

Review

Sustainable Agriculture—Enhancing Environmental Benefits, Food Nutritional Quality and Building Crop Resilience to Abiotic and Biotic Stresses

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Abstract: Feeding nutrition-dense food to future world populations presents agriculture with enormous challenges as estimates indicate that crop production must as much as double. Crop production cannot be increased to meet this challenge simply by increasing land acreage or using past agricultural intensification methods. Food production doubled in the past through substantial use of synthetic fertilizer, pesticides, and irrigation, all at significant environmental cost. Future production of nutrition-dense food will require next-generation crop production systems with decreased reliance on synthetic fertilizer and pesticide. Here, we present three case studies detailing the development of cover crops and plant-beneficial microbes for sustainable, next-generation small grain, tomato, and oilseed rape production systems. Cover crops imparted weed and pathogen control and decreased soil erosion and loss of soil nitrogen, phosphorus and carbon, while plant-beneficial microbes provided disease control and phosphorus fertility. However, yield in these next-generation crop production systems at best approximated that associated with current production systems. We argue here that to substantially increase agricultural productivity, new crop germplasm needs to be developed with enhanced nutritional content and enhanced tolerance to abiotic and biotic stress. This will require using all available technologies, including intensified genetic engineering tools, in the next-generation cropping systems.

Keywords: next generation cropping systems; plant beneficial microbes; nutritional quality; environmental stresses; plant biotic stress; antimicrobial peptides; genetic engineering; future agriculture

1. Introduction/Future Challenges Confronting Agriculture

The global population is expected to increase in number and affluence by the middle of this century, with estimates of the world's population in 2050 varying between 8 and 10 billion [1]. Feeding the world's future population will place unprecedented demands on agriculture. We will need to increase food production while at the same time decreasing the negative impacts of agriculture on land, water, and climate [2]. Estimates indicate agricultural production must as much as double to meet projected demands for food [2]. More food is needed and food quality must be improved, particularly regarding nutrient content [3].

Overarching the challenge of producing more food is global climate change [1,3]. Global climate change is expected to bring increased temperatures and increased concentrations of CO₂ and ozone in the atmosphere. Also predicted are altered patterns of weather and drought. Rising CO₂ concentrations may increase yields of certain crops such as wheat, although the extent of the benefit of CO₂ fertilization to crop yield is the subject of debate [3,4]. Offsetting any potential benefits due to global climate change are associated negative impacts leading to concerns about our ability to increase, or even maintain,

crop yields [3]. Increased temperatures will impact yield, as yields of most crops decline dramatically at temperatures much above 30 °C; the optimum temperature for photosynthesis being between 20 °C and 25 °C [5]. For example, yields of the major US crops, corn, soybean, and cotton, increase up to 29 °C, 30 °C, and 32 °C, respectively, with temperatures above these thresholds being very damaging [6]. Drought, salinity stress, and higher ozone levels due to global climate change, as well as the development of new pest and pathogen problems, are predicted to be a drag on crop yields [3,4]. Also of concern is that plants develop more quickly at higher temperatures, leaving less time to accumulate human nutrients such as sugars, fat, and protein [5]. Rising CO₂ levels are also predicted to lower the nutritional quality of certain crops, lowering mineral and protein content [7].

It is unlikely that crop production can be increased to meet the demands of future populations simply by increasing land acreage devoted to agriculture. Competition for land use with urbanization, and the loss of land to salination and desertification will reduce land available for conversion to agricultural production [5]. Additionally, conversion of land to agricultural production has serious environmental consequences. Transforming natural ecosystems to land in agricultural production impacts the global carbon and hydrological cycles, habitat biodiversity, and soil conditions [2,5,8–12]. Among other things, repurposing natural ecosystems for crop production can have substantial impacts on greenhouse gas emissions by releasing carbon stored in vegetation and soil biomass to the atmosphere [12].

It is also unlikely that we can increase productivity of current production systems to meet the demands of future populations by using the agricultural intensification methods of the past. Food production doubled worldwide over the past 35 years; largely due to the use of synthetic fertilizer, pesticides, and irrigation [2]. Nitrogen (N) and phosphorus (P) fertilizer inputs increased dramatically [13]. From 1960 to 2000, the use of N fertilizers increased approximately 800%, with corn, rice, and wheat accounting for about 50% of current fertilizer use. These crops typically have N use efficiencies below 40% leading to loss of N into the environment [8,11,13]. Worldwide, nearly 90% of N fertilizer is NH₄⁺ which is converted to NO₃⁻ by the soil microflora and easily leached into water systems. Under anoxic conditions, excess N in soil from fertilizer is transformed mainly into N₂, but also into the potent greenhouse gas N₂O [10,13]. Overuse of P fertilizer also has negative environmental impacts, causing eutrophication of water systems [11]. Pesticide use increased dramatically (15 to 20 times) over the past 40 years as well [14]. Pesticides used in agriculture are generally hazardous to human health and that of other species, with some pesticides accumulating in food chains [11]. Irrigated land doubled globally over the past 50 years to the point where 70% of freshwater withdrawals are now used to irrigate cropland [2]. Irrigation can result in nutrient loading into water systems and salinization of arable land. Clearly, agricultural intensification over the past decades has had negative impacts, such as increased soil erosion and decreased soil fertility, pollution of ground water and eutrophication of rivers, lakes, and coastal ecosystems, and increased atmospheric constituents that lead to global climate change and water resources with dramatic consequences for food production [2,10,11].

2. Current Cropping Systems

Crops are currently grown globally using various conventional production systems which use synthetic fertilizer and pesticides, and to a lesser extent with organic production systems that use 'natural' sources for maintaining soil fertility and pest control. Conventional production systems can be further categorized into those which incorporate tillage and those relying on a no-till or reduced-tillage strategy. Conventional no-till systems are typically considered more sustainable than conventional tillage systems as they sequester more carbon, have better soil erosion prevention, improve water and fertilizer use efficiency, have better soil nutrient cycling, enhance soil biological activity, and reduce energy, labor, and machinery inputs [15,16]. Organic production systems are designed and perceived to be less detrimental to humans and the environment than the conventional systems as organic production systems place a greater emphasis on managing ecological processes and eliminating inputs

that are, or perceived to be, harmful to humans and the environment such as synthetic fertilizers and synthetic pesticides [17–20]. A major principle of organic agriculture is building soil organic matter via the use of cover crops (non-cash crops grown for their environmental benefits) and animal manures and by-products. Organic soils with higher organic matter levels often have higher capacity to mineralize, capture, and store essential nutrients, such as N, and water resources [17,21,22]. Higher soil organic matter also leads to higher soil aggregate stability and is associated with richer food webs and higher biological activities that drive beneficial soil processes [23].

