Book Chapter

Advanced Breeding Tools in Vegetable Crops

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Abstract

Vegetables are key ingredients in a well-balanced nutritious diet. Their worldwide rising consumption reveals the awareness of their health benefits. The major biotic factors affecting vegetable production are pathogens causing diseases, insects and nematodes pests, and weeds. Vegetables are also sensitive to drought, flood, heat, frost and salinity. Plant breeding provides means for introducing host plant resistance, adapting crops to stressful environments, and developing cultivars with the desired produce quality. The genetic enhancement of vegetables aims

achieving the market-driven quality along with agronomic performance needed by growers. Trait heritability, gene action, number of genes controlling the target trait(s), heterosis and genotype × environment interactions determine the vegetable breeding method to use. Coupled with the use of dense DNA markers and phenotyping data, quantitative genetic analysis facilitates dissecting trait variation and predicting merit or breeding values of offspring. Genomics, phenomics and breeding informatics further facilitate screening of target characteristics, thus accelerating the finding of desired traits and contributing gene(s) in vegetables. Genomic estimated breeding values are used today for predicting traits, thus replacing the routine of expensive phenotyping with inexpensive genotyping. Genetic engineering protocols for transgenic breeding are available in various vegetables, and may be useful if target trait(s) are unavailable in genebank or breeding population. Transgenic cultivars could overcome some limiting factors in vegetable production such as pathogens, pests, and weeds, thus reducing pesticide residues, human poisoning and management costs in horticulture. Gene editing can be also a useful approach for improving traits in vegetables and speed breeding. Examples are taken from various vegetables (including root and tuber crops) to show how these advances translate in genetic gains and save time and resources in their breeding.

Keywords

Breeding; Vegetables; Vegetable Breeding Strategy; Conventional Selection; Genetic Engineering; Transgenic Breeding; Cisgenesis; Gene Editing; Genomics-Led Breeding; Marker-Assisted Selection; Genomic Prediction

Vegetable Worldwide Overview

Vegetables are key ingredients in a well-balanced nutritious diet since they supply bioactive compounds such as dietary fiber, essential vitamins and minerals, and phytochemicals [1-3]. They are associated with human disease prevention by improvement of gastrointestinal health, good vision, and reduced risk of chronic and degenerative diseases such as cardiovascular diseases,

certain cancers, diabetes, rheumatoid arthritis and obesity [3]. Their worldwide rising consumption reveals this awareness of their health benefits.

A world vegetable survey showed that 402 vegetable crops are cultivated worldwide, representing 69 families and 230 genera [4]. Leafy vegetables – of which the leaves or young leafy shoots are consumed– were the most often utilized (53% of the total), followed by vegetable fruits (15%), and vegetables with below ground edible organs comprised 17%. Many vegetable crops have more than one part used. Most of the vegetables are marketed fresh because they are perishable. Consumption shortly after harvest guarantees optimal vegetable quality. Asia produces and consumes more than 70% of the world's vegetables. The per capita consumption of vegetables in Asia, has increased considerably in last 20 years. The main factors for this increase were the rapid growth in mean per capita incomes, and awareness of nutritional benefits.

Vegetable production suffers from many biotic stresses caused by pathogens, pests, and weeds and requires high amounts of pesticides per hectare. Pest loads vary and are complex *vis-à-vis* field crops because of the high diversity of vegetable crops and due to their cultivation intensity. Until now the main method for controlling pathogens, pests, and weeds has been the use of pesticides because vegetables are high-value commodities with high cosmetic standards. Synthetic pesticides have been applied to vegetable crops since the 1950s, and have been highly successful in reducing crop losses to some insects, pathogens, and weeds. Vegetables account for a significant share of the global pesticide market. Insecticides are regularly applied to control a complex of insect pests that cause damage by feeding directly on the plant or by transmitting pathogens, particularly viruses. Despite pesticide use, insects, pathogens, and weeds continue to cause a heavy toll on world vegetable production. Pre-harvest losses are globally estimated as 15% for insect pests, 13% for damage by pathogens, and about 12% for weeds. Pest and viruses are particularly important in tropical and subtropical countries like many in Southeast Asia. Pesticide residues can affect the health of growers and consumers and contaminate the

environment. Vegetables are often consumed in fresh form, so pesticide residue and biological contamination is a serious issue. Consumers worldwide are increasingly concerned about the quality and safety of their food, as well as the social and the environmental conditions under which it is produced. Vegetables are also sensitive to drought, flood, heat, frost and salinity.

Plant breeding provides means for introducing host plant resistance, adapting crops to stressful environments, and developing cultivars with the desired produce quality. The genetic enhancement of vegetables aims achieving the market-driven quality along with agronomic performance needed by growers.

