Ornamental Horticulture
ISSN 2447-536X | HTTPS://ORNAMENTALHORTICULTURE.EMNUVENS.COM.BR/RBHO
SBFPO Sociedade Brasileira de Floricultura e Plantas Ornamentais

Review Article

Ornamental plant breeding: entering a new era?

Johan Van Huylenbroeck[1]* and Krishna Bhattarai[2]

[1]Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Melle, Belgium
[2]Gulf Coast Research and Education Center, University of Florida, Florida, USA

Abstract

During the last century, plant breeding of crops and ornamentals has become an important business. Mendel's principles of inheritance of traits laid the foundation of today's modern genetics. Although intra- and interspecific cross breeding, chromosome doubling and mutation breeding still form the cornerstone of ornamental plant breeding, the speed of plant breeding has significantly increased since Mendel's time. New genomic tools now offer possibilities for advanced precision breeding and selection towards more difficult to assess or quantitative traits. Today's ornamental plant breeding is a complicated exercise, characterized by new and rapidly-changing challenges. Partnerships between industry and research will be essential to implement available technologies in minor crops.

Keywords: interspecific hybridization, polyploidization, molecular markers, genomics, CRISPR/Cas, mutation breeding, Ri-technology.

Resumo

Melhoramento de plantas ornamentais: entrando em uma nova era?

No último século, o melhoramento de plantas de culturas e de ornamentais tornou-se um negócio importante. Os princípios de herança de características de Mendel lançaram as bases da genética moderna de hoje. Embora os cruzamentos intra e interespecífico, a duplicação de cromossomos e o cruzamento de mutações ainda constituam a base do melhoramento de plantas ornamentais, a velocidade do melhoramento de plantas aumentou significativamente desde a época de Mendel. Novas ferramentas genômicas agora oferecem possibilidades para precisão avançada de melhoramento e seleção para características mais difíceis de serem acessadas ou características quantitativas. Atualmente, o melhoramento de plantas ornamentais é um complicado exercício, caracterizado por novos e rápidos desafios. Parcerias entre indústria e pesquisa serão essenciais para implementar as tecnologias disponíveis em culturas menores.

Palavras-chave: hibridização interespecífica, poliploidização, marcadores moleculares, genômica, CRISPR/Cas, melhoramento de mutações, tecnologia Ri.

Introduction

Breeding and selection of many of the currently popular ornamental plants in Europe started in the 17th and 18th century when plant hunters, botanists or adventurers brought new plants from Asia and the Americas. One of the first descriptions of an artificial hybrid carnation dates to 1717 (Leapman, 2001) but most of the first developments in ornamentals were based on selection of natural mutations or selections from spontaneously generated seedling populations. Mendel's discovery of how traits are passed from one generation to the next started a new era of plant breeding activities. Plant breeding based on cross-breeding and subsequent selection of seedlings combining desired characteristics of both parents was also gradually applied to ornamentals. Starting in mid-1800s it became an industrial activity in private companies, resulting today in the rich diversity of ornamental species and cultivars.

Long et al. (2018) use extrapolations to estimate that as many as 85,000 to 99,000 species of ornamental plants now exist worldwide. These numbers include the wild relatives of cultivated plants. It demonstrates the huge potential of natural variations and germplasm as well as the necessity to conserve genetic resources of ornamental plants for breeding and future development. In recent decades legal systems as The Convention on Biological Diversity (CBD),

*Corresponding Author: johan.vanhuylenbroeck@ilvo.vlaanderen.be

https://doi.org/10.1590/2447-536X.v28i3.2516
Received: May 13, 2022 | Accepted: June 3, 2022 | Available online: June 28, 2022

Area Editor: Márkilla Zunete Beckmann-Cavalcante and Patrícia Duarte de Oliveira Paiva

the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and the Nagoya Protocol were established to govern the conservation and sustainable use of plants. These systems will have an impact on how genetic resources can be accessed and used by breeders. Equitable benefit-sharing will stimulate the improvement of conservation of ornamental plants and will conserve important genetic variation for future demands and challenges.

In this review paper on ornamental plant breeding, we focus briefly on the most commonly used breeding methods, namely intra- and interspecific cross breeding, chromosome doubling and mutation breeding. We describe the possibilities that new developments in genetic and molecular tools can offer for ornamental breeders and present some challenges that breeders will face in a quickly changing world.

Intra- and interspecific hybridization

Selection of novel phenotypes in progenies from controlled intraspecific crosses or even in open pollination seedling populations has been, and still is, a successful approach in many ornamental crops. The majority of ornamental species have high levels of heterozygosity, which generates enough variation in F1 populations to allow for selection of new valuable cultivars. However, in crops with a long breeding history, such as rose or chrysanthemum, the gene pool has narrowed due to the tendency of commercial breeding to build on recently released varieties. The restricted genetic variation in crossing parents makes it difficult to achieve truly innovative breakthroughs. New crossing combinations between different species (interspecific hybridization) in ornamental crops is therefore the most appropriate tool to increase variation and represents an excellent way to introduce new interesting genes into a breeding gene pool (Van Huylenbroeck et al., 2020). In many of the most popular ornamental crops, interspecific hybridization forms the foundation of the present assortment. In rose, only about 8 to 15 species contributed to the original germplasm of the modern cultivars (Leus et al., 2018). Chrysanthemum emerged from natural hybridizations between different wild species, leading to a wide-range cultigen complex (Spaargaren and van Geest, 2018). Completely new characteristics can be expected when breeding distant species, including non-domesticated species. A good example is lily, where over the last 50 years a range of techniques to overcome crossing barriers has successfully been used to create a completely new range of flower characteristics. This has led to a significant shift in the produced assortment (Van Tuyl et al., 2018). Other examples are described in Marantaceae (Van Huylenbroeck et al, 2018) *Asclepias* sp. (Lewis et al., 2021), *Pavonia* (Yue and Ruter, 2021), and many other crops. Asexual propagation makes it possible to propagate new types arising from interspecific hybridization even if they are sterile.