Conventional and organic cropping systems have been compared in numerous studies and have been found to result in different crop yields, impacts on the environment, and levels of sustainability [24]. Yield averages associated with organic production systems were found to be lower. However, these differences in yield were contextual, varying between 5% and 34% lower depending on crop, conditions, and management practices [19]. Yield differences between organic and conventional systems also differed dramatically with different regions of the world [19]. Some have argued that environmental benefits associated with organic production are diminished as lower crop yields lead to greater deforestation and loss of biodiversity when land is converted to agricultural use to maintain crop production at a certain level [19,25]. Results from one meta-analysis showed that organic farming generally had less negative impacts on the environment per unit land area, but not necessarily with respect to per unit product due to lower yields [25]. For example, organic farms tended to have higher soil organic matter and lower nutrient loss (N leaching, N₂O emissions, NH₄ emissions) per unit field area but higher NH₄ emissions, N leaching, and N₂O emissions per unit product [25]. Organic production systems also had higher eutrophication potential per unit product.

3. Development/Refining Crop Production Systems for Sustainable Intensification of Crop Production

3.1. Development of Next-Generation Cropping Systems

Next-generation cropping systems should include a combination of high yield potential and low negative environmental impacts drawing on the most sustainable aspects from organic and conventional crop production systems [12,24,25]. The emphasis on building soil health in organic production systems using cover crops and other organic materials will need to be combined with the development of new crop cultivars using traditional breeding or genetic engineering techniques. New crop cultivars need to be developed that have increased tolerance of abiotic stress; that offer higher yields but use less water, fertilizer, and other inputs; and have higher nutritional quality. More sustainable methods for pathogen, pest, and weed management also need to be developed [1]. The techniques used to increase crop yield and environmental sustainability will depend on the crop in question. The next sections illustrate development of next-generation sustainable crop production systems for the row crops corn, wheat, and soybean; the horticultural crop, tomato; as well as the development of environmentally friendly disease control methods. These next-generation cropping systems place emphasis on the use of cover crops and beneficial microbes for enhancing soil fertility (N, P), weed and disease control, and decreasing soil erosion. These case studies are being presented to illustrate the approaches as well as challenges encountered during development of these next-generation cropping systems. It should be noted that this review is not exhaustive in nature as only a limited number of applications of cover crops and plant-beneficial microbes are discussed. Finally, examples of the power of genetic engineering for development of crop cultivars with enhanced nutritional content, abiotic stress tolerance, and biotic stress resistance are included.

3.2. Development of Next-Generation Sustainable Grain Cropping Systems

The farming systems project (FSP) is a long-term agroecological research project that was established at the Beltsville Agricultural Research Center in Beltsville, Maryland, USA, in 1996 to evaluate the sustainability of the conventional and organic grain cropping

systems currently being used in the mid-Atlantic region of the United States. The five cropping systems being evaluated at FSP are a three-year conventional no-till corn-rye cover crop/soybean-wheat/soybean rotation (NT), a three-year conventional chisel-till corn-rye cover crop/soybean-wheat/soybean rotation (CT), a two-year organic hairy vetch/corn-rye/soybean rotation (Org2), a three-year organic hairy vetch/corn-rye/soybean-wheat rotation (Org3), and a six-year organic corn-soybean-wheat-alfalfa-alfalfa-alfalfa rotation (Org6). These five cropping system plots are co-located and incorporate large field-scale plots to address field variability and long timeframes, as some soil processes being compared occur very slowly [26]. Conventional systems were managed with herbicide and synthetic fertilizer programs and current GMO cultivars. Organic systems were managed using USDA National Organic Program Standards [27].

Crop yields were considerably lower with the organic systems, with yields 31% less for corn and 20% less for soybean than with conventional cropping systems in a ten-year analysis [27]. Certain environmental benefits were greater with the organic cropping systems than the CT or NT cropping systems. Soil organic carbon (SOC), measured to a depth of 1 m, was 11% greater in the Org3 than the NT cropping system indicating that tilling organic materials (manure) into soil may be a more effective means of increasing SOC than eliminating tillage [28]. SOC was greater in the NT cropping system at the 0–5 cm soil depth but greater in the Org3 cropping system at the 5–10 cm and the 20–25 cm depths. This increased SOC in the organic cropping systems led to greater N mineralization potential and biodiversity [27]. Increased SOC also decreased global warming potential of the organic cropping systems by sequestering carbon in soil [28]. Global warming potential was negative for Org3, indicating it was a net sink for CO₂ equivalents, and positive for NT and CT production systems. These differences in global warming potential were also driven by lower energy usage with the Org3 production system. Global warming potential per unit of grain yield was negative and significantly lower for the Org3 systems than the NT or CT systems despite lower grain yields [20].

Other environmental benefits associated with organic systems, such as minimizing soil erosion and sediment-bound nutrient transport from fields, were mixed when compared with the CT and NT cropping systems. It was found that predicted soil sediment loss was 33% less with Org3 than CT. N, P, and soil carbon loss was similarly less with Org3 than CT as N, P, and soil carbon are absorbed to the soil sediments lost in run-off [29,30]. However, when NT and Org3 cropping systems were compared, soil erosion and loss of N, P, and soil carbon were 80% less with NT than Org3. Tillage used in Org3 diminishes soil aggregate size and these smaller soil particles are more susceptible to erosion than macroaggregates [30,31]. Using cover crops and animal manure in Org3 built SOC, and had the associated enhancement of soil aggregation, which probably offset the negative impact of tillage on soil aggregation [32], resulting in soil erosion being less with Org3 than CT but greater than with NT. Meteorological influences on corn and soybean grain yields over an 18-year period were compared among the cropping systems at the FSP site as well [27]. Efficiency of grain yield per unit precipitation was greater for conventional than organic cropping systems. Precipitation and heat stress had a significant impact on organic production systems during this study with weed cover playing a significant role due to competition between weeds and the grain crops for water.