Crossbreeding Methods Summary

Introduction

As indicated by Ortiz [5], cultivars of self-fertilizing vegetables such as tomato may be inbred lines or hybrids. The methods for their crossbreeding are mass selection, pedigree, bulk, single seed-descent, doubled haploids, backcrossing hybridization and population improvement through recurrent selection. Pedigree is still the main breeding method though hybrid and populations improvement methods are also used. Inbred lines are nearly homozygous due to long inbreeding by forced self-pollination or sib-mating. These inbred lines can be used in genetic research (e.g. mapping genes and quantitative trait loci), allele discovery, and directly as cultivars in self-fertilizing vegetables or as parents of hybrids and synthetic cultivars. Outcrossing vegetables such as cucurbits or onions show mild to severe inbreeding depression and significant heterosis, which should be managed while developing composite, hybrid and synthetic cultivars [5]. Inbred line development, population improvement both facilitated today by DNA marker-aided breeding are used for the genetic enhancement of outcrossing vegetables. Mutation and genetic recombination allow breeding asexual root and tuber crops such as potato, cassava, sweet potato and yam [6]. Analytical breeding through ploidy manipulations leads to broadening of the genetic base of these crops.

Selection in a genetically variable population according to the

phenotype remains a key feature of vegetable breeding. It leads to adaptation in the local environment after selecting repeatedly for the target trait across growing seasons if the source breeding germplasm had genetic variability for it, particularly for characteristics significantly influenced by the environment such as edible yield, host plant resistance or produce quality. Quantitative genetics provides a model to study how many genes and non-genetic factors affect adaptive traits to climate change, thus assisting on their understanding for further use according to the various vegetable breeding methods. Trait heritability, gene action, number of genes controlling the target trait(s), heterosis and genotype × environment interactions determine the breeding method to use. Coupled with the use of dense DNA markers and phenotyping data, quantitative genetic analysis facilitates dissecting trait variation and predicting merit or breeding values of offspring. DNA marker-aided breeding and genomic selection may be also used for breeding self-fertilizing species such as tomato, which may be a model plant system for the genetic enhancement of other vegetables with alike breeding systems. *Ex ante* and *in silico* assessments may assist determining the best approach, method or technology before incorporating them into a vegetable breeding program.

The release of hybrid cultivars is among the main achievements of vegetable breeding based on exploiting heterosis, which led to significant edible yield increases [5]. In the F_1 hybrid the undesirable (often deleterious) recessive alleles from one parent are suppressed by the dominant allele of the other parent. An alternative theory regarding this outbreeding enhancement or hybrid vigour i.e, the heterozygote being superior to either homozygote parent. The biochemical, physiological and molecular basis of hybrid vigour remain however elusive. Genetic diversity and distance among breeding lines and their correlation with hybrid performance may define heterotic groups and assist predicting hybrid yield. When combining ability information lacks, knowledge on the relationship among genotypes aids to select parents for further crossing. There are some self-fertilizing vegetables with successful F_1 hybrid cultivars, e.g. tomato. Their use depends on the added value given by heterosis and efficient pollination mechanisms to justify

the development and production costs of hybrid seed. Cytoplasmic and genic male sterility or self-incompatibility provide means for producing hybrid seed in various self-fertilizing vegetables.

Hybrid-Enabled Line Profiling (HELP) is a new integrated breeding strategy for self-fertilizing crops, which combines existing and recently identified elements resulting in a strategy that synergistically exceeds existing breeding concepts. HELP integrates modern high-throughput versions of existing and new concepts and methodologies into a breeding system strategy that focuses on the most superior crosses, less than 10% of all crosses. This focus results in significant increases in efficiency, and can reverse the edible yield plateauing seen or feared in some of our major selfing food crops [7].

Orange-Fleshed Sweetpotato: A Success Story

Protein-energy malnutrition and micronutrient deficiency are among public health problems leading to learning disability, impaired work capability, illness, and death. Improving the nutrient content of staple food crops through crossbreeding represents a sustainable way to alleviate micronutrient malnutrition, e.g. corneal blindness owing to vitamin A deficiency. As noted by Ortiz [8], cultivars grown in distinct locations can be assessed for ß-carotene content to identify those with high in micronutrient content. A plant breeding program for vitamin A needs to assess the occurrence of its deficiency in target areas and provide the best germplasm to farmers in each location to address it accordingly. Breeding targets in the outcrossing hexaploidy root crop sweetpotato are increasing storage root yields, improving quality, enhancing host plant resistance to pathogens and pests, and bettering adaptation to drought. Poly-cross breeding is the most used population improvement method in sweetpotato, which allows increasing the frequency of favourable alleles in the population from which outstanding clones can be selected for cultivar development. Several dozen of sweetpotato cultivars were released in Africa in the last two decades, many of which have an orange flesh: 15 out of 56 cultivars releases from 1993 to 2003, and 62 out of 89 from

2004 to 2013, had orange flesh [9]. A cooperative breeding and cultivar delivery project involving 250 other partners in Mozambique was able, after multi-site and on-farm testing, to bring selected planting materials of orange-fleshed sweetpotato (OFSP) with high storage root yields to 122,216 households across the country by the end of 2001 after a devastating flood which displaced 450,000 people [8]. A preliminary impact assessment noticed a return rate of US$ 4 for each US$ 1 project grant just after two years of the scaling-up for technology exchange in this project. As a result of tireless and convincing public health campaigns –using bright orange clothing and trucks painted with slogans promoting the high β-carotene sweetpotato cultivars– OFSP are found today along the roads in Mozambique – a country where 70% children still suffer from vitamin A deficiency.

