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REVIEW ARTICLE

APPLICATION OF GENOMIC AND GENETIC ENGINEERING TOOLS FOR IMPROVEMENT OF GRAPEVINES

A. Mwamahonje,[1] Z. Maseta,[1] F. Mlalila[2] and T. Feyissa[3]

[1]Tanzania Agricultural Research Institute (TARI), Makutupora Centre, P.O Box 1676, Dodoma, Tanzania; [2]Rijk Zwaan Q-Sem Limted, P.O Box 12345, Arusha, Tanzania.
[3]Institute of Biotechnology, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia.
Corresponding author's email: andekelilem@gmail.com

ABSTRACT

Conventional breeding in grape is tedious and time-consuming. The long juvenile phase of grape delays first flowering and fruit setting. Use of genomic tools including, genetic engineering, functional genomics, genome wide association and bioinformatics facilitate exploitation of traits to shorten breeding cycles. Short breeding cycles can be achieved by selection and screening of the best cultivars in the genebank. However, it is time consuming to develop new cultivars. The incorporation of traits to the new grape cultivar is done through conventional breeding by crossing male and female parents with contrasting traits. In addition, application of molecular markers can easily identify Quantitative Trait Loci (QTLs) influencing traits of interest for fastening introgression into the recipient grape cultivars by backcrossing method. Genetic engineering is another tool that uses *Agrobacterium tumefaciens* and biolistic mediated transformation whereby targeted genes are inserted into a DNA of a new grape cultivar for regeneration for example, grape transgenes. In a hybridization study, in which a seedless cultivar is used as maternal genotype, embryo rescue technique is necessary. Some traits in consideration in grape breeding include, flowering time, yield, drought tolerant, diseases resistance, sugar content and wine quality. Therefore, the application of genomic and genetic engineering tools is inevitable for grape improvement.

Key words: Biotechnology, Breeding, Germplasm, Molecular Marker, QTL, Trait.

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INTRODUCTION

Grape (*Vitis vinifera*) is a valuable horticultural fruit crop in the world. Historically, cultivation of domesticated *Vitis vinifera* species started 6,000-8,000 years ago from wild progenitor *Vitis vinifera* subsp. *sylvestris* (Myles *et al*., 2011). It was further expanded to the south and western side of the Fertile Crescent, the Jordan Valley, Anatolia, Syria and Egypt 5,000 years ago and later spread to the rest of the world (Pierozzi and Moura, 2016). Selection and early improvement of grape were based on several parameters like yield, disease resistance, sprouting ability, quality of fruits and wine. Advances in genetic techniques have simplified screening and improvement of health qualities of grape and grape-derived products. China ranks the top producer (13,160,788 tonnes annually) worldwide, followed by Italy and United States producing 7,169,745 and 6,679,211 tonnes per year respectively (FAOSTAT, 2017). *V. vinifera* is in the genus *Vitis* (2n=38 chromosomes). The crop has about 60 species in the genus *Vitis*, family Vitaceae (Villano, 2015). Botanically, some grape species are hermaphrodite and others are monoecious. Grape is classified from the largest rank of domain Eukarya to the smallest which is genus *Vitis*. The genus *Vitis* is common in both wild and domesticated grape species. The genus includes *Vitis vinifera* L., *Vitis acerifolia* Raf, *Vitis aestivalis* Michx and *Vitis amurensis* Rupr. However, the species *Vitis vinifera* is the most cultivated in the world followed by *Vitis labrusca* (Villano, 2015). Grape is used for making wine, table grapes, juice, raisin and spirit (Villano, 2015). It is good source of protein, minerals, vitamin C, K, iron, potassium, zinc, calcium and manganese (Sousa, 2014). It plays an important role in the regulation of aging processes because of antioxidant produced by the fruits' resveratrol. It helps to increase efficiency of digestion and prevent infection in the body. It contains phenolic compounds like tannins, phenolic acids, anthocyanins which maintain human health (Yilmaz and Toledo, 2004). This review describes the genomic and genetic engineering techniques for grape improvement including conventional and molecular breeding methods.

Current Cultivation of Grape: Grape is grown in temperate and sub-tropical climate with high and erratic rainfall (Vivier and Pretorius, 2000). The crop generally reaches at full production after 5 to 8 years from planting. The life cycle is 50-100 years depending on the regular agronomic management and ecology. The rich genetic diversity of grapes provides wide adaptation in multi-

environmental conditions across the world. The crop can be developed in the tissue culture laboratory using parts of the grapevine or can be propagated by grafting technology. The convenient pH for viticulture ranges from 5.5–7.5 depending on the variety and ecology. Organic and inorganic fertilizers are recommended for grapes depending on the nutrient deficiency in vineyard. Pruning is done to control number of fruits, vigour and nutrient content. Weeds, diseases and pests are controlled by sustainable use of chemicals and other practices.

