Plant Science xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

Review

Precision breeding of grapevine (*Vitis vinifera* L.) for improved traits

Dennis J. Gray[a,*], Zhijian T. Li[a], Sadanand A. Dhekney[b]

[a] *Grape Biotechnology Core Laboratory, Mid-Florida Research and Education Center, University of Florida/IFAS, 2725 Binion Road, Apopka, FL 32703-8504 USA*

[b] *Department of Plant Sciences, Sheridan Research and Extension Center, University of Wyoming, 663 Wyarno Road, Sheridan, WY 82801 USA*

ARTICLE INFO

Article history:
Available online xxx

Keywords:
Grapevine
Vitis vinifera
Cisgenics
Intragenics
Genetic engineering
Disease resistance
Anthocyanin marker
Expression analysis

ABSTRACT

This review provides an overview of recent technological advancements that enable precision breeding to genetically improve elite cultivars of grapevine (*Vitis vinifera* L.). Precision breeding, previously termed "cisgenic" or "intragenic" genetic improvement, necessitates a better understanding and use of genomic resources now becoming accessible. Although it is now a relatively simple task to identify genetic elements and genes from numerous "omics" databases, the control of major agronomic and enological traits often involves the currently unknown participation of many genes and regulatory machineries. In addition, genetic evolution has left numerous vestigial genes and sequences without tangible functions. Thus, it is critical to functionally test each of these genetic entities to determine their real-world functionality or contribution to trait attributes. Toward this goal, several diverse techniques now are in place, including cell culture systems to allow efficient plant regeneration, advanced gene insertion techniques, and, very recently, resources for genomic analyses. Currently, these techniques are being used for high-throughput expression analysis of a wide range of grapevine-derived promoters and disease-related genes. It is envisioned that future research efforts will be extended to the study of promoters and genes functioning to enhance other important traits, such as fruit quality and vigor.

Published by Elsevier Ireland Ltd.

Contents

* Corresponding author. Tel.: +407 41 6946; fax: +407 814 6186.
E-mail address: djg@ufl.edu (D.J. Gray).

http://dx.doi.org/10.1016/j.plantsci.2014.03.023
0168-9452/Published by Elsevier Ireland Ltd.

Introduction

Genetic improvement of grapevine (*Vitis vinifera* L.) is one of the critical needs to enhance crop productivity and foster profitable wine industries throughout the world [1]. Although numerous unique hybrids were developed over the years, genetic improvement of elite hybrids, the mainstays of worldwide production, is deemed to be largely unsuccessful, especially in areas ravaged by severe disease/pest infestations and/or that require extensive chemical control to maintain. For example, the bacterial pathogen that incites Pierce's disease, *Xylella fastidiosa*, has no proven method of durable control other than well-known genetic resistance and the unsustainable mass spraying of pesticides to inhibit insect vectors, despite well over 50 million dollars expended, apparently unsuccessfully, by Federal and State governments since 1999 [2]. However, genetic resistance (tolerance) among native *Vitis* species was identified by 1958 [3]. The practical use of genetic resistance was subsequently confirmed through hybridization with *V. vinifera* cultivars to instill durable and near complete control of the pathogen [4–6]. Particularly urgent now is the introduction of specific traits for durable tolerance to diseases, pests, and abiotic stresses, while maintaining the essential quality of highly desired elite cultivars [7,8]. However, it is not possible to rely on conventional breeding to improve elite cultivars so that they can adapt to the production environment, while still meeting the strict expectations of oenophiles [9,10]. Conventional breeding cannot practically be used to add desired disease resistance traits to elite cultivars of *Vitis* because of a long lifecycle, severe inbreeding depression, and complex genetic control of enological qualities [11]. A majority of the relatively few elite grape cultivars currently cultivated worldwide are centuries-old and maintained primarily through a stringently managed system of vegetative propagation [12,13]. However, elite cultivars often lack other desirable traits such as durable disease and pest resistance that are demanded by today's intensive agricultural conditions. As such, producers rely on frequent use of pesticides to control diseases, particularly in areas of higher humidity; this is in spite of increasing public outcry against such practices and resulting environmental issues [14]. To mitigate such increasingly crucial agricultural and health concerns, modern biotechnology has advanced to the point where it is now possible to expedite genetic improvement of existing elite cultivars via precision breeding [7,15]. This review is intended to provide an overview of current technological advancement, particularly genomic analyses, for the development of resistance to biotic and abiotic stresses, as well as other traits, via precision breeding of elite cultivars.