Genetic Engineering for Improving Vegetables

Transgenic Breeding

Introduction

Recently Kyndt et al. [10] found that cultivated sweetpotato is a natural transgenic crop since the genome of cultivated sweetpotato contains *Agrobacterium* T-DNA with expressed genes. The fixation of foreign T-DNA into the sweet potato genome occurred during the evolution and domestication of this crop. The natural presence of *Agrobacterium* T-DNA in sweet potato and its stable inheritance during evolution is a nice example of the possibility of DNA exchange across species barriers. This finding could influence the public's current perception that transgenic crops are unnatural.

Some transgenic field crops such as maize, canola or oilseed rape, soybean and cotton, are grown today by, or available to, farmers, particularly in North America, the Southern Cone of South America, South Africa, South Asia, China, the Philippines and Australia [11]. Horticulture remains in the infancy regarding the use of transgenic crop technology because vegetables are minor crops compared to field crops, due to the lower resources invested –especially by the multinational private seed sector, and derived of the high costs of deregulation. [12,13]. Horticulturists

have been working on the genetic engineering of vegetables but many of them yet to reach end-users [12]. Dias and Ortiz [12] did a literature review based on 372 articles about the status of transgenic vegetables to improve their production and nutritional quality. They analysed the progresses and potentials in transgenic research until 2010 on tomato, potato, eggplant, summer squash, watermelon, cucumber, melon, brassicas, lettuce, alliums, carrot, cassava, sweet potato, sweet corn and cowpea. They observed that some experimental transgenic vegetables show enhanced host plant resistance to insects and plant pathogens (including viruses), slow ripening that extends the shelf-life of the produce, herbicide tolerance, high nutritional status, seedless fruit and increased sweetness, or can be use for vaccine delivery.

Transgenic cultivars could overcome some limiting factors in vegetable production as pathogens (bacteria, fungi, viruses), pests (insects and nematodes), and weeds, reducing pesticide residues, human poisoning and management costs in horticulture. Transgenic vegetables with tolerance to abiotic stresses or enhanced input efficiency could also provide various benefits to farmers and the environment. Consumers could also benefit further from the use of more nutritious transgenic vegetables. Likewise, food safety can be enhanced through transgenic approaches. This section highlights advances in breeding transgenic vegetables, and issues affecting their use, as illustrated by the case studies of tomato, potato, eggplant, summer squash and sweet corn.

Tomato: Delaying Fruit Ripening

The first commercially grown transgenic crop worldwide was Flavr Savr™ tomato, which was released in the USA by Calgene in 1994 [14]. This tomato contains an antisense version of the *poligalacturonase* (*PG*) gene. The use of this gene ensued after many years of research on several genes involved in fruit development and tomato ripening. They were identified, cloned, and characterized to breed transgenic tomato cultivars [15]. By suppressing enzyme activity, the tomatoes expressed delayed ripening, thus enabling them to be picked when vine-ripe. It was

sold with a clear label "Genetically engineered" and was initially a success story. At the same time (1996 to 1999) canned GM tomatoes sold by Zeneca, under licence of Calgene, were introduced in the United Kingdom as paste from these tomatoes [16]. The grocery chains Sainsbury and Safeway sold 1.8 million cans. Following publication of scare stories GMOs "Frankenfood" the cans were removed from the shelves. Production in the US market was later discontinued following purchase of the Calgene by Monsanto.

There has been further research conducted to manipulate fruit ripening, texture and nutritional quality using transgenic approaches. Many of the genes targeted include ethylene because of its role in fruit ripening. Enzymes that regulate ethylene biosynthesis in plants are S-adenosylmethionine (SAM) synthase, 1-aminocyclopropane-1-carboxylate (ACC) synthase, and ACC oxidase. The genes encoding these enzymes as well as those that metabolize SAM or ACC have been targeted in order to manipulate ethylene biosynthesis and thereby to regulate fruit ripening. It has been clearly demonstrated that modulation of ethylene biosynthesis using genetic engineering can yield tomato fruits with predictable ripening characteristics. However, ripening of tomato has been shown possible by introduction anti-ripening genes, *rin* and *nor*, in heterozygous form and these genes have been incorporated in many fresh and processing tomatoes.