Genetic Diversity: The centre of origin for genetic diversity of grape reported in Anatolia since 8000 BC (Vouillamo *et al*., 2006). The diversity spread into North Africa in 5th millennium BC and 1st millennium BC in Europe and later spread all over the world including Central Asia and United States of America (Vouillamo *et al*., 2006). Most studies using morphology and molecular markers (RAPDs, AFLPs, SSRs, ISSRs and SNPs) revealed high genetic diversity among wild and cultivated grape varieties (Bowers *et al*., 1999; Riaz *et al*., 2013; Mwamahonje *et al*., 2015; Da Costa *et al*., 2017). However, Myles *et al*. (2011) proposed that, the domestication of grape reduces the genetic diversity giving example of recent 950 genotyped *Vitis vinifera* and 59 *Vitis sylvestris* accessions using SNPs markers. The decrease of genetic diversity is contributed by cultivating the same varieties for a long period of time without releasing new cultivars. Consumers' preferences have an impact on the genetic diversity of grapes for instance religious ceremonies have specific varieties for wine making (Migicovsky *et al*., 2017). Lack of consistent genebank for grape species conservation accelerates loss of diversity (Zhou *et al*., 2017). On the other hand, the grapes have long life cycle which takes many years to release new varieties. Climate change has contribution to decrease of genetic diversity such that, some varieties lose their performance due to changes in temperature, pH of the soil and photoperiod. Genetic diversity plays a major role in the grape breeding by providing broad base of breeding materials. This increases the chances of exploiting the traits of preference which meet demands (Da Costa *et al*., 2017). Genetic diversity can be modified through a number of ways like breeding contrasting traits to introduce new varieties. It can be enhanced by mutation, plant tissue culture and germplasm collection (This *et al*., 2006; Da Costa *et al*., 2017).

Improvement Strategies: Grape improvement aims to address various constraints like diseases, low yield, sugar content, drought and pests. This is achieved by breeding improved varieties in relation to diseases and pests, drought tolerance, sugar content, taste and early maturity (Pierozzi and Moura, 2016). The methods used include conventional and molecular breeding methods. Conventionally, it is possible by breeding based on phenotyping only in the field. The molecular method is achieved using molecular markers, marker assisted selection and genetic engineering tools and phenotyping field data for accurate results. Both approaches entail backcrossing during incorporation of the traits of interest to the new varieties. Molecular breeding method is assisted by molecular markers. The method of traits transfer can be done using genetic engineering for instance *Agrobacterium tumefaciens* and gene gun (Laimer, 2007). Through this approach new varieties with high yield, disease resistance, sugar content, early maturity, standard antioxidants and other traits have been modified (Laimer, 2007). Use of disease free seedlings at young stage is recommended. Increase of wine, juice and raising industries will provide the opportunities for expansion of acreage of grapes production.

Traditional Breeding and Limitation in Grape: Traditional breeding of grapes involves crossing of the varieties with contrasting traits. It exploits traits of interest from one variety to another. Some of the traits which are considered for improvement include yield, diseases and pests resistance and biochemical content. These parameters help plant breeders to screen the useful traits for incorporation into new variety. Conventional plant breeding method may take 6-20 years to release new grape variety depending of the heritability of traits. This is due to long juvenile phase that takes 5-6 years from planting (McClure *et al*., 2014). In spite of grape to undergo out crossing still, the produced varieties lack genetic differences as evidenced in Cocoa (Schnell *et al*., 2007). Utilization of wild grape species in grape breeding provides potential traits which can improve the domesticated grape. It can be achieved through conventional breeding. For example, foxy smell in wild grape species *V. labrusca* shares the same ancestor with *V. vinifera* over 20 years ago (McClure *et al*., 2014). Hence, crossing to susceptible varieties may produce new improved varieties with combination of traits.

Role of Biotechnology in Grape Breeding: Use of biotechnology improves quality of grape products. It helps to introduce new varieties with combination of traits such as drought tolerance, diseases and pests' resistance and high yield from one genotype to another through gene transformation (Gascuel *et al*., 2017; Hvarleva *et al*., 2009). The technology is achieved using molecular markers for traits and QTLs identification which ultimately can be introgressed into potential variety through marker assisted backcrossing and genetic engineering (Migicovsky and Myles, 2017). Genetic engineering enhances rapid breeding programmes as it increases efficiency, performance and quality grapes produced within a short period of time. The technology involves both laboratory screening using markers and field phenotyping which together strengthen selection of

suitable heritability (Gascuel *et al*., 2017). Through this technology, the variety Chancellor has been introduced.

Germplasm Biodiversity and Conservation

Germplasm Diversity: Germplasm is validated regularly by adding new identified or collected cultivars. The grape germplasm contains wild and domestic species (Leão *et al*., 2011). Both have useful information for diversity studies. Some cultivars are originated by spontaneous or induced mutation. The diversity studies of grape germplasm use different tools including ampelography. Molecular markers are used in the study of DNA gene differences among germplasm (Leão *et al*., 2011). Genotyping helps to correct misnaming accessions by ampelography. The accessions may be the same but different name in different geographical location. The correcting naming of accessions can be achieved by using molecular markers like ISSR and SSR. These markers can identify the genetic differences and similarities within and between cultivars (Emanuelli *et al*., 2013). Both ampelography and molecular markers assist grape breeders to find individuals for grape improvement. Through this approach, drought tolerant, disease resistant and sugar content germplasm have been screened (Leão *et al*., 2009). Similar recommendations have been reported by a number of studies (Martínez *et al*., 2006). Despite the efforts made to maintain germplasm, the speed of releasing new grape varieties is Slow perhaps there is a need for further optimization of protocols used in each molecular marker.