Interspecific hybridizations have drawbacks as well, including a lower success rate, higher investment costs and require a longer time to commercialization, as backcrosses are often required before obtaining the final product. Successful interspecific or *in extensa* intergeneric crossing within a specific genus involves a number of hurdles (Eeckhaut et al., 2006). First, knowledge of the genetic relationship between the different species in the available genepool can help to predict the hybrid production rate. Existing taxonomic classification of species and taxa is useful to a degree, but the gold standard is genomic techniques, used either in combination or not with data on chromosome numbers and chromosome size. More and more studies are highlighting the importance of these data as an important indicator to predict crossing efficiency in breeding programs as has been shown for example in *Geranium* (Akbarzadeh et al., 2021), *Helleborus* (Meiners and Winkelmann, 2012) or *Hydrangea* (Granados-Mendoza et al. 2013). This does apply to all crosses, however: factors such as genetic distance, ploidy level and genome size did not hamper interspecific crosses in *Sarcococca* (Denaeghel et al., 2017). Breeding barriers are not limited to genetic distance either. In nature spatial distribution of species or differences in flowering period form either physical or season-dependent barriers which prevent interspecific crosses to take place. In a breeding program, some of these issues can be resolved by growing plants under controlled environmental conditions or by storing pollen. Other prezygotic barriers inhibit pollen germination or growth through the style. A number of tools including cut-style pollination, intrastylar pollination, use of mentor pollen or hormones, and in vitro pollination are available to resolve pollen-pistil incongruities. Many of these technologies have been applied in lily (Van Tuyl et al, 2018) and in other crops, but in genera with small individual flowers, some of these techniques are very challenging, and require highly skilled personnel.

Many interspecific crosses show problems after fertilization (postzygotic barriers), such as lack of endosperm development, embryo abortion, albinisms or reduced growth vigor. Embryo rescue, ovary culture or ovule culture protocols are proven techniques to address these challenges.

Fertility problems can also occur in the generated F1 hybrids, limiting further use of these plants in breeding. In such cases, chromosome doubling can restore fertility. When sexual crossing fails completely, protoplast fusion is the last resort, although it has succeeded in only a few ornamentals.

Chromosome doubling

Chromosome doubling or polyploidy still plays an important role in breeding of ornamentals. In natural populations, spontaneous chromosome doubling is frequently observed. Under stressful growing conditions such as high altitude or in the Far North, there is a higher percentage of plants with altered chromosome numbers.

Changes in ploidy levels not only create physiological changes that help plants to survive harsh growing conditions, they also frequently create morphological alterations. Natural polyploids arise from auto-polyploids, and in cases of interspecific hybridization, allo-polyploidy can occur. Historically, the naturally-occurring plants that had the most attractive features (= bigger flowers or leaves, darker leaf color, etc.) were selected by breeders and collectors who did not know the chromosome number of their selection. This explains why many of the current popular ornamentals as rose (tetraploid) or chrysanthemum (hexaploid) are polyploids.

In nature, new polyploids arise via the spontaneous occurrence of male or female gametes that possess the somatic chromosome number (or higher). This process is named gametic or meiotic polyploidization. The utilization of the so-called unreduced gametes has great potential for plant breeding. However, in most plants species unreduced gametes are only infrequently produced. In some cases, higher production is observed when flowers are formed under temperature stress or by treatment with chemicals or N_2O (Eeckhaut et al.,2018). Especially after interspecific hybridization, unreduced gamete production can help overcome fertility problems. Furthermore, this technique also yields a single-step procedure when breeding for triploid progenies.

The 20th-century discovery of the chromosome doubling effect of colchicine opened new horizons for ploidy breeding, and mitotic polyploidization became an important tool for plant breeders. Today, a number of other less toxic chemicals can be used to block mitotic division and create polyploids (Dhooghe et al., 2011). Induced mitotic polyploidization is a relatively easy and cheap way to generate new cultivars with phenotypic changes such as larger flowers, increased leaf thickness, increased width/length ratio of the leaves, more intense green color of the leaves, or more compact growth. This technology is therefore interesting to use, even in ornamental species that have not yet been intensively bred.

Mutation breeding/somaclonal variation

Genetic mutations are the primary source of genetic variability. In nature, new alleles arise continuously, some of which may enable the plant to evolve by coping more successfully with new environmental conditions. Spontaneously occurring mutations still form an important source of new variations, especially for ornamental crops without dedicated breeding activities. In many woody ornamentals, new introductions originate from mutations or bud/branch sports (Van Laere et al., 2018), such as variegated leaf forms or dwarf growing cultivars. Mutations in a number of popular genera of greenhouse grown plants, especially foliage plants, are the main driver to transform a wild species into a cultivated ornamental. Some examples can be found in genera such as *Aglaonema*, *Marantha*, *Monstera*, *Ficus*, *Hedera,* and so on. One special group of variegations, namely leaves with white or yellow cell lineages, are the result of plastid mutations.

In some ornamentals, i.e. chrysanthemum, orchids, carnation, induced mutation (either *in vivo* or *in vitro*) is used routinely to create new variations. Especially in chrysanthemum, this technology is used frequently and successfully to quickly generate new color variants. The success of induced mutation in ornamentals has two main reasons: (1) the main goals are easily-detected esthetic characteristics (color, plant habit, variegation patterns); and (2) most ornamentals have a high mutation frequency because they are heterozygous. Mutations can be stimulated using either chemical (i.e. EMS) or physical (X-rays, gamma, ion beam) techniques. Efficiency is largely dependent on different factors such as genotype, explant type, duration of treatment etc. In combination with tissue culture, mutants can be generated quickly and with great stability. Currently in some Asian countries as Malaysia, Japan, South-Korea still extensive research is being done to develop mutation technology for creating new varieties in ornamentals (Ibrahim et al., 2018).