Potato: Host Plant Resistance to Insects and Viruses and Less Acrylamide during Frying

Potato is the world's most important vegetable crop, with nearly 400 million t produced worldwide every year. *Bt*-potato cultivars, obtained from Russet Burbank cultivar and containing the CryIII gene, and expressing resistance to Colorado potato beetle (CPB; *Leptinotarsa decemlineata*) –the most destructive insect pest of potato in North America– and aphids –associated with *Potato virus Y* and *Potato leafroll virus*– were approved for sale in the United States in 1995. NewLeaf™, NewLeafY™, and NewLeafPlus™ were the trade names of the transgenic potato cultivars sold by NatureMark –a subsidiary of Monsanto [17].

About 600 ha were grown commercially when NewLeaf™ cultivars were introduced in 1995 in Pacific Northwest US, and the commercial acreage reached rapidly about 20,000 ha in 1998 because they quickly became popular among growers since the product was very effective at preventing CPB damage. Market success of the NewLeaf™, NewLeafY™, and NewLeafPlus™ potatoes could be attributed to the difficulty in controlling CPB, in regions like Pacific Northwest with mild winters, where CPB was a problem, and also where there are high pest populations of aphids associated with virus problems [18]. This virus resistance benefited seed producers, while commercial growers benefited from higher yields and reduced need for insecticides [18].

The reported profits in USA were on average US$ 55 ha^{-1} for *Bt*-potato [19]. Likewise, an ex-ante analysis suggested an average profit of US$ 117 ha^{-1} for virus-resistant potato in Mexico [20]. The processing industry and consumers benefited from improved quality. Potatoes were one of the first foods from a transgenic crop that was commonly served in restaurants. NewLeaf™ potato cultivars were the fastest cultivar adoption in the history of the USA potato industry [18], until potato processors, concerned about anti-biotech organizations, consumer resistance and loss of market share in Europe and Japan, suspended contracts for *Bt*-potatoes with growers in 2000 [21]. The North American fresh market continued to accept transgenic potatoes, but with processed potato markets closing growers became reluctant to take on the risk of planting biotech potatoes [22]. The major impact came when the leading fast food McDonald's chain, concerned about anti-biotech organizations, decided to ban transgenic potatoes from its servings. Surrendering to dwindling marketability for their products, Monsanto closed its NatureMark potato business in the Spring of 2001 [22].

At about the time that Monsanto withdrew from the biotech potato business, the Idaho-based J.R. Simplot Company (Simplot) began efforts on potato product development through genetic engineering, testing, and regulatory submissions [22]. Learning from the marketing difficulties encountered by Monsanto, Simplot focused on consumer traits rather than producer traits for its first biotech potato. Simplot also used only

potato genes for trait introgression (i.e., cisgenesis) in order to address the public's concerns regarding biotech food safety. One of the first consumer traits focused on by Simplot was potatoes that had a lower propensity for the formation of acrylamide, a substance linked to birth defects and cancer in mice and rats and so a probable human carcinogen [23]. Since 2002 it has been known that it can result during boiling or frying some kinds of starchy food [24]. So reduced levels of it in fried potato, cooked at high temperatures is desirable.

Anticipating the need for low-acrylamide raw product for its potato processing business [25], Simplot successfully developed potatoes with a lower potential for producing acrylamide. A second consumer trait of interest to Simplot was black spot bruise resistance, which could reduce food waste during processing and open new avenues for marketing fresh cut potatoes. The genetically modified Innate™ 1.0 potato, developed by J.R. Simplot Company, received deregulation from the USDA in 2014 and was approved by the US Food and Drug Administration in 2015 [22]. The cultivar Innate™ 1.0 it is designed to resist to blackspot bruising, browning and to contain less of the amino acid asparagine that turns into acrylamide during the frying of potatoes. The Innate™ 1.0 potato name comes from the fact that this cultivar does not contain any genetic material from other species (the genes used are "innate" to potatoes) and uses RNA interference to switch off genes (silencing target genes with the aid of RNAi methods). Simplot expects that by not including genes from other species will assuage consumer fears about biotechnology.Five different potato cultivars have been transformed, producing Innate second generation versions with all of the original traits, plus the engineered ones [22]. 'Atlantic', 'Ranger Russet', 'Russet Burbank' potato cultivars have all been transformed by Simplot, as well as two proprietary cultivars. Modifications of each five cultivars involved two transformations, one for each of the two new traits, thus there was a total of 10 transformation events in developing the different Innate cultivars. In May 2015, the Innate™ 1.0 potatoes entered the fresh and chip market channels as a limited commercial launch. Simplot implemented a directed marketing stewardship program to keep the biotech potatoes out

of the dehydration and frozen processing market channels. The company also submitted a petition to USDA for Innate™ 2.0 potatoes that have the same 1.0 traits but add late blight (*Phytophthora infestans*) resistance and cold storage capability. They achieved resistance to late blight by transfer of a gene from the wild American species *Solanum venturii*. McDonald's is a major consumer of potatoes in the US. The Food & Water Watch movement has petitioned the company to reject the newly marketed Innate potatoes. McDonald's has announced that they have ruled out using Innate™.