Grape Cultivars Characterization and Phylogeny: Grape cultivars are characterized based on physiological, biochemical and molecular markers. In ampelography, grape cultivars are characterized using descriptor lists. It entails number of autochthonous and hybrid cultivars which are involved in assessing the genetic variation within and among cultivars (Ates *et al*., 2011). The characterization of grape cultivars provides wide range of exploiting traits for improvement (Ates *et al*., 2011). Mutations have contribution in the formation of new cultivars that may or may not be useful for direct adoption. The best cultivars developed by mutations are selected and kept in the genetic resources centres for future research (Sefc *et al*., 2001). Each marker has advantage and drawback. Therefore, it depends on the objective of study. For instance, RAPD is less costy, easy to apply, time saving for genetic diversity studies in grape genotypes and rootstock variability (Sefc *et al*., 2001). Nevertheless, RAPD is not versatile to the environmental conditions, depend on quality DNA, it is difficult to standardize results in the laboratory. AFLP can show only one single band difference. SSR markers play a major role in the genetic diversity studies in grapes due to its ability to identify genetic similarities and pedigree construction (Mwamahonje *et al*., 2015). It is locus specific, co-dominant and reproducible. SSR cannot sometimes identify the diversity at intra-variety level; the cost is high and it is a time consuming tool during construction and screening of genomic libraries, design and optimization of PCR primers (Doulati-Baneh *et al*., 2013). Alternatively, ISSR markers can usually identify the diversity at intra-variety level and among grape accessions (Pierozzi and Moura, 2016). The knowledge of genetic diversity studies helps plant breeders to find the best combinations in breeding programs. SSR markers are commonly used in many genetic diversity study of grape. SNPs markers have well distribution in the grape genome, high resolution and powerful in confirming the parentage of crops genotypes (Myles *et al*., 2011). They can identify the genetic differences among genotypes to single base level (Bautista *et al*., 2008). Through SNPs, it is possible to identify traits which are compatible to high yield, disease resistant, drought tolerant, fruit quality and quality wine (Bautista *et al*., 2008). Emphasis is given by grape breeders to keep the new varieties and cultivars in the gene bank for future application. The genetic resources conservation of grape accessions remains unstable. Only few traits have been exploited from the point of origin. In the future, most of them may be depleted if not conserved. Wild species which are highly influenced by natural situation should properly be genotyped (Emanuelli *et al*., 2013).

Genetic Resources and Conservation Approaches of Grape: The conservation of grapes involves both domesticated and wild species. Genetic resources maintain the genetic diversity which is a key for using grape accessions for future traits exploitation. The advancement of good yield, quality, and disease resistance depends on availability of germplasm in the genetic resource conservation centres. It offers wide range of choosing the suitable cultivars for wine, juice and raisins processed products (Villano, 2015). Germplasm conservation involves both *ex situ* and *in situ* conservation methods. Conservation can be in the genebank and plant tissue culture laboratory (Tehrim and Saji, 2011). Distribution of germplasm to farmers helps to ensure stability of germplasm so that, in case and damage and loss from genebank, the germplasm can be recollected from the farmers' field (FAO, 2014), for instance, Genetic Identification and Conservation of Local Turkish Grapevine (*Vitis vinifera* L.). Genotypes on the edge of extinction must be considered in both *in situ* and *ex situ* germplasm conservation (Sabir *et al*., 2018). Grape varieties Cabernet franc, Semillon, Petit, Verelot, Carmenere and Cot have been achieved through this method (Roby *et al*., 2014).

Molecular Breeding Tool: Molecular breeding is a method of plant breeding which uses tools such as marker assisted selection, marker assisted backcrossing, genomic

selection, bioinformatics, genome wide association and genetic engineering for improvement of new crop varieties. These tools shorten breeding cycles compared to conventional method (De Lima *et al*., 2006). The markers are designed based on the objective of the study and used for exploitation of traits of interest (Töpfer *et al*., 2011). The tools are applied in grape to supplement conventional breeding. Grape breeders use molecular breeding tools to screen and evaluate the best breeding parents for different traits (Töpfer *et al*., 2011). The common traits which are considered in grape improvement include yield, diseases and pest resistance, quality and maturity of the breeding materials (Sánchez-Mora *et al*., 2017). In addition, the tools are applicable to detect the genes that are tolerance to drought due to rapid increase of the temperature that affects the crop.

Marker-assisted Selection: Marker assisted selection is an application of molecular markers to exploit traits of interest for improvement. The tool supplements conventional breeding method which uses many cycles to achieve new improved varieties (Mwamahonje *et al*., 2015). In grapes, marker assisted selection has been useful for addressing a number of problems (Dalbó *et al*., 2001). Powdery mildew, a fungal disease in grape, has been addressed using markers introgression which is able to identify QTLs associated with disease resistance (Dalbó *et al*., 2001). This is achieved through crossing contrasting traits thus, developing population for genotyping. The QTLs are introgressed to the susceptible varieties through marker assisted backcrossing. Marker assisted selection plays a major role in grape breeding. It helps to address different challenges pertaining crops for example, diseases, pests, low yield, drought and long juvenile phase. Marker assisted selection shortens breeding programmes in crops (Edge-Garza *et al*., 2015). Markers which are tightly linked close to QTLs controlling traits of interest are mapped. The mapped QTLs can be developed through Recombinant Inbred Lines (RILs) by marker assisted backcrossing. Commonly used molecular markers for QTLs identification in crops include SSR and SNPs. These markers have high throughput, efficient resolution, polymorphism and are co-dominant which help to identify useful QTLs influencing traits parameters in crops (Viana *et al*., 2016). The point to note, marker assisted selection cannot work alone thus, depends on comparison with phenotypic data from the field. The QTLs for resistance to grapevine powdery mildew have been tested using SSR markers and identified the QTLs influencing resistance on the chromosome number 18 (Migicovsky and Myles, 2017). Proposing that, molecular markers SSR can be used to upgrade more powerful tools for high efficiency QTLs to pyramid new grape varieties for multiple disease resistance (Riaz *et al*., 2009). Pyramid resistance genes *Run1* and *Rpv1* for powdery mildew and down mildew respectively, have been achieved through backcrossing to susceptible grape varieties (Eibach *et al*., 2007). The efficiency of QTLs from different traits is proved by phenotyping of breeding materials in the field (Kicherer *et al*., 2017). The population which retains the traits from the donor parents are screened for further evaluation and finally released as the new varieties (Kicherer *et al*., 2017). Thus, marker assisted breeding and phenotyping depend on each other for disease control. Phenotyping with good trait heritability include leaves, shoot, rachis and berries (Riaz *et al*., 2011). The best way to confirm QTLs for powdery mildew using molecular markers is to use the reference of published mapped QTLs. Powdery mildew is highly favoured in warm climate with temperature ranging from 25-30^{O}C. The grape genotypes which are resistance under this temperature range can be screened as good for powdery mildew resistance. Therefore, QTLs influencing these traits are identified in the chromosome position before introgression to the susceptible varieties for resistance improvement (Töpfer *et al*., 2011). Introgression of QTLs of interest should be subject to yield as the important parameter expected from the consumers and farmers. *Run 1* gene originated from mascadine grapes (*V. rotundifolia*) is resistant to powdery mildew1. It is found in the chromosome number 12. It has transferred resistance to the genetic engineered grape cultivars. However, recent investigation has detected sporulation of powdery mildew pathogen on introgressed grape lines with RUN1 locus which is contributed by breakdown of resistance of the pathogen (Cadle-Davidson *et al*., 2011). Through marker assisted selection, new seedless grape varieties have been introduced by crossing seed versus seedless. By crossing seed and seedless grape genotypes produce 1:1 that provides opportunity of reducing the cost for evaluation. For instance, Sultanina is one of the seedless grapes in the grape breeding possessing QTL SD1 which controls the seedless (Karaagac *et al*., 2012). In addition, marker VMC7F2 accounts for decreasing of progeny size enhancing seedless screening. Although marker assisted selection identifies QTLs influencing the traits, QTLs are influenced by the environmental condition and geographical locations. Therefore, using large population size in the screening of QTLs and genes associated with traits of interest simplify identification. Grapevine seedlings take 3-6 years to produce fruits, thus, the use of molecular tools may shorten the breeding cycle. The challenge to achieve this, it needs large space of vineyard establishment where management may not be efficient by plant breeders. In this regard, marker assisted breeding