In micropropagated plants, variations in plant habit, leaf morphology, flower color, flower shape and leaf variegation sometimes occur without the use of chemical or physical mutagens. These so-called somaclonal variants are the results of the genetic and epigenetic diversity that occurs during micropropagation. These variations arise due to various stress factors such as wounding, exposure to sterilizing agents, use of undifferentiated cells and callus cultures, high concentrations of plant hormones, presence of sugars, long-term culture cycles, light conditions and hyperhydricity. The exploitation of in vitro-generated somaclonal variation has been used in the routine breeding of different commercial crops as reviewed by Krishna et al. (2016). In *Rhododendron simsii* leaf explants increase the frequency of flower color bud sporting, which can be used to generate a color series (Samyn et al., 2002).

In some cases, somaclonal variation via cell cultures can be exploited for the selection of genotypes that are resistant to either biotic or abiotic stresses (salinity resistance, low temperature tolerance). Selection towards fungal resistance can be obtained by applying fungal toxins or culture filtrates as selective agents, as documented in carnation and other ornamentals (Thakur et al., 2002).

Undifferentiated cells like protoplasts or calli seem to be particularly prone to somaclonal variation. The potential of protoplasts as explant type has been demonstrated in *Dianthus* (Shiba and Mii, 2005) and *Cyclamen* (Winkelmann et al., 2008). In addition to morphological changes, altered ploidy levels have also been observed. Within protoplast regenerants of chrysanthemum, different types of somaclonal variation include changes in flower number and flower size, a reduction in plant height and altered flowering induction time and flower types and colors (Eeckhaut et al, 2020).

Marker assisted breeding and genomics technology

Since Mendel's great discovery, plant breeders have pursued better cultivars and faster breeding, resulting in the development of new tools throughout history. Molecular markers and application of marker assisted selection (MAS)

are part of that toolbox. Molecular markers are the DNA sequence that are tightly linked and inherit together with the genomic region of interest. These markers can be used in breeding to select the individuals containing the genomic region of interest with high precision. Simple sequence repeat (SSRs) and single nucleotide polymorphisms (SNPs) are the most used molecular markers in the present context because of their higher frequency and wider distribution across plant genomes. Although MAS do not lead directly to new cultivars or increased genetic diversity, they do greatly increase the efficiency of plant breeding by retaining the plants containing the trait of interest. Molecular markers have been reported for a wide range of ornamental crops and for numerous traits with a focus on qualitative traits, oligogenic traits and some quantitative traits with known loci. In ornamental crops, MAS has been particularly important to increase host disease resistance for bacterial, fungal and viral pathogens. Floral traits such as double flowers, flower color and flower longevity have also been selected using MAS (Smulders et al., 2012). MAS can be helpful for complex traits controlled by multiple loci or regions such as productivity and flowering time, as well as traits that are difficult to assess. MAS is effective in cultivar development when using germplasm that shares the same genetic resources. Besides conducting progeny selection, parental selection is also possible using markers. In crops with established marker information, parental selection can offer increased efficiency in cultivar development process, especially for qualitative traits.

Despite the advantages of molecular markers, within ornamental breeding they are only used routinely for cultivar development in economically important crops such as rose, carnation, chrysanthemum, petunia, and lily (Onozaki et al., 2004; Von Malek et al., 2000; Su et al., 2019; Tychonievich et al., 2011; Van Tuyl et al., 2018). High levels of heterozygosity, different genetic resources controlling the traits, involvement of inter- and intra-specific crosses and genome complexities across the world all limit the usefulness of molecular markers across wider germplasms in ornamental species. In addition, floral traits can be assessed relatively easily using visual phenotypic methods and hence do not require molecular markers when visual phenotyping is the most effective method.

The use of molecular markers depends on the availability of genetic and genomic resources. Next generation sequencing and analysis of big data has increased the availability and affordability of reference genomes, linkage maps, SSRs and SNPs for non-model crops. Reference genomes have helped in the identification of genes that control certain traits and the development of molecular markers associated with the genes. Availability of long-read sequencing and analyses technology have made sequencing of polyploid species with larger genome sizes easier. A significant number of ornamental crops are polyploid. Now, markers and their associated genes can be more accurately identified in the specific chromosome and deployed in the breeding program. Genome reduction techniques like transcriptome sequencing and genotyping by sequencing have facilitated identification of genes and SNPs and SSRs in multiple ornamental crops like gerbera, hydrangea, impatiens, caladium, and others (Bhattarai et al., 2020, Wu et al., 2021; Bhattarai et al., 2018; Cao et al., 2017). Techniques like genome resequencing help to compare genomic regions of the individuals across the species and identify the markers applicable in the respective germplasm at the global level. These techniques can resolve large QTL regions to candidate genes for which specific markers can be developed.

As SNPs become the choice of marker, SNP array technologies like the Axiom and Infinium array have been developed for horticultural crops to genotype plant samples. These techniques are used to genotype large number of individuals and identify a large number of SNPs to construct high-density genetic or physical maps and to develop markers for trait identification. With the increase in genome resolution and marker-trait association, arrays with a few thousand SNPs are becoming increasingly common in agronomic and horticultural crops. These arrays have been developed for rose and chrysanthemum (van Geest et al., 2017; Koning-Boucoiran et al., 2016). If these arrays would be developed in other ornamental crops, they could be used for gene or QTL identification, quality checks (retaining true to type) plants, protecting plant variety, and studying population structure in the germplasm. In ornamental crops without readily available genomic resources, genome reduction techniques can be used by relying on reference genomes of the closely related species in the same family and with similar ploidy and genome complexity based on synteny, especially for identification of a candidate gene or genomic region.