3.2.1. Increasing Organic Cropping System Complexity to Enhance Environmental Benefits and Yield

Comparisons of the organic production systems at FSP indicated that increasing cropping system complexity increased grain yield by decreasing weed competition [33]. Corn yield losses due to weed competition were estimated to be 35% in Org2 and decreasing to 14% in Org6, and for sake of comparison, 7% with the NT treatment. In Org2, weed mortality events occur at the same time each year as the two cash crops, corn and soybean, are sown at the similar times in the calendar year. This tends to favor the establishment of summer annual weeds in Org2 plots. With Org3, wheat is added to the rotation, resulting in decreased seed set by the summer annual weeds due to cutting at wheat harvest, or weed seed mortality, due to preparation of the soil for the cover crop used in the Org3 rotation [28]. With Org6, the addition of alfalfa to the rotation adds an additional layer of complexity

as alfalfa is cut three to five times a year, incorporating more weed mortality events into the cropping system [28]. All measures of nitrogen availability increased as cropping system complexity increased from Org2 to Org3 to Org6 [22], also possibly contributing to the increased grain yield associated with the Org6 rotation. Preliminary results also suggested that increased complexity decreased predicted soil erosion [28]. Although this approach enhances yield and environmental benefits associated with organic grain cropping systems it does not come close to the estimated doubling in yield needed to feed the future world population.

3.2.2. Organic No-Till Cover Crop-Based Small Grains Cropping System

A second approach for a next-generation sustainable grain cropping system is an organic no-till cover crop-based production system [34]. This strategy integrates the soil conservation benefits of no-till grain production [35–37], with the soil organic matter building practices of organic systems. Features of this system are the use of cover crop mulches for weed suppression and the contribution of N-fixing legume cover crops towards meeting N demand of the subsequent cash crop. Use of cover crop mulch for weed control allows the system to move away from the use of tillage for weed control, and its deleterious impacts on soil aggregation, biology, and erosivity [16,37–39]. These organic no-till systems rely on mechanical termination of winter annual cover crops with roller-crimper devices and no-till planting corn and soybean into the resulting cover crop mulches. Simulation models indicate that this approach has the potential to increase environmental benefits compared to current tillage-based organic grain production systems [40]. However, more research is needed [34].

Cereal rye is typically used as the cover crop preceding soybean in the rotation. Small grains cover crops, such as cereal rye, produce substantial biomass and provide reliable weed suppression as a living cover crop and after termination as a surface mulch [41,42]. These cover crops also prevent erosion and build soil organic matter [41]. Legume cover crops, such as hairy vetch, are used prior to the corn phase of the rotation as corn requires more exogenous N than soybean. These legume cover crops fix atmospheric N and release N during decomposition. However, hairy vetch grows slowly in the fall and decomposes rapidly after termination making it less effective than cereal rye for weed suppression. Recent research has switched focus to mixtures of hairy vetch and cereal rye, or another winter grain, since hairy vetch alone does not provide sufficient weed control. Mixtures of hairy vetch and cereal rye cover crops have been shown to provide greater above ground biomass and weed suppression than hairy vetch monocultures and release greater N content to the soil than cereal rye monocultures [42–44]. However, significant challenges remain regarding management of cover crop and cover crop mixtures to enhance weed control and soil fertility and minimize the cover crop itself competing for resources as a weed with the subsequent cash crop [34,45]. As with the preceding approach, this organic no-till cover crop-based system does not appear to come close to doubling grain yield.

3.3. Next-Generation Cover Crop-Based Sustainable Tomato Production System

Fresh-market tomatoes, like other vegetables in the US, are grown using high-input production systems to maximize yield and product quality. For tomatoes, these production systems use raised beds and depend heavily on synthetic fertilizers, black polyethylene plastic mulch, and tillage. N from synthetic fertilizer is essential to maintain fertility levels, while the black polyethylene plastic is used primarily to control weeds [46]. Fertilizer, black plastic, and tillage comprise a large proportion of the production costs for large-scale production of fresh-market tomatoes. Additionally, N recovery by the tomato plant from synthetic fertilizer is low, causing some growers to apply excess N to maximize yield, driving up production costs and potentially contributing to surface and groundwater pollution [46].

To increase sustainability of this, and potentially other vegetable production systems, the legume cover crop hairy vetch has been substituted for the black polyethylene plastic in the next-generation tomato production system. This system features no-tillage planting of tomato transplants into a killed, hairy vetch cover crop grown on raised soil beds. Importantly, in research conducted over a ten-year

period, this no-till hairy vetch mulch system resulted in greater yield and economic return than tomatoes grown using the conventional black polyethylene plastic system [46,47]. Economic advantage with the hairy vetch mulch system was greatest in years with abundant rainfall and less during droughty years.

Intended environmental benefits provided by this no-till hairy vetch system were a reduced need for synthetic nutrient inputs such as N, reduced soil erosion resulting from the no-tillage system, and increased soil water holding capacity [48]. As with the hairy vetch cover crop used in the no-till production system discussed above, hairy vetch was intended to utilize N in soil leftover from the previous crop and fix N from the atmosphere. Both the intercepted soil N and fixed atmospheric N were then expected to be converted into hairy vetch biomass and released to the soil upon decomposition for the subsequent tomato crop [49]. In a three-year field study, exogenous N requirements were reduced for tomato grown with the hairy vetch system compared with those grown using black polyethylene plastic. Minimum N rates necessary to achieve maximum yield were 80 lb/acre with the hairy vetch system and 170 lb/acre with the black plastic system [49]. As expected, soil erosion was less when hairy vetch was used. With the black plastic system, 50% to 75% of the field is covered with the water-impervious plastic enhancing run-off and lessening water retention in field soil. Losses of two to four times more water and five to fifteen times more soil sediment were observed with the black plastic system over a field season. Pesticide loads released from fields were also greater as pesticides intended for the tomato plant collected on the plastic surface and were loaded into run-off during rainfall events [50,51].

Another intended environmental benefit of the no-till hairy vetch production system was reduced weed competition and hence reduced need for herbicides. However, mixed results were obtained for weed control. As with the no-till small grains production system discussed above, better weed control may result from cover crop mixtures where the hairy vetch is mixed with cereal rye or another cover crop that provides more biomass for a weed-suppressive mulch and decomposes more slowly than hairy vetch. The hairy vetch system did, however, reduce disease on tomato due to *Alternaria solani*, the causal agent of tomato early blight [52,53]. Infective propagules of this fungal pathogen in soil are spread by splash dissemination of infested soil into the tomato canopy. Soil sediment detected in the tomato canopy was significantly lower with the hairy vetch production system than the black plastic production system after rainfall events. Development of tomato early blight disease was also slower on tomatoes grown with the hairy vetch production system. It was thought that more complete coverage of the soil surface with the hairy vetch mulch (hairy vetch mulch covers the entire field while black plastic covers the tomato beds but not the interspaced rows) physically obstructed splashed soil near the soil surface preventing soil infested with pathogen inoculum from entering the tomato canopy. The tomato crop grown in the hairy vetch mulch had negligible loss to early blight in the absence of fungicide relative to the fungicide treated controls [52,53]. There was also greater resistance of the tomato crop grown in the hairy vetch mulch to invasion and damage by the Colorado potato beetle [54]. Possibly contributing to disease and pest reduction was induction of disease resistance in tomato by the hairy vetch production system. As discussed below, certain genes functioning in plant defense had an altered expression profile in tomato grown in hairy vetch relative to these genes in tomato grown with the black plastic [55].