approaches become supportive. Previous studies have reported some of the useful QTLs for mapping berries in grape (Doligez *et al*., 2010). Through their effort, molecular markers linked to major loci for traits of interest in grape genotypes have been available for breeding enhancement. They have boosted conventional breeding method including establishment of seedless grape species which is influenced by SD1 QTLs (Karaagac *et al*., 2012). The optimization of protocol for different markers to increase efficiency is suggested (Riaz *et al*., 2009). Furthermore, marker assisted selection is very useful during pyramiding in breeding since it involves manipulation of many genes for disease resistance. Thus, it contributes to yield improvement. Fig.1 shows the application of marker assisted selection in grape improvement (Töpfer *et al*., 2011).

Functional GenomicS in Grape: Grapevine cultivars include many species adapted in different climate. The study of functional genomicS in these species is part of updating data in the web data source of the family *Vitaceae* (Doddapaneni *et al*., 2008). The database for annotated Expressed Sequence Tags (EST) and gene expression data involved in both *V. vinifera* and outside the *V. vinifera* species are available online (Doddapaneni *et al*., 2008). The database is controlled online for easily accessibility by every user globally. This enhances data availability in the world. Grape species information has been stored in the ExpDB database for referencing of above 320,000 EST sequences with high proportion of *V. vinifera* than other species. It includes grape species of both local and hybrid varieties. The online database for annotating grape species involves in the retrieval of putative homologous of the EST in various species with a long message of possession nucleotide identifies polygenic comparison and useful applicable primers (Doddapaneni *et al*., 2008). Functional genomicS contribute storing of functional genes of grape species for gene exploitation. EST use bioinformatics techniques to widen its efficient application for the specialization of genomic, marker design and gene annotation (Dong *et al*., 2005). Functional genomicS can regulate effect of excessive cold and chilling temperature affecting grape growth in most temperate countries. Coldness increaseS dormancy in grape, though, by maintaining gene sequences can assist in reduction of dormancy by screening method. It is possible to exhibit genetic variation of dormancy adaptation using genetic model. These mechanisms can be described by functional genomicS approach using molecular markers. This approach uses molecular markers for mapping biochemical and physiological processeS producing both induced short photoperiod and minimum temperature. This has provided plant breeders with grape species which are resistant (Fennell, 2014). They are regulating dormancy so that can be used on the required time. Studies have been conducted to exploit traits from seedless grape species (Sultani Cekirdeksiz *syn*. Sultanina) for developing new seedless varieties. This is achieved by sequencing for easy exploitation of the novel strategy for further studies (Di Genova *et al*., 2014). Other studies have concentrated deep in the heterozygosity genome and functional genomicS analysis (Di Genova *et al*., 2014). To accomplish this strategy, SNP markers are used for identification of seedless trait position in the chromosome. SNP catalogue is available for grape species. It is one of the powerful tools of functional genomicS analysis and very helpful for updating genome information in grapes (Di Genova *et al*., 2014). Functional genomicS, which is current tool in grape will enhance plant molecular breeding and therefore improving the production. Table 1 shows sources of *Vitis* species and the corresponding number of expressed sequence tags available in grape (Doddapaneni *et al*., 2008). It is time for plant breeders and molecular biologist to apply this tool for plant and animal genome storage in the database which have contribution for yield improvement.

