasional periods of drought without permanent damages. Accordingly, its yield level does not collapse but the plant is rapidly recovering after the dry fortnight.

Breeding for drought tolerance has been started in many crop plants particularly using genetic modification. Field tests are going on e.g. in maize and rice as well as in wheat, cotton and oilseed rape in various countries. Yield improvements of about 10–40 percent have been obtained in dry conditions hitherto.[46] First drought-tolerant varieties are estimated to be released for cultivation in 4–5 years' time.

In a breeding program in Egypt, a single gene for drought tolerance was introduced in wheat. The gene was isolated from barley in a purified form and transferred to wheat using genetic modification.

Field trials showed that the number of irrigations necessary in wheat cultivation can be reduced from eight to one using these drought-tolerant wheat lines. Consequently, based on such drought-tolerant varieties wheat cultivation could be extended to areas of low rainfall lacking adequate systems of irrigation.[47]

Water is the primary limiting factor in sugarcane production in many regions India included.[12]

Drought tolerance is under development in sugarcane in Brazil, Australia and Mauritius using genetic modification.[35,48] The bulk of the projected new sugarcane cultivations would be founded in worn-out pasture areas. These are notably drier than traditional sugarcane cultivation regions. Respectively, improvements in drought tolerance would be welcomed.[49]

Field trials with three different drought-tolerant GM sugarcanes are going on in Australia in 2007–2010. [43–44] Water use efficiency has been improved either by producing various extra sugars in sugarcane cells or utilizing a regulator gene controlling other genes' activities in the plant. Genes have been retrieved e.g. from thale cress (*Arabidopsis*) or apple.

Trehalose is a sugar protecting cell structures from damages caused by dehydration in many organisms. A gene necessary for trehalose production was introduced in sugarcane from a mushroom species in China. The GM sugarcanes grew well and accumulated high concentrations of trehalose in their cells. Trials in laboratory and in the field showed that these trehalose sugarcanes tolerate periods of drought better, recover faster thereafter, grow better than conventional ones in dry conditions, and produce higher concentrations of sugar than customary sugarcanes.[50]

In the above application, the tolerance gene is functioning non-stop in all plant cells. Another Chinese research group has modified sugarcane with marker genes controlled by a regulator sequence (promoter) which comes into operation only in dry conditions. The promoter was found from thale cress.[51] Such inducible genes may protect the plant against periods of drought more economically in certain cases, because they do not retard plant development in favorable conditions.

Water availability for the plant could probably be enhanced by improving the structure of plant root system as well. E.g. the bulk of sugarcane roots populate the uppermost 60 cm layer of soil, whereas a few roots may grow even to the depth of 5 meters. Deep rooting could be pursued by breeding so that water reservoirs deeper in the soil would become available for the plant in dry conditions.

Regarding water use – and its wastage – stomata are key actors in plants. Knowledge of their formation and control is accumulating, and accompanied by plant physiologists in University of Helsinki a scientific breakthrough was made recently, paving the way for the breeding of better drought-tolerant crops.[52]

The bulk of the higher plants apply the C3 system of CO_2 assimilation which works well in temperate and moist environmental conditions. However, C3 plants are devoid of a CO_2 storage system, and consequently these are condemned to lose much water by keeping their stomata open in sunlight for the acquisition of CO_2 for assimilation in real time. Therefore, plants with the C4 system of assimilation are better adapted to sun-baked conditions, thanks to their ability of loading their CO_2 reserves for assimilation in advance at night when water transpiration rates are lower.[53]

Therefore, a great international research consortium is developing rice to a C4 plant within a decade. Its estimated benefits are: 50 per cent higher yield level plus doubly better efficiency in water use.[54] Alas, such developments cannot be applied in sugarcane, because it already is a C4 species by its nature.

Breeding for salt-tolerance

Provided climate conditions change as forecasted, shortage of fresh water will limit crop production severely in hot regions in the world. About one half of the readily accessible fresh-water reserves are already in use.[55] That fact has to be taken into account especially in areas where remarkable increases in crop production are being planned, whether for food, feed, fibre of biofuel.

Fresh water constitutes only one per cent of all water in the Earth, and the same holds true for brackish water. Accordingly, 98 per cent of our water reserves are marine salt-water. One quarter of the global land area is salinized, and due to salinization the area of irrigated lands is reduced by 1–2 per cent annually.[56]

In coastal regions saline water could be utilized for irrigation – provided that our crops could be adapted to salinity. Though, the bulk of our staple crops cannot tolerate salinity (Table 3). Not more than one per cent of current land plants are able of growing and reproducing on saline soils, and only a few ones can tolerate the salt concentrations occurring in seawater.