3.3.1. Impact of Hairy Vetch Cropping System on Tomato Physiology

This no-till hairy vetch tomato cropping system had a broad and beneficial impact on tomato plant physiology. In field and greenhouse experiments gene transcripts of proteins involved in diverse processes that regulate metabolism and growth were found to be differentially up-regulated in leaves of hairy vetch-grown tomato plants relative to those grown under black plastic [55–57]. Up-regulated genes included the most abundant chloroplast protein, ribulose biphosphate carboxylase/oxygenase (Rubisco), important for carbon fixation; nitrogen-responsive glutamine synthase, regulating carbon/nitrogen signaling; nitrogen utilizing and nitrite toxicity

reducing nitrite reductase; nitrogen-use efficiency protein glucose-6-phosphate dehydrogenase; chaperone proteins (HSP70 and ER protein BiP) that stabilize native proteins; cytokinin- and gibberellin-related regulatory proteins; and plant defense anti-fungal proteins chitinase and osmotin. Up-regulation of these plant defense genes may contribute to disease resistance as, in these and other field experiments, hairy vetch-grown tomato had less disease than tomato grown under black plastic [52,53,55].

Other genes/proteins that promote senescence and aging, the ethylene biosynthesis gene ACC synthase and the senescence-regulated SAG12 gene, were more down-regulated in leaves from hairy vetch-grown tomato. Consistent with this, hairy vetch grown tomato had higher levels of the cytokinin indicator gene, cytokinin receptor protein kinase (CRK) [55,58]. A continued supply of cytokinin from the roots to the upper parts of a plant should delay senescence as cytokinin inhibits accumulation of senescence-enhancing gene transcripts [59]. Interestingly, senescence in hairy vetch-grown tomato was delayed relative to tomato grown with black plastic. Cytokinin signaling has also been found to regulate plant-microbe interactions [60]. Engineered accumulation of cytokinins led to the upregulation of defense-related genes including basic chitinase [61] and osmotin [62]. Therefore, accumulation of transcripts and protein of these two anti-fungal defense proteins, chitinase and osmotin, in hairy vetch-grown tomato [55] indicated that cytokinin signaling may regulate disease resistance as well as senescence. Implications of these results in the organism-to-organism interactions in the ecosystem have been proposed (www.glf.c.forestry.ca/frontline/bulletins/bulletin_no.18_e.html).

A field pot experiment was conducted to determine the impact of N released during decomposition of hairy vetch on hairy vetch-grown tomato physiology [63]. In this experiment, one treatment consisted of tomato grown in soil where hairy vetch was grown as the winter cover crop (including hairy vetch residue on the soil surface) while a second consisted of bare soil (no hairy vetch cover crop). Additional treatments consisted of the bare soil and hairy vetch treatments supplemented with varying amounts of inorganic N fertilizer. Tomato fruit yield, plant biomass, and photosynthesis were found to be higher in plants grown with the hairy vetch treatment than the bare soil treatment. Additionally, a parabolic response to inorganic N in the bare soil treatments containing supplementary N fertilizer was evident for tomato growth and photosynthesis, suggesting N toxicity in pots with the highest rates of supplemental N. There was also a decline in expression of several genes such as nitrate reductase and PEP carboxylase that regulate nitrogen and carbon metabolism associated with the high (200 N kg ha⁻¹) rate of supplemental inorganic N. Surprisingly, these parabolic responses were mitigated in the hairy vetch-grown plants, where higher photosynthetic rates were maintained at high supplemental inorganic N rates. Hairy vetch also mitigated the decline in expression of the genes regulating nitrogen and carbon metabolism. Consistent with prior experiments, the plant defense-related gene, osmotin, was up-regulated in tomato plants grown in the hairy vetch treatment relative to those grown in bare soil.

Results from this field pot experiment suggest that physiological cues released from the decomposing cover crop, other than N, have beneficial impacts on tomato plant physiology. These studies also suggest that tomato can distinguish between organic and inorganic sources of N, and that N management by on-site production of legume cover crops such as hairy vetch in sustainable cropping systems offer additional physiological advantages to cash crops than cropping systems utilizing inorganic N fertilizer alone [63]. Pertinent to these findings is a recent study that showed soil organic N influences plant growth as well as nitrogen use efficiency in plants, and that carbon cost of organic N assimilation, with its carbon content, into proteins is lower than with inorganic N [64]. Factors that contribute to higher nitrogen use efficiency in crops grown on organic N include nitrogen-based productivity and higher root:shoot ratio.

Another aspect of the physiology of tomato plants altered due to growth under different cropping systems (hairy vetch, black plastic, rye cover crop) compared to bare soil was the metabolome of the tomato fruit. Since metabolomics data for the fruit is relevant to the nutritional quality these studies affirmed that interaction between nutritional quality and growth environment [65].

3.3.2. Impact of Hairy Vetch Cropping System on the Soil Microbiome

Cover cropping with hairy vetch increased microbial biomass in the bulk soil as well as tomato rhizosphere when compared with the black plastic and bare soil treatments. The hairy vetch treatment also altered the microbial community structure [66,67]. Microbes are associated with healthy soil, nutrient cycling, soil organic matter, disease suppression, and therefore this altered microbial community structure may be at least partially responsible for the impact of hairy vetch on tomato physiology described above. There was indirect evidence suggesting that more readily available carbon in the vetch treatment may have caused the change in microbial community structure.