Advances in gene insertion technology

The methodology to insert specific genes into plants without inducing significant genetic rearrangement has been in development for over thirty years. Such technology is particularly attractive for perennial crops like grapevine that have severe genetic obstacles to conventional breeding and require multi-year evaluation for durability of desired traits due to their long lifecycle and longevity. In order to provide a reliable working platform for genetic testing, efficient cell regeneration systems were developed [16–20]. An increasing number of scientists used such regeneration systems to document insertion of single or few genes into grapevine [11]. The precise methods of gene insertion employed either biolistic particle bombardment [21,22] or, more commonly, *Agrobacterium*-mediated gene insertion into regenerative cells, followed by plant recovery [11,23–25]. Both methods have been meticulously refined and optimized over the years and are now capable of producing hundreds of genetically modified plants. The majority of plants modified via the *Agrobacterium* approach tended to harbor low-gene copy number and defined gene insertion [24,26]. A large number of modified plants is critical for identification of lines with a desirable level of gene expression and performance to meet overall improvement objectives [27]. The need to test many plant lines, as is the norm with conventional breeding, is critical in order to select outstanding individuals.

During the early years of technology development aimed toward precision breeding, it was necessary to test genetic elements from non-plant hosts, including animals and bacteria, due to the relatively primitive state of biotechnology. This approach was generally referred to as "transgenic" modification. This early discovery research was absolutely essential so that cell culture and gene insertion methods could be refined to the point of being fully functional [28]. Subsequently, as pointed out by Rommens [29], many non-plant genes and promoters with known functionality were utilized to display the technological marvel of biotechnology. This approach to crop improvement inevitably invited arguments and ongoing worries as to whether such plants with foreign genes and promoters were healthy, represented an environmental threat and/or were otherwise dangerous in some way. The use of foreign genetic material in food crops including grapevine remains to be the pivot of social and ethical public debate [7,29,30].

Along with the refinement of cell culture and gene insertion methods, the final technology required to enable precision breeding was completion of the draft genome of *V. vinifera* 'Pinot Noir' in 2007 and the relatively new-found and simplified availability of computational analysis [31,32]. It is now possible to identify grapevine genes, along with their associated genetic elements, isolate them, from sexually-compatible disease-resistant relatives, and insert them into elite cultivars. While still in its infancy, the application of precision breeding to grapevine improvement is well underway, with a number of modified plants in approved field trials and more on the way [1,33–35].

Application of precision breeding is the logical and biologically conservative extension of conventional breeding, made possible only by long-term scientific research. Studies have suggested that application of precision breeding will boost consumer's confidence and acceptance of improved crop products as well [36–38].

As we continue to refine precision breeding, more remains to be discovered. We require a better understanding of genome structure organization and sequence/function associations. It is estimated that grape genome contains over 30,400 genes, which is more than that found in most animals [39]. Many important agronomic traits are controlled by a complex network of regulatory sequences and factors and often influenced by dynamic sequence alterations, such as gene duplication, transposon insertion and loss- or gain-of-function mutations [40]. Environmental factors also play an important role in gene expression and interaction [41]. Thus, the actual function and sustainability of any isolated genetic material, whether a gene or a promoter, has to be confirmed within its intricate genetic milieu and then rigorously tested over a prolonged time in the environment; this is the fundamental way to determine durable structure/function relationships. Although we are making rapid progress in sequence analysis and functional annotation of the grapevine genome [31,32,42–44], progress in functional characterization of important genes/promoters is slow, creating a significant obstacle to the practical utilization of the genetic resources already available. Since precision breeding is biologically consistent with conventionally bred crops and, indeed, the entire plant lifecycle, it constitutes a technical refinement of existing breeding methodologies. The solution to accelerating the crucial functional analyses needed to both test the technology and produce improved cultivars is to not regulate evaluation of precision bred grapevine, so that individual lines can be tested in quantity and in grower fields, as has always been the manner for conventionally bred crops. As with all crop breeding, many progeny must be

Please cite this article in press as: D.J. Gray, et al., Precision breeding of grapevine (*Vitis vinifera* L.) for improved traits, Plant Sci. (2014), http://dx.doi.org/10.1016/j.plantsci.2014.03.023