Quite the opposite was true in the far-off past. The first plant species grew in the sea, and consequently all of these were halophytes, i.e. adapted to high salt concentrations. Notably, in addition to salt, seawater contains richly of all the indispensable micro and macro nutrients that are often lacking in the fields.

Sensitive plants (such as papaya, mango and banana) are affected at about $EC_e = 2$, whereas tolerant ones (e.g. coconut, tamarind) are only affected at 8–10 or more.[58]

A chromosomal region connected with salt-tolerance during seedling stage has been localized in wild rice. The region has been transferred to several cultivated rice varieties using traditional species crosses followed by backcrosses with cultivated rice.[59] Though being fairly slight, such tolerance can help rice cultivation in the soils (such as in Pakistan) that are only temporarily salinized for short times during seedling stage, e.g. following sea flooding, but are thereafter rapidly desalinized thanks to monsoon rains.

In permanently salinized soils, additional genes for salt-tolerance would be needed. African rice varieties are being developed for tolerating irrigation with saline water.[40]

Salt-tolerance is being bred in cultivated plants by bringing in tolerance genes from naturally salt-tolerant plant species using genetic modification. Tolerance genes have been obtained from e.g. a seashore succulent (*Sueada salsa*, Fig. 1) in China and a mangrove species in India.

The salt-tolerance gene from *Suaeda* has been introduced in rice, tomato and soybean in Shandong University in China. Such salt-tolerant crops decontaminate salinized soils by taking in salt from the soil and storing it harmless in the “waste containers” (vacuoles) of its leaf cells. Though, their seeds and fruits do not accumulate salt.[60–61]

A gene for salt-tolerance has been cloned from a mangrove tree growing in brackish water in river deltas and introduced in certain cultivated rice varieties in India. According to news articles such GM rice lines are able of tolerating salt concentrations exceeding those of seawater.[62–63]

Sugarcane

The salinized and acidic soils are widespread in sugarcane growing areas of the world.[13] Irrigation waters with high salt concentrations are a commonplace in semidry areas of Brazil.[64] Those areas could be utilized fairly productively for sugarcane cultivation provided salt-tolerant varieties were available (Fig. 2).

Regarding sugarcane, breeding for salt tolerance would most probably call for genetic modification methods. A score of the currently best sugarcane varieties should be chosen for starting materials. In genetic modification these popular varieties will largely retain their assured characteristics and be only supplemented with the novel salt-tolerance trait, because their superior genotypes are not broken apart as is the rule in meiosis.

In addition to the ones mentioned above, more than a dozen of other genes influencing salt-tolerance in experimental plants have been found in studies.[61] Some of these may prove feasible in developing salt-tolerance in sugarcane.

Salt-tolerant sugarcane is under development in Mauritius in cooperation with Queensland University in Australia.[65]

Environmental consequences of genetic changes in the fields

Ecosystem manipulation by crop choice

Farmer's fields constitute an artificial ecosystem where one or a few plant species are favored at the expense of other plants. Consequently, it is the choice of the dominating plant species, i.e. the crop plant species to be grown, that is the by far most influential factor regarding environmental effects due to crop production. That has much greater influence than any choice between different varieties within one crop species, whether having been bred with or without genetic modification.[*]

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[*] There may be one exception, however. New pest-resistant varieties may often bring considerable benefits to the surrounding ecosystem due to their better focused, selective and point wise way of controlling crop pests, in comparison to growing susceptible varieties with the help of frequent control sprayings.

For example, nectar-producing and insect-pollinated plant species offer much better resources for the populations of pollinating insects than wind-pollinated crops such as cereals. Any increase in the cultivation area of such pollinator plants would promote the numbers and diversity of pollinating insect colonies in the neighborhood.