CRISPR genome editing technology

Application of clustered regularly interspaced short palindromic repeats (CRISPR) is one of the most rapidly emerging gene-editing technologies in crop improvement in recent years. It is a low-cost, widely applicable, simple and highly efficient technique. CRISPR can precisely modify the genome sequence in an organism to achieve the desired phenotype. A complex of single guide RNA (sgRNA) and Cas9 protein is formed in which sgRNA directs the complex to the specific target site adjacent to protospacer-adjacent motif (PAM) and Cas9 proteins creates a double strand cleavage at the targeted site (Jinek et al., 2012). Repair of the cleaved site(s) occurs by two methods: Non-homologous end joining (NHEJ) and homology-directed repair (HDR) (Symington and Gautier, 2011). NHEJ, which helps to create small insertions or deletions, can also be used when inserting donor sequences and could be used to stack multiple genes and perform gene functional analysis (Lieber, 2010). Mutations created by CRISPR-Cas in plants have been demonstrated to stably transmit changes to subsequent generations following classic Mendelian genetics (Feng et al., 2014). Multiple sites in the genome can be edited using one nuclease enzyme and site specific sgRNAs (Salsman et al., 2016). In contrast, HDR can be used to create precise mutations or insert/delete desired sequences at the targeted site using homology-

based sequences. This biotechnological technique makes it possible to both create genetic diversity and speed up the breeding process using rapid trait modification or transfer (Chen et al., 2019). With the possibility of multiplexing, CRISPR can insert multiple traits into the target individual. If the genome is sequenced and protospacer adjacent motif (PAM) sequence is available, CRISPR technology can be applied to either mutate the existing gene or deploy the gene in the targeted region in the genome.

In the past, biotechnological tools like mutagenesis breeding and genetic transformation have been used in ornamental breeding to pursue novel traits like flower color or disease resistance (Shibata, 2008; Van de Wiel et al. 2017). However, in these cases it was difficult to predict flower color prior to mutagenesis, as the treatment generates random mutations. CRISPR-Cas9 system, the most popular CRISPR-Cas system at present, has been successfully used in ornamental plants to engineer flower color modifications from purple violet to pale purplish pink by targeting specific genes, the flavanone 3′-hydroxylase (*F3H*)*A* and *F3HB* coding genes, in *Petunia* (Yu et al., 2021). In Japanese morning glory, *I. nil*, CRISPR-Cas9 was used to target an anthocyanin biosynthesis enzyme encoding gene, *dihydroflavonol-4-reductase-B* (*DFR-B*), thus generating loss of function mutants that resulted in white flowers. Flower longevity is another important trait in the floral industry. Delayed petal senescence in *I. nil* has been achieved by knocking out the *EPHEMERAL1* locus (Shibuya et al., 2018). The gene that inhibits the deposition of carotenoids in *I. nil* petals, *carotenoid cleavage dioxygenase 4 (CCD4)*, was knocked out using CRISPR-Cas9, resulting pale yellow petals in the mutated lines instead of white petals (Watanabe et al., 2018). CRISPR-Cas9 based gene knock outs have been used in multiple crops to develop a male sterile maternal line in rice (Zhou et al., 2016). This technique could be used in invasive ornamental plants to develop sterile cultivars, thus checking their spread in order to protect native flora. Male sterility could also be beneficial in flowers on which pollen production is not desired by consumers, e.g. in cut gerberas (Deng and Bhattarai, 2018).

CRISPR-Cas plays a significant role in advancing precision breeding techniques and has opened plethora of avenues for altering and developing plant phenotypes or desirable traits. Currently, however, application of CRISPR-Cas9 in ornamental plants has been limited to a few crops, because genome sequences of ornamental species need to be available and functionally annotated before this system can be applied. Given the high heterozygosity and outcrossing nature of ornamental crops, the challenge of implementing the CRISPR-Cas9 system in numerous ornamental crops is not trivial. Despite the increasing affordability of sequencing, the minor economic significance of most ornamental crops hinders development of reference genomes for these species. Despite the availability of the reference genome, optimization of the plant transformation system has been a limiting factor in several crops including rose (Fang et al., 2021). The plant transformation system protocol must be optimized per plant species. This creates a major bottleneck for some ornamental crops, as tissue culture and plant transformation have never been performed on many of them. Although the list is likely to increase rapidly, only a handful of ornamental crops like petunia, morning glory, chrysanthemum, orchids to name a few have successfully undergone genome editing (Zhang et al., 2016; Kishi-Kobashi et al., 2017; Kui et al., 2017; Watanabe et al., 2017). The optimization of the plant transformation system and availability of plant genome sequences will drive development and application of CRISPR-Cas9 in ornamental crops.

Ri technology, a valuable tool to create compact growth

Genetically modified flowers have been created in crops including Dianthus, rose and chrysanthemum. Most of the targeted traits were flower color or enhanced vase life. However, there are strict regulatory procedures for the release of genetically modified plants which differ according to country or region. The significant cost of regulatory compliance hampers the potential commercial return of GMO plants, leading to a nearly complete lack of commercialization of GMO ornamentals. An interesting alternative transformation strategy only uses wild type strains of *Rhizobium rhizogenes*. This group of pathogenic bacteria carry a root-inducing (Ri) plasmid and thus cause the so-called crazy root disease in many dicotyledonous plant species. Co-cultivation of the bacteria and plants under lab conditions results in natural transformation. Regenerated Ri phenotype plants are considered as non GMO and can thus be commercialized without regulatory constraints. (Desmet et al., 2020a).

These Ri phenotypes can express morphological alterations in leaves, flowers, flowering time and root growth, as well as the most interesting trait: growth habit. Compact growing phenotypes are frequently observed after Ri transformation, which makes this technology an interesting alternative approach to obtain more compact growing plants. In many ornamental crops, compact growth is an important economic factor. In current practice, compactness is usually obtained through the application of chemical plant growth regulators. This approach is under pressure as many toxic chemical products are now being banned. Alternative methods include alterations in light quantity or quality, temperature differences between night/day, water gift, and fertilization, but these are less robust and are more difficult to manage. Genetically compact growing cultivars are clearly preferable. Ri technology delivers pre-breeding material which is subsequently used in conventional cross breeding programs. Efficient tissue culture protocols for transformation and regeneration ensure the creation of many Ri lines in a specific genus. Successful use of the technology has been demonstrated in *Kalanchoe blossfeldiana* (Christensen et al, 2008), among others. In *Osteospermum fruticosum* the combined decrease in internode length and plant height with increased branching resulted in very attractive compact and bushy plants (Desmet et al., 2021). Although the Ri phenotype

was inherited in next generations, a non-Mendelian inheritance was observed in *Sinningia* (Desmet et al., 2020b). These observations reveal that the Ri phenotype is a complex trait influenced by the genetic background of the Ri line. For accurate application in breeding programs it is essential to get better insights in the exact segregation ratio and its accompanying phenotype. In the future, thorough genetic identification of lines from unique transformation events will facilitate the implementation of this technology on a larger scale in combination with conventional and molecular plant breeding.