Bioinformatics Tool: Bioinformatics is the branch of biology which deals with the study of the application of computing methods during analysis of biological information which are related to biomolecules on the large scale (Knowles *et al*., 2013). It provides a coverage of the targeted topic like the study of genomic and gene expression. It compares biological data of different plants and animals. This tool is used in the gene sequence prediction on the gene protein and the gene sequence of different plant genome (Luscombe *et al*., 2001). It works with the data stored in the gene bank repository of nucleotide sequence and database responsible protein arrangement. Because of high demand by researchers, there has been rapid increase of information for storage (Benson *et al*., 2000). Grape regulates part of sequencing reads using FASTA or FASTQ format techniques which works in high efficiencY using bioinformatics tool. Grape can run the set with high quality control producer followed by aligning readings to the genome. During the alignment, part of it is removed from read order. Grape can approximate the gene and the efficiency of transcript expression findings the exon and new transcripts identification. Grape genome can be run in computer with mapping specification and identification tools. But, there must be modular design for guiding. There are tools which play a major role in controlling data exchange formats which can be integrated (Knowles *et al*., 2013).

Genetic Engineering

Methodologies of Genetic Engineering in Grape: Genetic engineering in grapes is the transformation of genes from one plant species to another purposely for modification. One of the recommendations in genetic engineering is that the foreign gene should not negatively affect the genes in the recipient plant. The methodologies used in this tool for transformation is achieved through the application of *Agrobacterium tumefaciens* whereas the gene of interest is transferred from one species to *Agrobacterium* for multiplication. Thereafter, they are transferred to the target new plant species through Transfer DNA followed by DNA replication and gene expression. The second approach is biolistic bombardment which directly inserts the gene of interest from a donor to a grapevine genotype. It needs large number of DNA copies because of low multiplication compared to *Agrobacterium* mediated transformation. Grape industries utilize these technologies for maximization of production through diseases control in the vineyards (Vivier and Pretorius, 2000). *V. vinifera* was the first species to be transformed in grape (Mullins *et al*., 1990). The number of explants of different grape species has been successfully transformed followed by regeneration for gene expression of optimized protocol through somatic embyogenesis (Kim *et al*., 2013). The variety Chancellor was developed through introgression of *tfdA* gene which is tolerant to 2, 4-D herbicides (Mulwa *et al*., 2007). Testing the combination of technologies like functional genomics, QTLs and their position in chromosome, marker assisted selection; cloning and genetic engineering enhance tackling of problems in crops (Vivier and Pretorius, 2000). Conventional breeding through introgression of genes in crops like grape, potato, banana, apple and strawberry is tedious. Therefore, Cisgenes are transferred to another plant species with no linkage drag as in conventional method (Holme *et al*., 2013).

Transgenic Grape Cultivars: A number of grape varieties have been developed through genetic engineering to address various problems in grape production (Costantini *et al*., 2007). Transgenic grape cultivars Chardonnay (Vouillamo *et al.* 2006), Thompson Seedless, Silcora (Mezzetti *et al*. 2002), Chancellor and Koshusanjaku (Perl and Eshdat 1998) have been developed through genetic engineering. Fig. 2 illustrates the phenotypic differences between transgenic and non-transgenic grape varieties (Costantini *et al*., 2007).

Mutation Breeding

Conventional Mutagenesis in Grapes: Conventional mutagenesis plays an important role in grape production. It involves diversification of grape through spontaneous mutation which occurs naturally (Hensz, 1981). Spontaneous mutation in grape increases genetic diversity however, it takes long time to occur. Application of grape conventional mutagenesis can lead to production of seeded or seedless grape. However, spontaneous mutagenesis has been useful for development of seedless grapevine which is formed by parthenocarpy or stenospermocarpy (Vardi *et al*., 2008). Conventional breeding in grape has little genetic base which do not allow sufficient genetic diversity materials for screening due to long duration of spontaneous mutation and long life cycle (Hensz, 1981; Ulukapi and Nasircilar, 2015). Application of spontaneous mutation facilitates creation of variability for introduction of new seeded or seedless grape cultivars (Pathirana, 2011). Cultivars at this stage may need further screening to obtain the best genotypes for future improvement. Nevertheless, for seedless grape, induced mutation is among the methods which supplement spontaneous mutation to fasten genetic diversity to enhance production in grapes (Ahloowalia and Maluszynski, 2001). The promising mutants originated by spontaneous mutation are screened and treated with induced mutagens like gama-rays, x-rays and thermal neutrons which create new mutants.

In vitro Mutagenesis and Selection: Mutation induction technology is one of the tools used in grape improvement. Application of in vitro-induced mutagenesis such as chemical, x-rays, gama-rays fasten improvement of grape by producing mutants which may respond well in response to yield, disease resistance, quality wine and nutrient contents (Khawale *et al*., 2007). Improvement is done through screening the best traits and growing in the controlled tissue culture laboratories depending on the objective of the study (Khawale *et al*., 2007). This helps production of potential varieties. In vitro mutagenesis in plant is achieved by various methods like somatic embryogenesis, embryo rescue, and micropropagation. These tools increase the genetic variation. The *in vitro* mutagenesis in combination with tissue culture can enhance variability in grape breeding (Sinski *et al*., 2014). This is done by multiplying large number of explants, which later are managed in the screen house for acclimatization before planting in the field. This technology has simplified the availability of seed in grape production programs. Effort has increased breeding for new seedless grape because of tremendous increase of demand. This has widened the market for these species (Bergamini *et al*., 2013). The challenge is small size of the fruit which is influenced by damage of embryo stenospermocarpic grape species that affect signalling performance (Sweetman *et al*., 2012).

Hybridization

Conventional Hybridization of Grape: Conventional hybridization is the method of plant breeding which involveS screening the best genotypes for improvement (Hansen, 2000). It is useful for generation of new grape genotypes with quality wine, raising, juices and table grapes. Conventional hybridization is achieved by transferring the pollinizer pollens to the stigma of the recurrent maternal parents to develop new varieties. The progenies created after crossing contrasting traits are screened to obtain the best for further evaluation (Hansen, 2000). Only small portion of the target trait is transferred to the genotype which is lacking. The best populationS are selfed or backcrossed to the recurrent for trait enhancement. Selfing allows segregation of progenies which helps breeders to select the promising population by phenotyping method. Population that remain stable until final stage of evaluation are released the new varieties for adoption. Though, practically it is difficult to achieve conventional hybridization through conventional approach (Aazami, 2010).