3.4. Use of Plant-Beneficial Microbes for Sustainable Crop Production

Biological control agents, plant-beneficial microbes that control plant pathogens and pests (invertebrates, pathogens, and weeds), are being developed to replace, or to be used in combination with reduced levels of, synthetic pesticides to enhance sustainability of crop production systems. Agricultural production systems are heavily reliant on pesticides for control of invertebrates, pathogens, and weeds as estimated losses to these pathogens and pests were 48% to 83% in the absence of some form of crop protection [14]. When there is no effective plant resistance, biological controls must be developed if pesticide inputs are to be reduced. Cultural practices such as crop rotation, alteration of planting date, etc. certainly play a role in pathogen and pest management but the level of control is often inadequate or economically nonviable [68]. For example, to decrease fungicide use in oilseed rape production an effective biological control strategy must be developed for the important soil-borne pathogen *Sclerotinia sclerotiorum* as the other forms of disease control tactics for this pathogen can be inadequate. Traditional breeding strategies for plant resistance to *S. sclerotiorum* are challenging due to limited gene pools and the need for multigenic resistance [69]. Crop rotation is limited in effectiveness as *S. sclerotiorum* has a broad host range and lengthy persistence [70].

Toward this end, biological control agents have been developed that can be applied at strategic points during the disease cycle of *S. sclerotiorum* on oilseed rape. *S. sclerotiorum* overwinters as sclerotia in soil, which upon germination, produce apothecia or directly produce mycelia. Ascospores produced from apothecia are the primary inoculum for most diseases of *S. sclerotiorum* and typically germinate on senescing flower petals. The pathogen then infects healthy leaf and stem tissue ultimately killing the plant [71,72]. Application points for biological control agents in this disease cycle are as a seed treatment, where the biological control agent is expected to colonize the developing oilseed rape plant and persist for the growing season, as a foliar spray at oilseed rape flowering to position the biological control agent so that it can prevent infection by germinating ascospores in the plant canopy, and as a spray on the field prior to planting oilseed rape so that the biological control agent can colonize and kill sclerotia of this pathogen prior to production of apothecia. Two seed treatment formulations of *Bacillus subtilis* BY-2 and of *B. subtilis* Tu-100, and spray applications of these two isolates at flowering, resulted in significantly lower disease incidence than the non-treated control and a significantly greater yield than this control in field trials conducted at a few locations. *B. megaterium* A6 was also shown to control *S. sclerotiorum* on oilseed rape at a few field locations when applied as a seed treatment formulation [73–75]. Control by these strains at these field locations compared favorably to that provided by the carbendazim chemical spray control applied at flowering. Fungal mycoparasites of sclerotia of *S. sclerotiorum* have also been isolated and developed for control of this pathogen on oilseed rape [76,77]. Spray application of one mycoparasite, *Aspergillus aculeatus* Asp-4, to the soil prior to sowing rice in a rice-oilseed rape rotation resulted in a significant reduction in incidence of *Sclerotinia* stem rot on oilseed rape compared with the non-treated control in two field trials. This application of *A. aculeatus* Asp-4 also resulted in a significant reduction in formation of apothecia on sclerotia relative to the non-treated control in these field trials, suggesting that colonization and degradation of sclerotia by Asp-4 and subsequent reduction in sclerotial germination led to disease control [77].

However, for these beneficial microbes to be widely acceptable to farmers as an alternative to pesticides they must be improved in reliability and efficacy so that they compare favorably to

synthetic pesticide compounds over a large range of fields [78,79]. Unfortunately, biological control efficacy is often inconsistent due to the inherent complexity of the interaction between the biological control agent, the pathogen, and the environment where this interaction occurs [80]. For example, *A. aculeatus* Asp-4 up-regulated numerous genes and proteins involved in mitigation of environmental stress and dissolution of sclerotial compounds during colonization and degradation of sclerotia of *S. sclerotiorum* [81]. Omic studies of other biological control interactions revealed equally complex gene and/or protein expression profiles by biological control agents during interactions with pathogens or the environment [82–84]. In turn, soils and plant surfaces, where biological control agents are expected to effect disease control, are highly heterogeneous regarding compounds and conditions that impact expression of genes important to biological control; ultimately impacting expression of these genes and reliability and efficacy of disease control (reviewed in [80]). One approach to improving reliability and efficacy of disease control is to combine biological control microbes. These microbes can be combined in individual formulations or through multiple treatments targeting strategic points in the disease cycle. It is thought that a combination of microbes, with different ecological adaptations and mechanisms of disease control, are more likely to express traits important for disease control over a wider range of environmental conditions than an individual microbe.

This concept for improving efficacy and reliability shows promise. For example, when isolates BY-2, Tu-100, and A6 were applied as oilseed rape seed treatment formulations in various combinations there was increased seed yield and decreased disease incidence with increased number of isolates in seed treatment preparations in pot and field experiments conducted in four different soils (Hu et al., unpublished). Further, the treatment containing the three isolates BY-2, Tu-100, and A6 resulted in an incidence of disease on oilseed rape that was significantly lower than that associated with seed treatments containing individual isolates most of the time. Pieces are in place to develop and test an integrated disease control strategy to increase performance (level of disease suppression, reliability of disease suppression) of biological control of *S. sclerotiorum* on oilseed rape. Application of seed treatment formulations containing multiple *Bacillus* isolates, can be combined with application of formulations of the mycoparasites of sclerotia of *S. sclerotiorum*. These two application methods would reinforce each other regarding disease control. The application of mycoparasites prior to planting oilseed rape would reduce initial inoculum of *S. sclerotiorum*, resulting in less disease to be controlled with the *Bacillus* seed treatment or spray formulations. Likewise, the follow-up use of the *Bacillus* seed treatments or spray formulations would minimize disease caused by pathogen inoculum that escaped the treatment with the mycoparasite.

A second approach to increasing sustainability of disease control is to combine microbial biological control agents with reduced rates of synthetic pesticides. For example, formulations of the mycoparasite *Trichoderma* sp. Tri-1 were tested in combination with reduced application rates of the chemical pesticide carbendazim for control of *S. sclerotiorum* on oilseed rape [77]. The treatment containing the recommended rate of carbendazim provided the greatest reduction in disease when compared with treatments containing individual applications of lower rates of this pesticide or the formulated Tri-1 treatment in all field experiments. Encouragingly, treatments containing formulated Tri-1 combined with carbendazim applied at 75% the recommended rate reduced incidence of disease to levels to those obtained with the treatment containing carbendazim applied at the recommended rate in field trials.