Sustainable ornamental horticulture: the breeder's challenge

The traditional breeding goal has been to develop cultivars with improved esthetic traits such as floral attributes (color, shape, perfume, enhanced vase life), leaf characteristics, or plant habit. In cut flowers, increased productivity (stems per m²) is another important goal. Growing environmental awareness is leading to a shift in legislation, which in turn will drive growers and breeders to pay much more attention to sustainable production. New breeding goals include enhanced disease and pest resistance and increased tolerance against abiotic stresses. This is a big challenge and is expected to become an increasingly important breeding target (Winkelmann et al, 2020).

In Europe, the publication of the European Green Deal (document 52019DC0640, COM (2019) 640 final) presents a roadmap towards a sustainable economy, including concrete targets that affect horticulture directly. These include a reduction by 50% of the use and risk of pesticides and a reduction by at least 20% of the use of fertilizers by 2030. It is clear that these targets will lead to further bans on chemical pesticides and plant growth regulators. Naturally-occurring disease and pest resistance will therefore become an important selection criteria for growers to decide which cultivars they will produce in their nurseries. Reduced fertilizer application may also have an impact in ornamental plant production. Nutrient use efficiency has traditionally only been a target in agricultural crops but will likely also become an issue in ornamentals.

Climate change requires selection for outdoor plants that can withstand a broader range of environmental conditions. Cultivars with better drought tolerance, i.e. plants that require less water input and can withstand more extreme weather conditions, are needed for application in gardens and public green. The traditional assortment of garden plants in more southern latitudes is now becoming popular in more northern areas, thus cultivars with enhanced cold tolerance or that can tolerate big temperature shifts in spring are desired. In the greenhouse, cold tolerance or selection towards chilling tolerant plants will also become more important in a context of increasing heating costs and stricter laws regarding greenhouse gas emissions.

Meeting all of these medium-long term targets will be a tremendous challenge, one that will require adequate selection methods and bio-assays in minor crops, combined with molecular genetics and related technologies. This will only become possible if industry and research collaborate, as most breeding programs are performed by small-scale breeding companies that lack the knowledge and financial resources to achieve these goals on their own. Appropriate IP protection, via plant breeders rights or plant patents, should ensure appropriate return on investments.

Conclusions

Ornamental plant breeding has become an important industrial activity. The most common methods of creating new, more attractive cultivars from wild plant species are cross breeding, interspecific hybridization and chromosome doubling. Newer breeding tools such as molecular tools and the possibilities for targeted mutagenesis offer great opportunities for improvements in ornamentals, especially for the more difficult selections for non-esthetic traits. Today, breeding of ornamental plants has become a complicated exercise requiring far more flexibility than the traditional medium-long term targets. Using the latest technologies and making huge investments are not always the best response to today's dynamic world, where short-term global changes require innovative and flexible responses. Indeed, often the passionate "traditional" breeders and even amateur plant lovers are producing the most innovative outcomes in minor crops. This apparent contradiction can only be overcome by more intensive collaboration by the different actors in industry and research.

Author contribution

JVH and **KB** both equally contributed to the writing of the manuscript.

References

AKBARZADEH, M.; VAN LAERE, K.; LEUS, L.; DE RIEK, J.; VAN HUYLENBROECK, J.; WERBROUCK, S.P.O.; DHOOGHE E. Can knowledge of genetic distances, genome sizes and chromosome numbers support breeding programs in hardy geraniums? **Genes**, v.12, n.5, 730, 2021. https://doi.org/10.3390/genes12050730

BHATTARAI, K.; CONESA, A.; XIAO, S.; PERES, N.A.; CLARK, D.G.; PARAJULI, S.; DENG, Z. Sequencing and analysis of gerbera daisy leaf transcriptomes reveal disease resistance and susceptibility genes differentially expressed and associated with powdery mildew resistance. **BMC Plant Biology**, v.20, n.1 p.1-17, 2020. https://doi.org/10.1186/s12870-020-02742-4

BHATTARAI, K.; WANG, W.; CAO, Z.; DENG, Z. Comparative analysis of impatiens leaf transcriptomes reveal candidate genes for resistance to downy mildew caused by *Plasmopara obducens*. **International Journal of Molecular Sciences**, v.19, n.7 p.2057, 2018. https://doi.org/10.3390/ijms19072057

CAO, Z.; DENG, Z. De novo assembly, annotation, and characterization of root transcriptomes of three caladium cultivars with a focus on necrotrophic pathogen resistance/defense-related genes. **International Journal of Molecular Sciences**, v.18, n.4 p.712, 2017. https://doi.org/10.3390/ijms18040712

CHEN, K.; WANG, Y.; ZHANG, R.; ZHANG, H.; GAO, C. CRISPR/Cas genome editing and precision plant breeding in agriculture. **Annual Review of Plant Biology**, v.70, p.667-697, 2019. https://doi.org/10.1146/annurev-arplant-050718-100049

CHRISTENSEN, B.; SRISKANDARAJAH, S.; SEREK, M.; MÜLLER, R.P.B. Transformation of *Kalanchoe blossfeldiana* with *rol*-genes is useful in molecular breeding towards compact growth. **Plant Cell Reports**, v.27, n.9, p.1485-1495, 2008. https://doi.org/10.1007/s00299-008-0575-0