***In vitro* Embryo Rescue:** *In vitro* embryo rescue is a tissue culture technology used to promote weak immature ovule embryo which fails to develop into full plants after fertilization (Li *et al*., 2015). It is grown *in vitro* tissue culture using media that contain nutrient concentrations specific for particular plant variety. Embryo rescue is commonly applied in weak embryo, interspecific hybrid plant species and breeding seedless grapes of various *Vitis* species where the embryo fails to develop after fertilization. Therefore, it needs boosting to grow into complete plant (Li *et al*., 2015). Uses of seedless grape varieties as female parent in grape breeding produce less than 15% proportion of viable seed due to abortion of fertilized ovule embryo (Yang *et al*., 2007; Zhang *et al*., 2013). Interestingly, uses of *in vitro* embryo rescue technique helps recovery of aborted ovule embryo during early growth stage which allows growth into complete plant (Guo *et al*., 2015). In ovulo embryo rescue technique is applicable under *in vitro* culture condition with controlled environment. It involves aseptic excision of weak ovule embryo allowing ovule culture in the culture medium. Depending on the varieties some embryo may be *in vitro* cultured without excising (Sharma *et al*., 1996). This facilitateS recovery of embryo which grows into normal complete plants same as in conventional approach (Valdez, 2005). *In vitro* embryo rescue promotes the development of the aborted ovule embryo from diploid and tetraploid cross varieties. Only few seed amount is produced depending on the genotypes crossed, medium concentration and growth regulators (Zhao and Guo, 2004). Fig. 3 shows embryo rescue and plant regeneration of seedless grape from ovule embryo by *in vitro* culture using recommended media concentration in comparison with the controls (Guo *et al*., 2015).

Somatic Cell Hybridization: Somatic embryogenesis is a propagation method for improving the genetic variation through the use of cellular techniques (Mezzetti *et al*., 2002). It forms non-zygotic embryos from the single somatic cell or group of somatic cells with enhancing developmental pathways which helps asexual grape embryo to develop into complete plant. Somatic hybrid can be tested from a number of genotypes using *in vitro* tissue culture. This technique involves use of media composed Murashige and Skoog medium containing 2, 4-dichlorophenoxyacetic acid (2, 4-D) and 6-benzylaminopurine in addition of casein hydrolysate (Prado *et al*., 2010). The somatic hybrid grape varieties show callus development in the medium while those without somatic cells will not be expressed (Ola´h *et al*., 2009). The source of plant tissue somatic embryo callus development is anther; nevertheless, other tissue parts of young grape plants like immature leaf, nucellar cells, vegetative cells of mature plants, and embryonic tissues are recommended (Perl and Eshdat, 1998). It is one of the methods of crop breeding achieved by interspecific and intragenic hybrid. It is achieved by fusing protoplasts of two different cultivars and screen the targeted hybrid cells and thus, generating into full hybrid plant (Grosser and Gmitter Jr, 2011). Protoplasts are produced from embryonic grape callus or suspension cultures of one cultivars and another protoplast from next cultivar. Protoplasts are fused, however in *in vitro* culture, at least some protoplastS must be embryonic to enhance regeneration of the new cultivars of fusing protoplast cultivars (Grosser and Gmitter Jr, 2011). Protoplast fusion is used for genetic transfers of traits of interest from one plant species to another thus, enhancing breeding in crops. The explants of grapes such as shoots, embryo, leaves, petioles and flowers produce somatic embryos (Mezzetti *et al*., 2002). To make the cultivar stable, explants are tested from the top reserve part of the cultivars. Some explants become active within a short time and are generated easily in culture medium with high generation of somatic embryogenesis (Perl and Eshdat, 1998). They maintain genetic stability of cultivar which enhances somatic embryogenesis for propagation and avoids rapid genetic variability to maintain genotype for a long time.