However, due to diseases such as clubroot, oilseed rape cannot be grown but once in five years in many regions. Accordingly, its cultivation area remains low and could not provide sufficiently of raw materials e.g. for biofuel production. In order to get its cultivation area increased manifold, with consequent benefits for the environment, oilseed rape varieties reliably resistant to clubroot should be developed. Until now, the occasional clubroot resistance available in oilseed rape is rather prone to genetic breakage so that any shortening of the established rotation period is not advisable.[66]

Ecosystem manipulation by alien biocontrol species

Ecological basic knowledge and amply of practical experience show that the environmentally most risky exercise is ecosystem manipulation aiming at biocontrol by introducing aggressive alien species outside their natural distribution area. It is a common disaster that the predator or pest species introduced for controlling troublesome plant pests in crop cultivation escapes from its aimed task and turns to destroying rare endemic species in the Nature instead.

One well-known example is the introduction of the New World carnivorous snail *Euglandina rosea* with the intention of controlling an agricultural pest: the giant African snail *Achatina fulica*. However, the biocontrol snail has preferred to extinguish many species of endemic snails of forested habitats in the Tahitian and Hawaiian islands.[67]

Another typical case is the harlequin ladybird (*Harmonia axyridis*). In recent years this alien predator, spread by man for a biocontrol agent against aphids, has appeared to be “the most invasive ladybird on Earth”. It is an aggressive predator that turns its teeth against other ladybirds, spoils apples and pears by nibbling and contaminates wine products with its alkaloids.[68]

Even then, new biocontrol organisms are being taken in large-scale use based only on scanty or practically lacking ecological studies.

For example six billions individuals of a parasitic wasp species (*Trichogramma brassicae*) have been spread to corn fields in Europe annually for decades to control the damages by European corn borer (*Ostrinia nubilalis*), despite that nobody knew what the alien parasite actually did in the fields – except that it parasitizes eggs of more than 200 insect species in all. When its European menu was finally studied in Switzerland, the parasite appeared to attack endangered butterflies at quite high rates in field cage studies.[69] Accordingly, these butterfly species may be waved goodbye once this billion-scale selection business finally succeeds in providing the parasite with somewhat better winter tolerance (or if the European winters are warming), allowing the parasite populations to become established in full scale in the Nature.

Neo-domestication and introduction of tropical tree species

Another ecological gamble proceeds with the efforts of introducing a wide array of novel wild tree species to cultivation. Neo-domestication of nitrogen-fixing tropical trees for use in agroforestry, aimed at providing crop cultivations with nitrogen and shelter, is even coined as if an alternative to the strengthening of crop breeding.[70] Though, some of the most infamous tropical weeds have originated from such introductions of nitrogen-fixing trees which have thereafter turned out to be highly invasive in certain tropical ecosystems.

It is the trait that makes the difference

The risks in species-level ecological manipulations are much greater than the ones due to old or new plant breeding. Genetically modified varieties are no new species but ordinary crops only improved in one or two traits relevant for us in exploiting plant resources better.

Contrary to common beliefs it is not the methods of breeding but the traits bred in the plant variety that determine its benefits or disadvantages in regard to man or Nature. That was first stated by the European Association of Plant Breeding Research two decades ago,[71] and it is constantly confirmed by the life science community in a broad consensus.[72]

Plants adopt useful traits

Wild plants only adopt traits advantageous to them. Contrary to the common claims of anti-biotechnology activists,[73–74] a trait bred in a cultivated crop variety cannot endanger its wild relatives in the Nature.

If the trait is disadvantageous to the wild relative in natural ecosystems – as is the case with most traits bred into plants to make these better suited for human use – the trait will not become common in the species but is fairly rapidly selected away from its populations. Its frequency cannot keep high only based on a low gene flow arriving from cultivated fields.

Higher sugar content in cultivated sugarcane varieties is a good example. The trait would not make wild sugarcane better but worse adapted in the Nature – a sweeter plant would only be a more attractive resource and consequently become more hard attacked at by interested herbivores. Similarly, sugarcane bred to self-degrade its cellulose cannot ever become common in the Nature.

Correspondingly, if a gene for a trait beneficial for a plant species is being added into its gene pool, such an addition does not threaten the species, contrary to the perpetual claims in anti-biotechnology campaigns. One classic example, regarding the alleged gene flow in Mexican corn, is the ecologically absurd claim widely circulated in world media by Greenpeace: “The world is at risk of losing unique diversity of maize to genetic pollution”.[74]

Namely, contrary to popular stories, such genetic addition could only increase the genetic diversity and enhance the adaptation potential of the wild species. According to ecological-genetic foundations, resistance against an alien pest (European corn borer) could only benefit the wild progenitor of maize, teosinte, in its struggle against extinction. Similarly, resistance against stem borer could only aid and not harm wild rice populations, just as the trait helps cultivated rice.[75]

Resistant plants take care of themselves

Pest-resistant plants may often prove an optimal solution regarding environmental effects, because these offer the best focused, point wise control of plant pests. As a rule only the pests attacking on the crop plant shall suffer, and other organisms in the field, e.g. weed-eating ones, remain unaffected, unlike when insecticides are being sprayed in the field.