Eggplant: Resistance to Fruit and Shoot Borer

Eggplant, or brinjal as it is called in South Asia, is one of the most important and popular vegetables in South and Southeast Asia where it is grown by hundreds of thousands of smallholder farmers. Eggplant is attacked by a number of insects including thrips, cotton leafhopper, jassids and aphids. But the most devasting and economic damaging pest is the eggplant fruit and shoot borer (FSB, *Leucinodes orbonalis*). The caterpillar damages eggplant by boring into the petiole and midrib of leaves and tender shoots, resulting in wilting and desiccation of stems. Larvae also feed on flowers, resulting in flower drop or misshapen fruits. But the most serious economic damage is to the fruit, because the holes, feeding tunnels, and larval excrement may make the fruit unmarketable and unfit for human consumption. FSB poses a serious problem because of its high reproductive potential, rapid turnover of generations and intensive damage during the wet and dry seasons. Losses have been estimated to be between 54 and 70% in India and Bangladesh and up to 50% in the Philippines, even after repeated insecticide sprays [26]. There are no known eggplant cultivars resistant to FSB, so the use of insecticide sprays continues to be the most common control method used by growers. The borer is vulnerable to sprays only for a few hours before they bore into the plant, which explains why growers often spray every other day, particularly during the fruiting stage. In Bangladesh conventional brinjal farmers can spray as many as 84 times during the cropping [27]. Consumers have generally no choice to buy insect-damage and infested fruits or those with high

pesticide residues. Application of frequent insecticide sprays results also in a high pesticide exposure for farmers and sometimes this can be associated with recurring health problems.

FSB-resistant *Bt* eggplant was genetically engineered by Mahyco (Maharashtra Hybrid Seed Company, India) under a collaborative agreement with Monsanto and the first *Bt* transgenic eggplant with resistance to FSB was produced in 2000. This GM eggplant incorporates the *cry1Ac* gene expressing insecticidal protein from *Bacillus thuringiensis* (the same protein has long been used by organic growers), that confers resistance against FSB. This *Bt*-eggplant was effective against FSB, with 98% insect mortality in *Bt*-eggplant shoots and 100% in fruits compared to less than 30% mortality in non-*Bt* counterparts [28].

In 2005, to help give farmers another option instead of insecticide sprays, the Bangladesh Agricultural Research Institute (BARI) and partners (Mahyco, Cornell University, USAID, and public sector partners in India, Bangladesh and the Philipines) began the hybridization of nine Bangladeshi brinjal cultivars with *Bt*-eggplant of Mahyco. The resulting F_1 seeds were collected and a backcrossing programme was initiated in 2006. Multi-location confined field trials evaluating the performance of *Bt*-lines were made at seven locations of Bangladesh. In 2014, four *Bt*-brinjal lines were released and distributed to 20 farmers in four districts making Bangladesh a pioneer in the world to allow the commercial cultivation of a genetically engineered vegetable crop developed in the public sector [29]. In two-year field trials (2016 and 2017) scientists compared the four released *Bt*-brinjal cultivars with their non-*Bt* equivalents [27]. The results showed that the *Bt* gene is almost 100% effective in protecting against FSB without any need for insecticide sprays. Research reported 0 to 2% infestation in *Bt*-brinjal cultivars, as compared to from 37 to 46% infestation in non-*Bt* isolines (i.e., the same cultivars but without *Bt* gene). Results after field trials show that Bangladeshi smallholder farmers can be better off economically using *Bt*-brinjal cultivars than the conventional alternative.

An economic analysis [27] revealed that all *Bt*-brinjal cultivars had higher gross returns than their non-*Bt* isolines. The non-sprayed non-*Bt* isolines resulted in negative returns in most cases. Even when the non-*Bt* lines were sprayed, only two of them showed a profit, but it was lower than the *Bt*-brinjal cultivars. Another socio-economic study was conducted in 35 districts of Bangladesh during 2016-2017 [30]. It showed that farmers using Bt-brinjal have 13% higher yield compared to farmers not using this technology, and that farmers growing B*t*-brinjal also had significantly higher gross return (21%) and net income (83%) than farmers not using such a gemplasm. The total variable cost and fixed costs were also lower for farmers growing *Bt*-brinjal compared to those not using it. Based in these results adoption of growing *Bt*-brinjal cultivars has increased dramatically since 2014 (four farmers) with more than 27,000 farmers across Bangladesh in 2018. When comparing the arthropod communities in *Bt* and non-Bt brinjal, there were any differences in either non-target pest species or beneficial species, thus suggesting that the four *Bt*-brinjal cultivars control FSB, without disrupting arthropod biodiversity [27]. Hence, it appears that arthropods such as whiteflies, mites, jassids and aphids, none of which are susceptible to *Cry1Ac*. However, insecticide sprays did have a disruptive effect on some species of beneficial arthropods [27].Overall, research-for-development results support the case for utilizing *Bt*-eggplant to control FSB and dramatically reduce insecticide use while increasing the economic return for resource-poor farmers in Asia. Pesticide residues will therefore be much lower on the *Bt*-brinjal crop. Besides farmers can keep their own seeds for next season because cultivars are not hybrid.