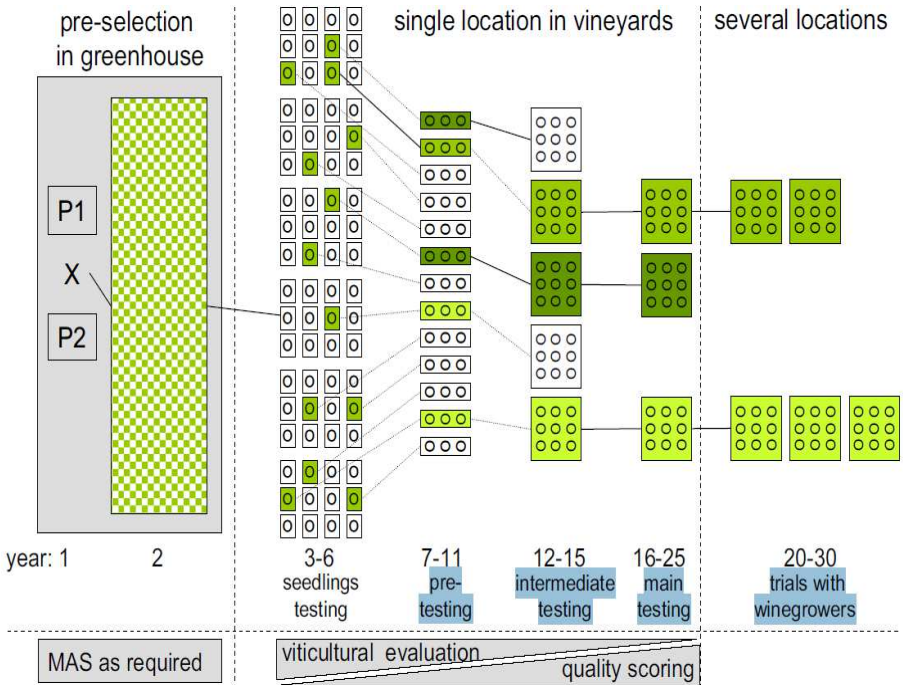

Fig. 1. Steps indicating time scale of a typical wine grape breeding programme. A pre-selection eliminating e.g. highly mildew susceptible is conducted in the greenhouse followed by MAS for traits difficult to evaluate prior to planting in the vine yard. MAS will receive increasing importance during the next couple of years. The various stages of testing seedling- (1 vine), pre- (10 vines), intermediate- (50 vines) and main stage (500 vines), with increasing number of vines are followed by trials in viticultural practice. Usually developing a new cultivar requires 25 to 30 years. Acceleration of the breeding process of up to 10 years is expected by the use of MAS and by merging pre- and intermediate testing to one testing phase as planting material becomes available. Source: (Töpfer *et al*., 2011)

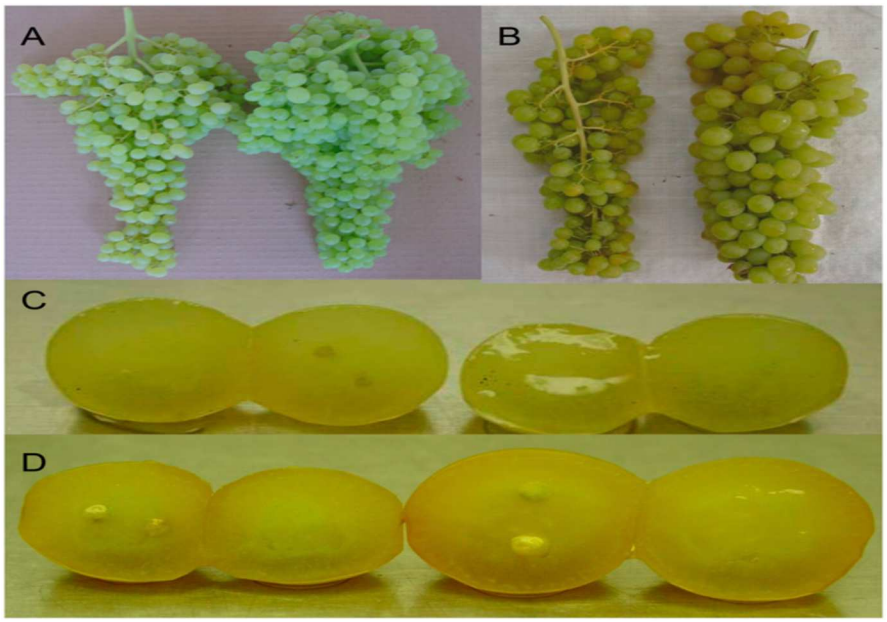

Fig. 2. Grape bunches of Thompson Seedless control (A, left) and genetically modified (GM; A, right), Silcora control (B, left) and GM (B, right). Berry section of Thompson Seedless control (C, left) and GM (C, right) and Silcora control (D, left) and GM (D, right).
Source: (Costantini *et al*., 2007).

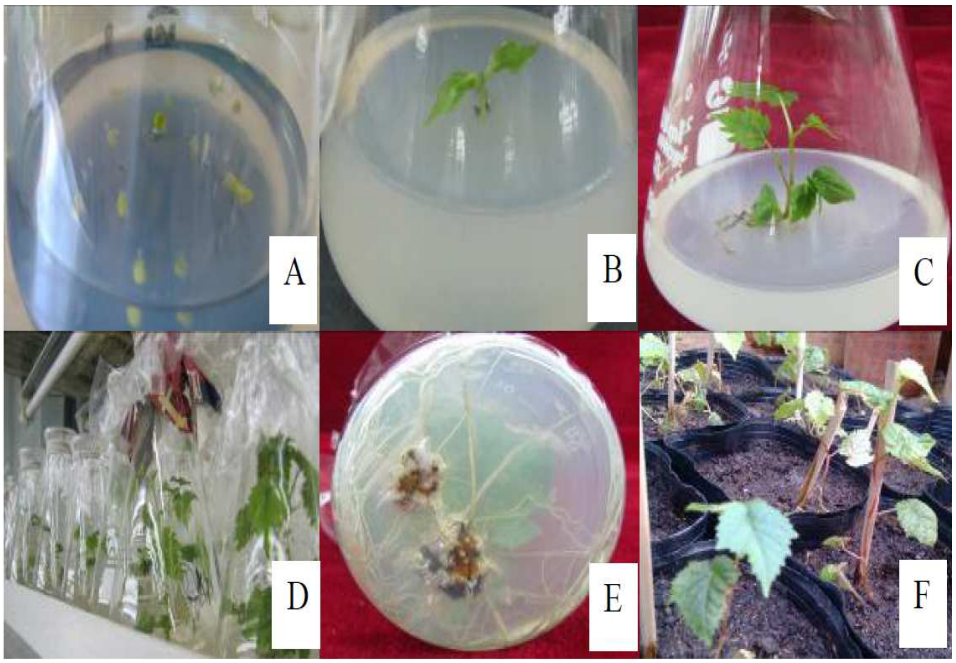

Fig. 3. Embryo rescue and plant regeneration of Venus seedless grape. Note: A. Ovules cultured; B. Embryo germination; C,D. Seedling survival; E. Rooting; F. Transplanted into the greenhouse. Source: (Guo *et al*., 2015).