Revolutionary new prospects in specificity in all branches of plant protection are provided by the new method of RNA interference which won Nobel-prize in Medicine in 2006. The first application in plants has already been bred, namely the newest generation of rootworm-resistant corn. Another ongoing project is the enhancement of cotton's own protection against cotton bollworm by damping down one of the pest's important enzymes using RNA interference.[76] Such better focused pest resistances mean a win-win situation to man and environment.

May super plants conquer Nature?

Might the improved varieties become too strong, however? Could vigor, greater biomass or resistance to environmental stresses such as drought or salinity change sugarcane to a nuisance in the wild? That is quite unlikely. Though, such potentials are always evaluated in biological studies in the field before any new GM varieties are released for commercial cultivation.[77–78]

It is not credible in general that any cultivated sugarcane, with its considerable sugar production and other man-oriented traits could exceed the nasty wild cane species in competitiveness in their particular environments. Even if that could ever be achieved, however, the main result would be replacing current weed canes in part with somewhat sweeter ones. Consequently, also wild or weedy cane populations could be harvested to certain extent for sugar production unlike today.

Could sugarcane research be applied to the development of other bioenergy crops?

Can the achievements in sugarcane be adapted to the breeding of other crops as well? The answer is: likely yes. Though, resistance to drought or salinity may not prove useful in areas lacking such problems even in the future. Unlike in traditional breeding, the progress achieved using genetic modification methods can often be transferred to many other crop species as such or suitably adapted to their specific conditions where necessary. Certain new traits enhancing the carbon and eco-efficiency as well as fuel productivity of the future sugarcanes, self-degrading cellulose included, could probably be introduced successfully also in other bioenergy plants such as switchgrass in North America[6] or reed canary grass in Finland.[7]

Prospects in the near future

It has become customary to generate an array of loose speculations of future developmental alternatives, called ‘scenarios’. That is not science, however.

Even so, it is possible to make a couple of general inferences. The above mentioned breeding efforts may probably result in an array of more efficient plant varieties to be released for cultivation within a decade. Though great enhancements may be achieved, these novel varieties still usually represent single trait improvements.

During the subsequent decade, however, the established new traits are being combined together, both using traditional crosses and *de novo* GM events. For example, varieties combining isomaltulose/trehalose or high-sucrose traits with drought and salt tolerance, successful lignin constitution or cellulose-degrading capacity may be commonly cultivated in various niches of sugarcane production area in the world.

Such trait combinations may in some instances show multiplicative effects and result in quantum leaps in biofuel crop efficiency. Such sustainable production would allow for retaining our food security even if the production conditions may widely deteriorate.

Breakthrough in precision and efficiency of genetic modification in plants

The age-old hopes in plant breeding came true in April 2009, when the development of an efficient and precise method for targeted genetic modification of plant genes *in situ*, i.e. in their native location in plant chromosomes, was announced by two independent research groups.[79–80] Double-strand DNA breaks are generated in breeder-specified loci in plant genome, and the plant is stimulated to make the desired genetic modification itself with the help of its own DNA repair enzymes. The use of specific selection markers goes out, because modification rates are so high (up to 4 %) that the successfully modified plant individuals can be selected from the progeny simply by screening their DNA for the occurrence of the desired gene form.

In the near future, more efficient gene forms e.g. for drought or freezing tolerance need not be any more added to plant chromosomes but the plant's endogenous (unfavorable) gene form can be *replaced* precisely and efficiently with the desired one. In addition, the fine structure of any endogenous gene can be optimized *in situ*, or the gene can be easily blocked from being expressed.

Meanwhile, the European Community is altogether lost with its "antique" and non-science based GM legislation (built on popular beliefs in 1987). Nobody is able of explaining what it may try to mean with 'genetic modification' – and why. The mere definition of the concept, based on various lists of included, excluded or omitted cases (all without safety justification), covers a full page in printing, and it seems to have no intelligible relationship with biological risk evaluation.[30] Directorate General Environment has recently founded an expert working group for puzzling out whether the recent quantum leaps in precision, efficiency and command in plant improvement should be still punished with overly burdensome and fatally costly GM regulation (EC No.