DENAEGHEL, H.; VAN LAERE, K.; LEUS, L.; VAN HUYLENBROECK, J. Interspecific hybridization in Sarcococca supported by analysis of ploidy level , genome size and genetic relationships. **Euphytica**, v.213, p.149 2017. https://doi.org/10.1007/s10681-017-1934-0

DENG, Z.; BHATTARAI, K. Gerbera. In: VAN HUYLENBROECK, J. **Ornamental Crops**. **Handbook of Plant Breeding**, vol 11. Cham: Springer, 2018. p.407-438. https://doi.org/10.1007/978-3-319-90698-0_17

DESMET, S.; DHOOGHE, E.; DE KEYSER, E.; QUATAERT, P.; EECKHAUT, T.; VAN HUYLENBROECK, J.; GEELEN, D. Segregation of *rol* genes in two generations of *Sinningia speciosa* engineered through wild type *Rhizobium rhizogenes*. **Frontiers in Plant Science, v11**. 2020b. https://doi.org/10.3389/fpls.2020.00859

DESMET, S.; DHOOGHE, E.; DE KEYSER, E.; VAN HUYLENBROECK, J.; GEELEN, D. Compact shoot architecture of *Osteospermum fruticosum* transformed with *Rhizobium rhizogenes*. **Plant Cell Reports**, v.40, n.9, p.1665-1678. 2021. https://doi.org/10.1007/s00299-021-02719-z

DESMET, S.; DHOOGHE, E.; DE KEYSER, E.; VAN HUYLENBROECK, J.; MÜLLER, R.; GEELEN, D.; LÜTKEN, H. Rhizogenic agrobacteria as an innovative tool for plant breeding; current achievements and limitations. **Applied Microbiology and Biotechnology**, v.104; p.2435-2451. 2020a. https://doi.org/10.1007/s00253-020-10403-7

DHOOGHE, E.; VAN LAERE, K.; EECKHAUT, T.; LEUS, L.; VAN HUYLENBROECK, J. Mitotic chromosome doubling of plant tissues in vitro. **Plant, Cell, Tissue and Organ Culture**, v.104, p.359-373, 2011. https://doi.org/10.1007/s11240-010-9786-5

EECKHAUT, T.; VAN DER VEKEN, J.; DHOOGHE, E.; LEUS, L.; VAN LAERE, K.; VAN HUYLENBROECK, J. Ploidy Breeding in Ornamentals. In: VAN HUYLENBROECK, J. **Ornamental Crops**. **Handbook of Plant Breeding**, vol 11. Cham: Springer, 2018. p.145-173. https://doi.org/10.1007/978-3-319-90698-0_7

EECKHAUT, T.; VAN HOUTVEN, W.; BRUZNICAN, S.; LEUS, L.; VAN HUYLENBROECK, J. Somaclonal variation in *Chrysanthemum* × *morifolium* protoplast regenerants. **Frontiers in Plant Science**, v.11, n.607171, 2020. https://doi.org/10.3389/fpls.2020.607171

EECKHAUT, T.; VAN LAERE, K.; DE RIEK, J.; VAN HUYLENBROECK, J. Overcoming interspecific barriers in plant breeding. In: SILVA, J.A.T **Floriculture, ornamental & plant biotechnology: advances and topical issues.** London: Global Science Books, 2006. p.540-551.

FANG, P.; ARENS, P.; LIU, X.; ZHANG, X.; LAKWANI, D.; FOUCHER, F.; CLOTAULT, J.; GEIKE, J.; KAUFMANN, H.; DEBENER, T.; BAI, Y. Analysis of allelic variants of RhMLO genes in rose and functional studies on susceptibility to powdery mildew related to clade V homologs. **Theoretical and Applied Genetics**, v.134, n.8, p.2495-2515, 2021. https://doi.org/10.1007/s00122-021-03838-7

FENG, Z; MAO, Y; XU, N; ZHANG, B.; WEI, P.; YANG, D-L.; WANG, Z.; ZHANG, Z; ZHENG, R.; YANG, L.; ZENG, L.; LIU, X.; ZHU, J-K. Multigeneration analysis reveals the inheritance, specificity and patterns of CRISPR/Cas-induced gene modifications in Arabidopsis. **Proceedings of the National Academy of Sciences**, v.111, p.4632-4637, 2014. https://doi.org/10.1073/pnas.1400822111

GRANADOS-MENDOZA, C.; WANKE, S.; GOETGHEBEUR, P.; SAMAIN, M. S. Facilitating wide hybridization in Hydrangeas. l. cultivars: A phylogenetic and marker-assisted breeding approach. **Molecular Breeding**, v.32, p.233–239. 2013. https://doi.org/10.1007/s11032-012-9822-8

IBRAHIM, R.; AHMAD, Z.; SALLEH, S.; HASSAN, A.A.; ARIFFIN, S. Mutation Breeding in Ornamentals. VAN HUYLENBROECK, J. **Ornamental Crops**. **Handbook of Plant Breeding**, vol 11. Cham: Springer, 2018. p.175-211. https://doi.org/10.1007/978-3-319-90698-0_8

JINEK, M.; CHYLINSKI, K.; FONFARA, I.; HAUER, M.; DOUDNA, J. A.; CHARPENTIER, E. A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. **Science**, v.337, n.6096 p.816-821, 2012. https://doi.org/10.1126/science.1225829

KISHI-KABOSHI, M.; AIDA, R.; SASAKI, K. Generation of gene-edited Chrysanthemum morifolium using multicopy transgenes as targets and markers. **Plant and Cell Physiology**, v.58, n.2, p.216-226. 2017.

KONING-BOUCOIRAN, C.F.; ESSELINK, G.D.; VUKOSAVLJEV, M.; VAN'T WESTENDE, W.P.; GITONGA, V.W.; KRENS, F.A.; VOORRIPS, R.E.; VAN DE WEG, W.E.; SCHULZ, D.; DEBENER, T.; MALIEPAARD, C.; ARENS, P.; SMULDERS, M.J.M. Using RNA-Seq to assemble a rose transcriptome with more than 13,000 full-length expressed genes and to develop the WagRhSNP 68k Axiom SNP array for rose (*Rosa* L.). **Frontiers in Plant Science**, v.6, p.249, 2015. https://doi.org/10.3389/fpls.2015.00249

KRISHNA, H.; ALIZADEH, M.; SINGH, D.; SINGH, U.; CHAUBAN, N.; EFTEKHARI, M.; SADH, R. Somaclonal variations and their applications in horticultural crops improvement. **3Biotech**, v.6, n.1, p.54, 2016.