Plant-beneficial microbes are also being developed to replace, or be used in combination with reduced levels of, synthetic phosphatic fertilizers to increase cropping system sustainability. The ability to solubilize P from compounds in soil is fairly wide-spread amongst soil microbes and certain biological control agents have been shown to control disease and solubilize P [85]. All three *Bacillus* isolates, *B. subtilis* Tu-100, *B. subtilis* BY-2, and *B. megaterium* A6 solubilized phosphate from inorganic and organic sources (Hu et al., unpublished; [86]. In addition to controlling disease, combinations of all three isolates in treatments of oilseed rape seeds resulted in promotion of growth relative to the nontreated control in five different soils (Hu et al., unpublished) indicating that these plant-beneficial

microbes contribute multiple environmental benefits. As with biological control, environmental conditions influence the ability of these microbes to solubilize P [85]. More research is needed regarding colonization and mode of action of these plant-beneficial microbes to facilitate their use in enhancing sustainability of crop production systems.

4. Development of New Crop Cultivars for Sustainable Intensification in Agriculture

4.1. Improving Nutritional Quality of Crops

Until recently, classical breeding strategies were focused on developing crop cultivars for mechanical harvesting, yield, size, and disease control while improving nutritional quality was mostly unexplored. Lack of knowledge regarding metabolic pathways and their genetic components likely contributed to slow progress on developing strategies to improve nutritional quality of crops. Lately, attention has focused on developing nutrient-rich crops in response to consumer demand for high nutritional quality foods. Although subject to debate [87]; and references therein], a number of nutrition studies have linked diet with certain human health maladies—cancer, osteoporosis, diabetes, hypertension, cardiovascular health, and age/lifestyle-related diseases [88–91]. Diet fortification with off-the-shelf vitamins and antioxidants is a solution to enhancing human nutrition, but it is the bioavailability of a nutrient in food that determines the extent to which the potential of a nutrient is realized [92,93].

Improving nutritional quality of food will require new strategies and approaches. Currently available crop cultivars developed with classical breeding approaches have been diminished in certain health-promoting and flavor-enhancing molecules while the important agronomic attributes, yield and time to harvest, have been optimized [94–98]. Significant reductions in minerals, protein content, and vitamins over time were found in 43 garden crops in a US Department of Agriculture (USDA) survey conducted between 1950 and 1999 [94]. Also, a recent study combining biochemical and genomic analyses with consumer tasting panel data of approximately 400 varieties of tomatoes showed that modern commercial varieties were significantly reduced in many flavor chemicals relative to older varieties [99]. These and other studies clearly illustrate the need to use new approaches to recover not only the flavor-imparting genes/compounds but also other important nutritional traits that may have been lost due to intense classical breeding and selection.

Biotechnological approaches, using refined genomics, proteomics, and metabolomics tools are available and capable of engineering crops for enhanced nutritional content. In particular, progressive genetic engineering allows specific and controlled introduction of more efficient and novel genes while RNA-programmable genome CRISPR/Cas9 technology has great potential for editing inefficient or unwanted genes for development of crop germplasm with higher concentrations of nutrients [100]. For example, engineered 'golden' rice that synthesizes and accumulates pro-vitamin A, β -carotene, and protein is playing an important role in conquering malnutrition in the developing world, particularly with children [101,102]. Similarly, transgenic multivitamin corn was developed by introducing four different cDNAs encoding enzymes in the biosynthetic pathways of the vitamins β -carotene, ascorbate, and folate. The resulting transgenic corn endosperm was shown to accumulate β -carotene ($\sim 59.32 \mu\text{g/gDW}$), the anti-cancerous lycopene ($22.78 \mu\text{g/gDW}$), vitamin C (ascorbate) ($106.94 \mu\text{g/gDW}$), and zeaxanthin ($35.76 \mu\text{g/gDW}$) [103]. Additionally, tomato fruit with enriched nutritional content (choline, lycopene, amino acids, sugars and organic acids) and enhanced shelf-life was developed using ripening-specific expression of the polyamines spermidine and spermine [104,105]. Unanticipated enrichment in lycopene levels in the engineered tomato by 2- to 3.5-fold as compared to the conventional tomatoes was substantial, exceeding that achieved by classical breeding methods [104]. Transcriptomic analysis of high polyamine-accumulating tomato fruits has shown that increased content of lycopene and flavonoids is consistent with upregulation in the transcription profiles related to carotenoid and flavonoid biosynthesis pathways (Fatima T. et al., unpublished). Other examples of genetic engineering-mediated enhancement of specific food crops,

including fruits and vegetables, with higher or novel doses of nutritional and disease-preventing molecules have been collated and reviewed [91,98,106–108].

It is understood that genetic events, as well as environmental and crop production system factors, impact both the type and quantity of cellular metabolites in crops [109,110]. In this regard, cross talk between carbon metabolism and nitrogen sensing was detected in the polyamine-accumulating, transgenic tomatoes described above when these plants were grown with hairy vetch as a cover crop but not when grown with black plastic or in bare soil [105]. In these polyamine-accumulating, transgenic hairy vetch-grown tomatoes expression of genes for PEP carboxylase (PEPC) and cytosolic isocitrate dehydrogenase (ICDHc) was upregulated; these gene transcripts generally being activated in response to nitrogen assimilation [111,112]. Increased high polyamine levels in tomato fruits grown in hairy vetch were associated with a higher respiration rate, decreased concentrations of sucrose and glucose, and increases in the aspartate family of amino acids, as well as malate, fumarate and citrate relative to the other treatments [105] suggesting that additional nutritional benefit may be obtained by combining transgenic tomato with a cropping system tailored to optimize nutritional content of tomato fruit. There is a good probability that approaches used in this tomato model system can be applied towards enhancing the levels and composition of health-related nutrients into other crops such as grains [113].

4.2. Improving Abiotic Stresses Tolerance in Crop Cultivars

Crops are exposed to one or more abiotic stresses daily that impact crop yield and quality. These include drought, temperature extremes, high light intensities and UV radiation, and salinity. For example, high temperatures are known to reduce the grain filling period in wheat, oat, and field corn [114–117]. Also, suboptimal environmental conditions resulting from abiotic stress are a major cause for crops not achieving their full genetic potential for yield and crop quality, with yield losses being as high as 50% [118–120]. Clearly, the development of stress-tolerant crops is important if we are to increase agricultural productivity.