1829/2003) – whereas dirty old methods of breeding are self-evidently glorified and fully freed from regulatory and financial burden.

Clearly, the cutting edge of plant breeding still stays in other continents. One of the above research groups is making its gene targeting method available publicly and will be offering training sessions in the technique. Consequently, brand new GM plant varieties invaluable for our changing world, in regard to both bioenergy, food security and more balanced nutrition,[81] may start pouring from small plant laboratories in the Third World in 10–15 years' time.

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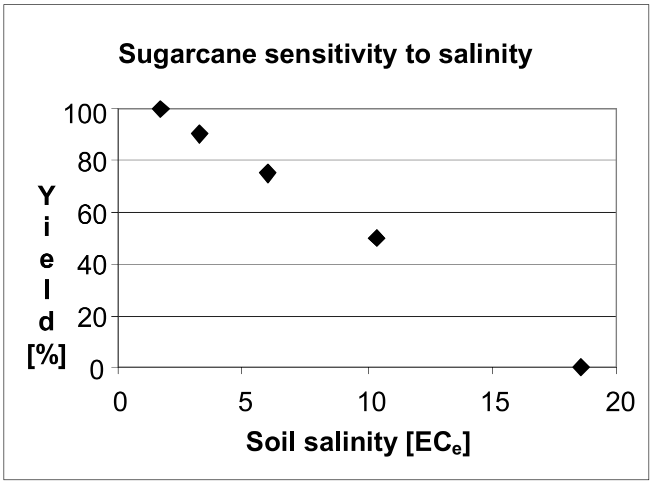
Figure legends

Figure 1. *Sueada salsa* is a halophyte which is even able of growing on the floor of salt-collection basins. Golden Sands, Bulgaria. ©J.Tammisola 2006

Figure 2. Sugarcane is moderately sensitive to soil salinity, and its yield is rapidly reduced with increasing salt concentrations in the soil.[17]


Sugarcane sensitivity to salinity
Yield [%]
100
80
60
40
20
0
0
5
10
15
20
Soil salinity [EC$_e$]

Jussi Tammisola May 27, 2009

Tables

Table 1. Sugarcane water requirements in various countries.[13]

Country	Water requirement (mm)
Australia	1522 (Drip)
Burundi, Central Africa	1327 to 2017 (Furrow)
Cuba	1681 to 2133 (Plant)
Hawaii	2000 to 2400 (24 months)
Jamaica	1387
Mauritius	1670 (Drip)
Philippines	2451 (Furrow)
Pongala, South Africa	1555
Puerto Rico	1752
South Africa	1670
Subtropical India	1800 (Furrow)
Taiwan	1500 to 2200 (Furrow)
Tropical India	2000 to 2400 (Furrow)
Venezuela	2420 (Furrow)
Thailand	2600 (Furrow)

Table 2. Summary of the decision process leading to the release of sugarcane cultivar CP 00-1101 in Florida.[24]

Year	**Month**	**Stage and selection decision**	**Genotypes in stage**	**Locations**
1998	Jan.	Cross made at USDA-ARS Sugarcane Field Station	–	Canal Point, FL
1999	May	Germinated true seed transplanted into field (seedlings)	100,000	Canal Point, FL
2000	Jan.	Advanced from plant-cane seedlings to Stage 1	15,000	Canal Point, FL
2000	Nov.	Advanced from plant-cane Stage 1 to Stage 2	1,238	Canal Point, FL
2001	Nov.–Dec.	Advanced from plant-cane Stage 2 to Stage 3	135	4 farms in Florida
2003	Nov.–Dec.	Advanced from first-ratoon Stage 3 to Stage 4	14	11 farms in Florida
2007	Sept.	Cultivar release	1	

Table 3. Soil Salinity classes in terms of electrical conductivity (EC_e).[57]

Salinity class	**EC_e (dS/m)**	**Salinity effects on crops**
Non-saline	< 2	Salinity effects are negligible
Slightly saline	2–4	Yields of very sensitive crops may be restricted
Moderately saline	4–8	Yields of many crops restricted
Very saline	8–16	Only tolerant crops yield satisfactory
Extremely saline	> 16	Only a few very tolerant crops yield satisfactorily