Summer Squash: Multiple Virus Resistance

Viruses cause 20 to 80% of yield losses in summer squash in the USA [31]. Three of the most important viruses affecting summer squash production are *Zucchini yellow mosaic virus* (ZYMV), *Watermelon mosaic virus* (WMV), and *Cucumber mosaic virus* (CMV). Summer squash cultivars with satisfactory resistance to CMV, ZYMV, and WMV are yet to become available from cross-breeding [32]. Two lines of squash expressing the coat

protein (*CP*) gene of ZYMV, WMV and CMV were deregulated and commercialized in 1996. Subsequently, many squash types and cultivars have been bred, using crosses and backcrosses with the two initially deregulated lines. This material is highly resistant to infection by one, two or all three of the target viruses [33-38]. Virus-resistant transgenic squash limits virus infection rates by restricting challenge viruses, reducing their titers, or inhibiting their replication or cell-to-cell or systemic movement. Therefore, lower virus levels reduce the frequency of acquisition by vectors and subsequent transmission within and between fields. Consequently, virus epidemics are substantially limited. The adoption of virus resistant squash cultivars has steadily increased in the United States since 1996. This adoption rate was estimated at 12% (approximately 3,100 ha) across the country in 2005 [39]. Virus-resistant transgenic squash has allowed growers to achieve yields comparable to those obtained in the absence of viruses with a net benefit of US$ 22 million in 2005 [39]. Engineered resistance has been so far the only approach to breed summer squash cultivars with multiple sources of resistance to CMV, ZYMV, and WMV.

Sweet Corn: from *Bt* to Triple "Stack" Transgenic Cultivars

The global retail value of sweet corn, baby corn and green maize is US$ 13 to 32 billion, thus ranking second after tomato (US$ 56 billion) among vegetables, and compares favourably to watermelon, onions and Brassicas –each worth about US$ 18 billion [40]. Sweet corn appears as the most popular specialty maize due to its high sugar content, conferred by the homozygous recessive *sugary-1* (su_1) genes, in the kernels at the milky stage, which allows its harvest as vegetable. Sweet corns combining the recessive allele *sugary-enhancer* (*se*) together with su_1 can show twice the sugar content and phytoglycogen levels, thereby conferring a creamy texture.

Sweet corn, expressing *Cry1Ab* endotoxin, was introduced commercially in the United States in 1998 into an industry that is highly sensitive to damage to corn ears from lepidopteran pests [41]. This endotoxin was very effective against the European corn borer (*Ostrinia nubilalis*) in the state of New York,

providing 100% clean ears when no other lepidopteran species were present and in excess of 97% when the two noctuids, corn earworm *(Helicoverpa zea)* and fall armyworm *(Spodoptera frugiperda)*, were also present [42]. Studies in other states in the USA have shown that *Bt*-sweet corn provided consistently excellent control of the lepidopteran pest complex and the potential for 70 to 90% reductions in insecticide requirement [41-46]. About 5% of the 262,196 ha of sweet corn (fresh and processing) grown in the United States in 2006 was with *Bt*-sweet because corn processors have avoided growing *Bt*-sweet corn due to concerns about export markets [47]. Since then it has been grown only as a fresh market vegetable crop.

An economic assessment in Virginia found a gain of US$ 1,777 ha^{-1} for fresh-market sweet corn vs. non-*Bt*-sweet corn sprayed up to six times with pyrethroid insecticides [45]. *Bt*-sweet corn was also much better at preserving major predators of *O. nubilalis* while controlling the European corn borer than were the commonly used insecticides lambda-cyhalothrin, indoxacarb and spinosad. *Bt*-sweet corn hybrids can be therefore truly integrated into a biological pest control program. Speese et al. [45] concluded that *Bt* sweet corn is an effective and economically sound pest management strategy for growers in Virginia.

In 2011, Monsanto announced the release, through its vegetable seed brand Seminis, of a "triple-stack" transgenic sweet corn with host plant resistance to insects and that also tolerates glyphosate sprays for weed control [48]. They expect that this transgenic sweet corn provides protection against damage by European corn borers, corn earworms, fall army worms and corn rootworm larvae, and reduces insecticide sprays up to 85% (vis-à-vis. a non-transgenic sweet corn). The use by farmers of this insect-resistant sweet corn that tolerates glyphosate can also result in eco-efficiency because of less tractor trips across the field that help farmers to save fuel, thereby reducing greenhouse gas emissions and decreasing the carbon footprint per ear of corn grown.