Developing stress-tolerant crops will require new strategies and approaches as classical breeding techniques have been largely unsuccessful, possibly due to the involvement of multigenic traits. Genetic transformation of crops by introducing genes that provide tolerance to one or more abiotic stresses is likely needed. A medley of genes, including specific transcription factors that interact directly or indirectly with genes associated with the abiotic stress signaling, have been identified by techniques such as comparative transcriptomics and validated [108,121,122]. Some transcription factors, belonging to a number of transcription factor families, enabled protection against multiple stresses, including cold, drought, and excess salt [120,122–124]. Additional, important candidate molecules that directly or indirectly impart tolerance to abiotic stresses include proteins with protective functions (dehydrins, heat shock proteins [HSPs], late embryogenesis abundant proteins), osmolytes (proline/trehalose/sugars), glycine betaine, signaling molecules (polyamines), and hormones (abscisic acid, ethylene, and methyl jasmonate). With regard to transcription factors, it is important to characterize which transcription factors among a family of transcription factors actually confer stress tolerance, rather than simply being stress responsive, in order to successfully utilize them for genetic transformation of a crop for improving resistance against one or more abiotic stresses.

Plants have been successfully enhanced in stress tolerance using genetic engineering. Overexpression of wheat transcription factor TaHsfA2d, similar to rice OsHsfA2d, infused tolerance of the transgenic Arabidopsis plant to a number of abiotic stresses—elevated temperatures, salinity and drought [125]. Also, gene shuffling improved the function of Rubisco activase, a critical protein in carbon fixation, and the resulting transgenic Arabidopsis plants performed better at moderately high temperatures [126]; the chloroplast chaperone, a HSP cpn60b chaperone with Rubisco activase acclimatized photosynthesis to higher temperatures [127]. HSPs accumulate in diverse plant cells in response to even a short exposure to high temperature, and are normally synthesized to maintain homeostasis of plant processes during these conditions. HSPs maintain functional conformation

of other proteins and moonlight as chaperones in the assembly and transport of nascent proteins, normally as well as in response to abiotic stresses [118,128–131]. Additionally, engineering crop plants has resulted in resistance against various abiotic stresses and industry has successfully generated drought resistant germplasm for farmers.

Genetic evidence has validated that plants synthesize small molecules for protection against extreme environmental conditions. Small molecules that act as osmoprotectants include glycine betaine, sarcosine, trimethylamine-*N*-oxide, glycine, proline, glutamate, mannitol, and trehalose. These compounds correct the cytosolic imbalance caused by stress exposure [132,133], and references therein]. For instance, salt-tolerant alfalfa plants roots accumulate two-fold more proline than the salt-sensitive plants [134,135]. Additionally, different strategies used to develop transgenic rice plants that accumulate proline and soluble sugars resulted in plants that were protected against drought and osmotic stress [136–138].

Transgenic approaches have confirmed that polyamines, small molecules discussed above with regard to their role in the fruit metabolome, play an important role in plant responses to various abiotic stresses [120,139–142]. Polyamine pathway-deficient *Arabidopsis* mutants were more sensitive to salt stress [143–147] while heterologous overexpression of polyamine genes generated transgenic plants—*Arabidopsis*, rice, tobacco, and tomato, which were tolerant to abiotic stresses [148–154]. Also, transgenic tobacco, rice, and tomato plants engineered to express heterologous *S*-adenosylmethionine decarboxylase gene, a rate-limiting enzyme/gene in polyamine biosynthesis [100], showed tolerance against salt, osmotic, and heat stresses [139,155–158], while overexpression of spermidine synthase gene—in *Arabidopsis*, pear and potato plants—made these transgenic plants tolerant against drought, salt and oxidative stresses due to the higher polyamine levels [152,153,159].

Thus, the success in developing novel germplasm/genotypes against abiotic stresses provides a positive spin and encouragement for conducting bon-a-fide field trials of the engineered crop plants. Such validation will catalyze producing super new high-yielding crops with durable resistance to harsh environmental stresses to meet the food demands of world population.

4.3. Plant Biotic Stress Resistance and Antimicrobial Peptides (AMPs)

Traditional breeding strategies for plant resistance to pathogens have successfully mined ‘R’ (resistance) genes from related plant hosts and incorporated them into high yielding cultivars [68,160]. Research carried out to understand plant-pathogen interactions helped the discovery of plant ‘R’ genes that contained pathogen attack via ‘innate’ immunity. Elite breeding lines became sources of disease-resistant germplasm that helped contain crop diseases with additional help from chemical pesticide application. Such strategies are inherently time consuming in nature, pathogen species-specific, and tend to be of short duration as new race(s) of pathogens develop/evolve continuously, eventually overcoming R-gene plant resistance.

Antimicrobial peptides (AMPs) are a promising alternative to the use of ‘R’-genes for plant resistance to plant pathogens [161,162]. AMPs are structurally diverse, small proteins consisting of 20–100 amino acids with the potential of imparting durable immunity to plants against pathogens. Being small molecules, changing their chemical structure to enhance potency is relatively easy. The combination of hydrophobic regions and net positive charge of AMPs allows electrostatic interactions with the negatively charged polar heads and hydrophobic core of microbial pathogen membranes [163]. Structural parameters other than conformation and charge include hydrophobicity, hydrophobic moment, amphipathicity, and polar angle that make them toxic and target specific. After binding their target, they permeabilize the pathogen membrane and impair pathogen cellular functions. AMPs establish interactions at the surface of these microbes in spite of the fact that prokaryotic organisms have significant structural differences, while their broad spectrum toxicity also involves targeting intracellular components necessary for the survival and proliferation of the pathogen. Structural disparity of prokaryotic and eukaryotic membranes contributes towards the

AMP selectivity [164–166]. They are also amenable to alteration for higher potency via production in higher amounts in heterologous systems using genetic biotechnology.

In vitro studies and in vivo expression of AMPs through transgenic approaches suggest that their antimicrobial activity is taxonomically very broad, likely because they bind to commonly specific domains that occur across great phylogenetic distances. AMPs are not cytotoxic to the host cells, thus enhancing their scope and application in disease management. Natural selection favors a defensive apparatus with a minimal maintenance cost plus a high deterrence value, which make AMPs fitting tools in plant defense strategies. It is noted here that AMPs are also a tool used by microorganisms including bacteria and fungi, likely for their defense [167].

AMPs Have Moonlighting Functions

Advancing research toward defining roles of AMPs in plant responses to abiotic and biotic stress has led to new information that suggests they also catalyze other important functions related to plant development. Some of these include roles in self-incompatibility—OCP-A1 and SP11 AMPs [168,169], pollination—LUREs, DEFL, ZmES-1 and DEF2 AMPs [170–172], and root nodule-specific secretory pathway [173,174]. Lipid transfer proteins, LTPs, are a class of cysteine-containing AMPs that function in the synthesis of cuticular wax [175], pollen adhesion [176], guiding pollen tube towards fertilization [177]—enabling enhanced tolerance to bacterial pathogens [178]. Molecular genetic manipulation of some AMPs confirmed their other cellular function(s). Such manipulation of DEF2 demonstrated its role in pollen viability, seeding and morphology of tomato [179] and inhibiting endogenous production of snak-in-1 impaired potato plant development [180].