KUI, L.; CHEN, H.; ZHANG, W.; HE, S.; XIONG, Z.; ZHANG, Y.; YAN, L.; ZHONG, C.; HE, F.; CHEN, J.; ZENG, P. Building a genetic manipulation tool box for orchid biology: identification of constitutive promoters and application of CRISPR/Cas9 in the orchid, *Dendrobium officinale*. **Frontiers in Plant Science**, v.7, p.2036. 2017.

LEAPMAN, M. **The ingenious Mr. Fairchild:** The forgotten father of the flower garden. St. Martin's Press, 2001. 280p.

LEUS, L.; VAN LAERE, K.; DE RIEK, J.; VAN HUYLENBROECK, J. Rose. In: VAN HUYLENBROECK, J. **Ornamental Crops**. **Handbook of Plant Breeding**, vol 11. Cham: Springer, 2018. p.719-767. https://doi.org/10.1007/978-3-319-90698-0_27

LEWIS,M.;CHAPPELL,M.;THOMAS,P.A.;MAYNARD, R. C.; AND GREYVENSTEIN, O. Development and verification of an interspecific hybridization protocol for asclepias. **HortScience**, v.56, n.7, p.831–837, 2021. https://doi.org/10.21273/HORTSCI15770-21

LIEBER, M.R. The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. **Annual Review of Biochemistry**, v79, n.1 p.181-211. 2010. https://doi.org/10.1146/annurev.biochem.052308.093131

LONG, C.; CHEN, Z.; ZHOU, Y.; LONG, B. The Role of biodiversity and plant conservation for ornamental breeding. In: VAN HUYLENBROECK, J. **Ornamental Crops**. **Handbook of Plant Breeding**, vol 11. Cham: Springer, 2018. p.1-12. https://doi.org/10.1007/978-3-319-90698-0_1

MEINERS, J.; WINKELMANN, T. Evaluation of reproductive barriers and realisation of interspecific hybridisations depending on the genetic distances between species in the genus *Helleborus*. **Plant Biology**, v.14, p.576-585, 2012. https://doi.org/10.1111/j.1438-8677.2011.00542.x

ONOZAKI, T.; TANIKAWA, N.; TANEYA, M.; KUDO, K.; FUNAYAMA, T.; IKEDA, H.; SHIBATA, M. A RAPD-derived STS marker is linked to a bacterial wilt (*Burkholderia caryophylli*) resistance gene in carnation. **Euphytica**, v.138, p.255-262, 2004. https://doi.org/10.1023/B:EUPH.0000047099.48469.3c

SALSMAN, J.; DELLAIRE, G. Precision genome editing in the CRISPR era. **Biochemistry and Cell Biology**, v.95, p.187-201. 2016. https://doi.org/10.1139/bcb-2016-0137

SAMYN, G.; DE SCHEPPER, S.; VAN BOCKSTAELE, E. Regeneration of explants of several azalea (*Rhododendron simsii*) cultivars in view of the appearance of sports. **Plant, Cell, Tissue and Organ Culture**, v.70, p.223-227, 2002. https://doi.org/10.1007/s13205-016-0389-7

SHIBA, T.; MII, M. Plant regeneration from mesophyll- and cell suspension-derived protoplasts of *Dianthus acicularis* and characterization of regenerated plants. **In Vitro Cellular and Developmental Biology Plant**, v.41, n.6, p.794-800, 2005. https://www.jstor.org/stable/4293937

SHIBATA, M. Importance of genetic transformation in ornamental plant breeding. **Plant Biotechnology**, v.25, n.1 p3-8. 2008. https://doi.org/10.5511/plantbiotechnology.25.3

SHIBUYA, K.; WATANABE, K.; ONO, M. CRISPR/Cas9-mediated mutagenesis of the EPHEMERAL1 locus that regulates petal senescence in Japanese morning glory. **Plant Physiology and Biochemistry**, v.131, p.53-57. 2018. https://doi.org/10.1016/j.plaphy.2018.04.036

SMULDERS, M.J.M.; VUKOSAVLJEV, M.; SHAHIN, A.; VAN DE WEG W.E.; ARENS, P. High throughput marker development and application in horticultural crops. **Acta Horticulturae**, v.961, p.547-551. 2012. https://doi.org/10.17660/ActaHortic.2012.961.72

SPAARGAREN, J.; VAN GEEST, G. Chrysanthemum. In: VAN HUYLENBROECK, J. **Ornamental Crops**. **Handbook of Plant Breeding**, vol 11. Cham: Springer, 2018. p.319-348. https://doi.org/10.1007/978-3-319-90698-0_14

SU, J.; JIANG, J.; ZHANG, F.; LIU, Y.; DING, L.; CHEN, S.; CHEN, F. Current achievements and future prospects in the genetic breeding of chrysanthemum: a review. **Horticulture research**, v.6, p.109, 2019. https://doi.org/10.1038/s41438-019-0193-8

SYMINGTON, L.S.; GAUTIER, J. Double-strand break end resection and repair pathway choice. **Annual Review of Genetics**, v.45, n.1 p.247-271. 2011. https://doi.org/10.1146/annurev-genet-110410-132435

THAKUR, M.; SHARMA, D.; SHARMA, S. In vitro selection and regeneration of carnation (*Dianthus caryophyllus* L.) plants resistant to culture filtrate of *Fusarium oxysporum* f.sp. *dianthi*. **Plant Cell Reports**, v.20, p.825-828, 2002. https://doi.org/10.1007/s00299-001-0412-1