Gene Editing

Gene editing or genome editing, is a new type of genetic engineering based in editing or deleting a genetic sequence. It can revolutionize vegetable breeding since it makes possible to precisely alter DNA sequences. At least five technological variants have been developed. Currently the most popular gene editing is CRISPR-Cas9 system [49,50], due to its ability to more accurately and efficiently insert and turn off desired traits. CRISPR, which stands for Clustered Regularly Interspaced Short Palindromic Repeats, is a natural bacterial defense system. CRISPR defends cells by identifying the DNA of invading viruses and, together with a protein made by bacteria Cas9, slicing parts out of the virus to deactivate it—like a pair of DNA-cutting scissors [51]. These systems, have been likened to a "biological word processing system" that allows scientists to cut and paste DNA sequences almost as easily as if they were editing a journal article in a computer. Hence, gene editing is similar to conventional breeding, since it can be used to introduce genetic variation, but faster, cheaper and more precise. Scientists believe that gene editing will enable numerous useful applications in agriculture and will speed breeding since they can, more accurately and efficiently pinpoint, remove genes or insert desired traits like drought and disease-resistance already found elsewhere in a plant species. Although gene editing can involve transgenics (the moving of genes from one species to another) it usually does not, thus diminishing the criticism from some anti-biotechnology experts who believe transgenics violate the 'natural order'.

A briefing on Genomics-Led Breeding

Introduction

Microsatellites (SSR) and single nucleotide polymorphisms (SNPs) are today amongst the most widely used DNA marker systems, while new generation sequencing starts providing access to more DNA landmarks for vegetable breeding [5]. DNA markers allow selecting directly or indirectly genes rather than solely based on phenotypes, and may reduce the time for assembling favourable alleles in doubled-haploids (DHs), near-

isogenic lines or recombinant-inbred lines. DHs along with DNA marker-aided breeding offer a short cut for backcrossing because they are fertile and homozygous at all loci in a single step [52]. Moreover, genomic prediction based on genotyping and along with genome-wide SNPs, pedigree and phenotypic data is a very powerful tool to capture small genetic effects dispersed over the genome, which allows predicting an individual's breeding value. Furthermore, this approach of genomic prediction to estimate breeding values (GEBV) for selection may decrease time, increase intensity, and enhance efficiency for low heritability traits [53]. GEBV will also allow screening in early generations, improving the precision of field trials, selecting across segregating hybrid offspring, adapting computation breeding, and catalyzing the reorganization of vegetable breeding. GEBV are used today for predicting traits, thus replacing the routine of expensive phenotyping with inexpensive genotyping. GEBV are based on both the reference (or training) population and a target population of environment used for evaluating this training set, and on including data from diverse geographical locations and genetic clusters. Genomic prediction may be also integrated into the evaluation of germplasm with a broad genetic base to identify in genebanks useful genetic diversity for further use in vegetable breeding. Predictive models will assist in selecting genebank accessions for introgressing useful genetic variation into breeding populations.

High-throughput precision phenotyping is rapidly becoming popular in crop breeding for its ability to facilitate measurement of data points from a wide spectrum of light reflectance wave lengths. The obtained data points if correlate well with a phenotypic trait of interest enables the use of this technology in evaluation of several horticultural important plant phenotypes. Vegetable breeding should therefore move from phenotyping screening to phenomics, high throughput field omics and e-typing in managed environments for accurate and fast genetic gains when pursuing knowledge-intensive, genomic-led approach. Partnerships are a must because sensor-based phenotyping or image analysis, and data standards are still under development, thus networking is a key activity for both learning and sharing facility use for vegetable research and breeding.

Greenhouse or growth chamber-based methods that enable 4 to 6 crop generations within a year will facilitate genomic selection in vegetable breeding.

DNA markers Facilitate Diversity Analysis and Breeding in Tomato

Genomics, phenomics and breeding informatics facilitate screening of target characteristics, thus accelerating the finding of desired traits and contributing gene(s). Research based on both genome and phenome led to understanding the evolution and domestication trends of tomato (Ortiz, [5] and references therein), and provided further insights on how to breed tomato. Tomato should be also regarded among the first plant species for understanding the genetics and molecular biology of quantitative trait variation. DNA marker-aided breeding was used for introducing and pyramiding host plant resistance in tomato, while next generation sequencing led to identifying SNPs for their further use in high throughput genotyping on tomato species, cultivars and segregating offspring or to identify tomato cultivars, testing hybrid purity, compare genetic and linkage maps, and to verify phylogenetic relationships. of the phenotypic variability and genetic diversity are important steps for the utilization of genetic resources by tomato breeding aimed at sustainability and resilience. Integrative genomic research facilitates the management of useful variation for genetic improvement of tomato. Diagnostic DNA markers enabling tomato breeders to predict phenotype from seeds or seedlings will be very valuable tools.