The promise and potential of employing AMPs not only in defense against pathogens but also in modulating and enhancing plant developmental processes can be immense in contributing to global food security. A test case that involved specific modification of the structure of the AMP, *msrA3*, and its introduction into potato via genetic engineering [181] brought to light important features about such a strategy which bear not only on disease-resistance phenomenon but also crop production. *msrA3*-transformed potato lines were found to be resistant to the necrotrophic pathogen *Fusarium solani*. Surprisingly, these transgenic potato plants mitigated normal plant defense responses such as the hypersensitive response (HR) and production of reactive oxygen species (ROS). Other characteristics of these transgenic potato lines included delayed leaf senescence and altered timing of bud development. Additionally, these transgenic plants did not elevate ROS (H₂O₂) levels in response to temperature stress or upon wounding, compared with the non-transformed wild-type potato. These properties were accompanied by dampened levels of gene markers for HR, ROS and senescence in the *msrA3*-transgenic plants [181]. Also apparent was that these transgenic potato plants were mitigated in normal jasmonic acid and H₂O₂ signaling observed in the control plants. It was concluded that the lack of oxidative burst, reduced H₂O₂ levels, and early suppression of gene transcription in response to different stressors is an indication that *msrA* functions upstream of these processes, consistent with the suggestion that the stress response pathways converge downstream of the stress recognition patterns [182]. Coincident to these observations was the finding that the potato vegetative phase was prolonged and bud development delayed. Of important agronomic consequence was the fact that the *msrA3* transgenic plants had significantly higher yield (52–57%) compared to the control plants.

The expression of *msrA3* in potato suppressed ROS (and HR) and prevented the induction of a number of gene transcripts, characteristics associated with extended vegetative growth, delayed floral development, and higher tuber yield. By extrapolation to studies in the literature, the delayed allocation of resources for reproductive growth seemed to translate into an increased tuber yield in the transgenic potato. Therefore, a dual action of *msrA* involving containing pathogen growth and maintaining a lower basal oxidative stress may contribute to enhanced productivity in plants. Since resource reallocation involves a global shift in the levels of hormones IAA and GA and/or nutrient balance, one can assume that *msrA* function may influence these processes. A stress environment induces a higher threshold of ROS, which in plants modulates development, signaling the stressed

plant to grow rapidly, flower early and even shorten the grain filling period in field crops to complete the life cycle. Such a redirection of nutrient flow from vegetative organs to reproductive growth seems to be the norm during a plant's transition from vegetative to reproductive growth. It is also known that generation of ROS-mediated HR (as a response to a stress or a pathogen attack) causes a shift in cellular metabolism for resource re-allocation, involving global changes in gene expression. Thus, a heightened defense response of a plant contributes to the fitness cost, as seen during JA-dependent defense against herbivores and pathogenesis.

5. Conclusions

Feeding the world's future population will place unprecedented demands on agriculture, necessitating a dramatic increase in food production while at the same time decreasing the negative impacts of agriculture on land, water, and climate [2]. Next-generation cropping systems employing sustainable aspects of existing conventional and organic cropping systems can decrease the impact of agriculture on the environment. Cover crops, in addition to environmental benefits such as reducing erosion, can be used for weed and pathogen control decreasing the need for synthetic pesticides and tillage. Cover crops can also be used for managing soil fertility decreasing the need for synthetic N fertilizer inputs. Likewise, beneficial microbes can be used for control of pathogens and for improving soil fertility. As illustrated here, next-generation cropping systems can also have beneficial impacts on crop plant physiology. Vetch-grown tomatoes were delayed in senescence, producing fruit for extended periods, and induced in expression of genes involved in plant disease resistance. Unfortunately, the use of these cover crops and plant-beneficial microbes only leads to yields that approximate, or are attempting to approximate, yields already obtained using synthetic pesticides and fertilizer. To increase yields we will need to develop better performing crop cultivars using all available technologies, including genetic engineering.

Genetic improvement of plants through biotechnology to tolerate, or be resistant to, abiotic and biotic stresses will be a key component of future global food security as these stresses can result in substantial yield losses globally each year [183]. Current high-yielding crop varieties only yield well under ideal environmental conditions with high pesticide and fertilizer inputs. A second Green Revolution is needed where crops are developed that yield well under environmental extremes with low input of pesticide and fertilizer [184] for use in sustainable crop production systems. Genetic improvement of crops via genetic engineering and other biotechnology approaches will be needed to complement, or replace, traditional breeding efforts as they make available a broader range of genes such as AMPS, etc. and can be used in a more precise manner than traditional breeding [184,185]. In addition to being more time consuming and limited by diminished gene pools, traditional crop breeding methods are subject to linkage drag. As illustrated here, novel approaches are available using genetic engineering of crop plants to enhance abiotic stress tolerance and increase resistance to biotic stress. Examples of commercial success of genetic engineering for crop resistance to stress include Bt cotton, corn, and other crops [186].

More nutritious foods are also needed. Although there appears to be an impact of cropping system on nutritional content of food the perception that organic foods are more nutritious than conventionally produced food is the subject of debate. Some reviews and meta-analyses found some evidence of organic food being more nutritious in certain cases, however, there were questions as to whether the differences in nutritional content were meaningful. Other studies concluded that there were no meaningful differences in nutritional content between organically produced and conventionally produced food [24,187]. Therefore, substantial improvement of the nutritional content of food will likely require new germplasm generated using advanced biotechnological approaches. As pointed out with the tomato-hairy vetch system added nutritional benefit can be obtained combining genetically modified crops with the appropriate sustainable crop production strategy.

It should be noted that approaches for sustainable intensification described here relate primarily to regions of the world with sophisticated agricultural production systems. Countries that have not

yet achieved food security face different challenges than the those with sophisticated agricultural production systems. In these countries crops are typically produced by farmers on small parcels of land not suitable for advanced agricultural technologies. However, increasing yield may be as simple as obtaining improved seed and fertilizer, or outreach providing informed advice on appropriate rates of fertilizer [188]. Clearly, to meet the challenge of sustainably feeding the future world population many different regional-specific approaches need to be developed and implemented.

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