TYCHONIEVICH, J.; WANGCHU, L.; BARRY, C.; WARNER, R. M. Utilizing wild species for marker-assisted selection of crop timing and quality traits in Petunia. **Acta Horticulturae**, v.1000, p.465-469. 2011. https://doi.org/10.17660/ActaHortic.2013.1000.65

VAN DE WIEL, C.C.M.; SCHAART, J.G.; LOTZ, L.A.P.; SMULDERS, M.J.M. New traits in crops produced by genome editing techniques based on deletions. **Plant Biotechnology Reports**, v.11, p.1-8. 2017. https://doi.org/10.1007/s11816-017-0425-z

VAN GEEST, G.; BOURKE, P.M.; VOORRIPS, R.E.; MARASEK-CIOLAKOWSKA, A.; LIAO, Y.; POST, A.; VAN MEETEREN, U.; VISSER, R.G.; MALIEPAARD, C.; ARENS, P. An ultra-dense integrated linkage map for hexaploid chrysanthemum enables multi-allelic QTL analysis. **Theoretical and Applied Genetics**, v.130, n.12 p.2527-2541. 2017. https://doi.org/10.1007/s00122-017-2974-5

VAN HUYLENBROECK, J.; CALSYN, E.; VAN DEN BROECK, A.; DENIS, R.; DHOOGHE, E. *Calathea* . In: VAN HUYLENBROECK, J. **Ornamental Crops**. **Handbook of Plant Breeding**, vol 11. Cham: Springer, 2018. p.301-318. https://doi.org/10.1007/978-3-319-90698-0_13

VAN LAERE, K.; HOKANSON, S.C.; CONTRERAS, R.; VAN HUYLENBROECK, J. Woody Ornamentals of the Temperate Zone. In: VAN HUYLENBROECK, J. **Ornamental Crops**. **Handbook of Plant Breeding**, vol 11. Cham: Springer, 2018. p.803-887. https://doi.org/10.1007/978-3-319-90698-0_29

VAN TUYL, J. M.; ARENS, P.; SHAHIN, A.; MARASEK-CIOLAKOWSKA, A.; BARBA-GONZALEZ, R.; KIM, H. T.; LIM K-B. Lilium. In: VAN HUYLENBROECK, J. **Ornamental Crops**. **Handbook of Plant Breeding**, vol 11. Cham: Springer, 2018. p.481-512. https://doi.org/10.1007/978-3-319-90698-0_20

VON MALEK, B.; WEBER, W.E.; DEBENER, T. Identification of molecular markers linked to Rdr1, a gene conferring resistance to blackspot in roses. **Theoretical and Applied Genetics**, v.101, n.5 p.977-983. 2000. https://doi.org/10.1007/s001220051570

WATANABE, K.; KOBAYASHI, A.; ENDO, M.; SAGE-ONO, K.; TOKI, S.; ONO, M. CRISPR/Cas9-mediated mutagenesis of the dihydroflavonol-4-reductase-B (DFR-B) locus in the Japanese morning glory *Ipomoea* (*Pharbitis*) nil. **Scientific Reports**, v.7, n.1, p.1-9. 2017.

WATANABE, K.; ODA-YAMAMIZO, C.; SAGE-ONO, K.; OHMIYA, A.; ONO, M. Alteration of flower colour in Ipomoea nil through CRISPR/Cas9-mediated mutagenesis of carotenoid cleavage dioxygenase 4. **Transgenic research**, v.27, n.1 p.25-38. 2018. https://doi.org/10.1007/s11248-017-0051-0

WINKELMANN, T.; BRAUN, P.; DHOOGHE, E.; VAN HUYLENBROECK, J. Advances in conventional breeding techniques for ornamentals. In: REID, M. **Achieving sustainable cultivation of ornamental plants**, v.82, Cambridge: Burleigh Dodds Science Publishing. https://doi.org/10.19103/AS.2020.0066.03

WINKELMANN, T.; PRANGE, A.; SPECHT, J.; SEREK, M. Morphological characterization of plants regenerated from protoplasts of *Cyclamen persicum* Mill. **Propagation of Ornamental Plants**, v.8, n.1, p.9-12, 2008. https://eurekamag.com/research/021/371/021371796.php

WU, X.; HULSE-KEMP, A. M.; WADL, P. A.; SMITH, Z.; MOCKAITIS, K.; STATON, M. E.; RINEHART, T.; ALEXANDER, L. W. Genomic resource development for hydrangea (*Hydrangea macrophylla* (Thunb.) Ser.)-A Transcriptome assembly and a high-density genetic linkage map. **Horticulturae**, v.7, n.2 p.25. 2021. https://doi.org/10.3390/horticulturae7020025

YU, J.; TU, L.; SUBBURAJ, S.; BAE, S.; LEE, G.J. Simultaneous targeting of duplicated genes in *Petunia* protoplasts for flower color modification via CRISPR-Cas9 ribonucleoproteins. **Plant Cell Reports**, v.40 n.6 p.1037-1045. 2021. https://doi.org/10.1007/s00299-020-02593-1

YUE, Y.; RUTER, J.M. *Pavonia x rufula* (Malvaceae): An interspecific hybrid between *Pavonia lasiopetala* and *Pavonia missionum*. **HortScience**, v.56, n.6, p.732-735, 2021. https://doi.org/10.21273/HORTSCI15711-21

ZHANG, B.; YANG, X.; YANG, C.; LI, M.; GUO, Y. Exploiting the CRISPR/Cas9 system for targeted genome mutagenesis in petunia. **Scientific Reports**, v.6, n.1, p.1-8. 2016.

ZHOU, H.; HE, M.; LI, J.; CHEN, L.; HUANG, Z.; ZHENG, S.; ZHU, L.; NI, E.; JIANG, D.; ZHAO, B.; ZHUANG, C. Development of commercial thermo-sensitive genic male sterile rice accelerates hybrid rice breeding using the CRISPR/Cas9-mediated *TMS5* editing system. **Scientific Reports**, v.6, 37395. 2016. https://doi.org/10.1038/srep37395