Genomic resequencing of various landraces or cultivars and pangenomics lead to discovery of novel alleles using bioinformatics along with genetics. It also allows genes that may have been lost during domestication to be identified and used in the development of new breeding lines, including genes restricted to exotic germplasm. For example, a tomato pan-genome based on genome sequences from 725 phylogenetically and geographically representative accessions, permitted recently to find a rare allele in the TomLoxC promoter selected against during domestication [54]. Further research shows role for

TomLoxC in apocarotenoid production, which contributes to desirable tomato flavor.

Genomic Prediction for Selection in Potato

Potato is a model plant for the genetic enhancement of polysomic polyploid vegetables, including root and tuber crops. Potato is a vegetatively propagating crop in which each tuber is identical with its mother plant, thus allowing that favorable traits are fixed in the F_1 hybrid generation. Potato breeding is a phenotypic process combining market-driven quality with agronomic performance and host plant resistance needed by growers [55]. It takes 10 years to select a cultivar, and 5 to 10 years to select parents for a potato breeding program. The probability that a cultivar becomes registered is 1:10000 seedlings/year after crossbreeding within the cultigen pool, and 1:100000 seedlings year^{-1} if crossing involves wild species. Marker-aided breeding in potato has been used for selecting a few host plant resistance genes with major effects, e.g. for cyst nematode and *Potato virus Y* (Ortiz, [5] and references therein). It also looks very promising for tuber quality features. Marker-aided selection along with estimating breeding values for simplex and complex traits may improve efficiency in potato breeding since it will likely reduce significantly the time for identifying superior germplasm. Dense genetic maps based on SNPs give details about quantitative trait loci (QTL) location and their genetics. The potato genome sequence provides further means for genome-wide assays and tools for gene discovery and enables the development of marker haplotypes spanning QTL regions. They will be very useful in introgression breeding and a whole-genome approach such as GEBV, thus improving the efficiency of selecting elite clones and enhancing genetic gain over time.

GEBV for selection could be incorporated into selection for quantitative traits within the most promising hybrid offspring in potato. Combining marker-aided selection and estimating breeding values for may improve potato breeding efficiency by significantly reducing the cycle length to identify elite germplasm. For example, each year several thousand clones may

become available after raising few hundred seedlings of each of the best dozens of crosses in sufficiently large pots to progress them straight to small plots in the field the following year [56]. This early testing could translate in genetic gains and save time and resources in potato breeding. GEBV models were initially developed for predicting yield in potato with a prediction accuracy of just 20 to 40% [57,58] but a model including additive and dominance effects increased it to 50–80% [59,60]. Although genomic prediction of breeding values appears to be feasible in potato [59,61,62], these predictions across breeding populations still remain unreliable [63], perhaps due to the high allelic diversity in this crop that calls for enlarging the training sets.

Outlook

The approach and methods for the genetic enhancement of vegetables are moving from Breeding 1 (selection with unknown loci) and 2 (selection by controlled crosses) to Breeding 3 (DNA marker-aided breeding), particularly for tomato, potato and cassava. Breeding 4 (ideotype-based selection and transformation), which may increase efficiency, is in its infancy and led by genetic engineering in tomato, eggplant, squash and sweet corn, and genomic selection, especially in cassava, potato and tomato.A stepwise approach for sustainable genetic gains in vegetable crop improvement begins by defining breeding objectives with end-users and developing the ensuing product profile that guides such an undertaking. Identify useful character(s) in breeding population(s) or genebank(s) according to the product profile will be the next step. Thereafter, managing the genetic variation of such useful trait(s) should be based on both genetics and “omics” knowledge with the aim of putting gene(s) into a usable form(s) [i.e., lines, clones, populations] for further use in crossbreeding of target vegetable. Genetic engineering for transgenic breeding or genome editing may be pursued if target trait(s) are unavailable in the genebank or breeding population(s) but where biosafety regulations for GMOs and sound intellectual property management are in place.

The Breeding Program Assessment Tool (http://plantbreedingassessment.org) facilitates the appraisal of *plant breeding* program with the aim of both increasing its efficiency and achieving higher rates of genetic gain. This tool uses a structured evaluation process for evaluating the management and organization of a plant breeding program with a questionnaire and an evaluation visit by a team of cultivar development experts. The evaluation report and scorecard ensuing from this process are thereafter used by the breeding program to develop an improvement plan.

Modern methods and tools to assess and exploit functional diversity in gene pools in allows innovative vegetable breeding under a changing climate. Plant genetic resources remain as raw materials for mining allelic variations associated with target traits. Crop improvement will continue to rely on combining diversity in crop populations via genetic recombination. Unlocking functional diversity using omics, precise high throughput phenotyping and e-typing for key agronomic traits such as crop phenology, plant architecture, edible yield, resilience to changing climate, host plant resistance and input-use efficiency, plus "speed breeding" may further assist germplasm use in genetic enhancement of vegetable crops for this 21st century.

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