Table 1. Sources of *Vitis* and the ESTs sequences available in the database.

Sr. No.	*Vitis species/Hybrids*	Number of ESTs
1	*V. vinifera (wine grape)*	303,054
2	*V. shuttleworthii*	10,704
3	*V. hybrid cultivar*	6,533
4	*V. rupestris × V. arizonica*	5421
5	*V. aestivalis*	2,101
6	*V. riparia*	1,910
7	*V. pseudoreticulata*	122
8	*V. cinerea × V. rupestris*	61
9	*V. cinerea × V. riparia*	58

Source: (Doddapaneni *et al*., 2008)

Conclusion: Conventional and molecular grape breeding methods play a significant role to address biotic and abiotic stresses for grape improvement. Germplasm collection ensures availability of breeding materials. The gene banks for conservation of different germplasm need to be established, repaired and maintained for future use. Breeding broadens varieties which may adapt into multi-environments. Breeding should involve phenotyping, conventional method and molecular breeding tools such as molecular markers, marker assisted selection, genomic, genome wide association, genetic engineering and tissue culture to shorten duration for release of new varieties. The breeding should involve wild grape species which carry important traits for instance, drought tolerance, and disease resistance, quality of wine and high content of medicinal substances for preventing the disease. Introgression of QTLs associated with traits of tolerance like, abiotic stress (salinity, drought, temperature and deficiency of soil fertility) and biotic stress (diseases and pests) to susceptible grape cultivars may enhance production.

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REFERENCES

Aazami, M.A. (2010). Effect of some growth regulators on *in vitro* culture of two *Vitis vinifera* L. cultivars. Rom Biotechnol Lett. 15(3): 5229–5232.

Ahloowalia, B.S. and M. Maluszynski. (2001). Induced mutations - a new paradigm in plant breeding. Euphytica. 118(2): 167-73.

Ates, F., H. Coban, Z. Kara and A. Sabir. (2011). Ampelographic characterization of some grape

cultivars (*Vitis vinifera* L.) grown in south-western region of Turkey. Bulg J Agric Sci. 17(3): 314–324.

Bautista, J., G.S. Dangl, J. Yang, B. Reisch and E. Stover. (2008). Use of genetic markers to assess pedigrees of grape cultivars and breeding program selections. Am J Enol Vitic. 59(3): 248–254.

Benson, D.A., I. Karsch-Mizrachi, D.J. Lipman, J. Stell, B.A. Rapp and D.L. Wheeler. (2000). Genbank. Nucleic Acids Res. 28(1): 15–18.

Bergamini, C., M.F. Cardone, A. Anaclerio, R. Perniola, A. Pichierri, R. Genghi, V. Alba, L.R. Forleo, A.R. Caputo, C. Montemurro, A. Blanco and D. Antonacci. (2013). Validation assay of p3_VvAGL11 marker in a wide range of genetic background for early selection of stenospermocarpy in *Vitis vinifera* L. Mol Biotechnol. 54(3): 1021–1030.

Bowers, J., J.M. Boursiquot, P. This, K. Chu, H. Johansson and C. Meredith. (1999). Historical Genetics: The parentage of chardonnay, gamay, and oher wine Grapes of Northeastern France. Science. 285(5433): 1562–1565.

Cadle-Davidson, L., S. Mahanil, D.M. Gadouri, P. Kozma and B.I. Reisch. (2011). Natural infection of Run1 -positive vines by naïve genotypes of *Erysiphe necator*. Vitis. 50(4): 173-175

Costantini, E., L. Landi, O. Silvestroni, T. Pandolfini, A. Spena and B. Mezzetti. (2007). Auxin synthesis-encoding transgene enhances grape fecundity [OA]. Plant Physiol. 143: 1689–1694.

Da Costa, A.F., P.E. Teodoro, L.L. Bhering, F.D. Tardin, R.F. Daher, W.F. Campos, A.P. Viana and M.G. Pereira. (2017). Molecular analysis of genetic diversity among vine accessions using DNA markers**.** Genetics and Molecular Research. 16(2).

Dalbó, M.A., G.N. Ye, N.F. Weeden, W.F. Wilcox and B.I. Reisch. (2001). "Marker-assisted Selection for Powdery Mildew Resistance in Grapes," *J*. Amer. SOC. Hort. Sci. 126(1): 83–89.

De Lima, J.C., A. Crippa, L. Garrido, U.A. Camargo and P.R. Dias de Oliveira. (2006). Molecular analysis of the seedlessness character in grape using RAPD markers. Crop Breed Appl Biotechnol. 6: 209-214.

Di Genova, A., A.M. Almeida, C. Muñoz-Espinoza, P. Vizoso, D. Travisany, C. Moraga and A. Maass. (2014). Whole genome comparison between table and wine grapes reveals a comprehensive catalog of structural variants. BMC Plant Biol. 14:7.

Doddapaneni, H., H. Lin, M.A. Fwalker, J. Yao and E.L. Civerolo. (2008). Vitis expdb: A database resource for grape functional genomics. BMC Plant Biol. 8(1): 23.

Doligez, A., Y. Bertrand, S. Dias, M. Grolier, J.F. Ballester, A. Bouquet and P. This. (2010). QTLs for fertility in table grape (*Vitis vinifera* L.). Tree Genet Genomes. 6(3): 413-422.

Dong, Q., L. Kroiss, F.D. Oakley, B.B. Wang and V. Brendel. (2005). Comparative EST analyses in plant systems. Meth Enzymol. 395: 400–418.

Doulati-Baneh, H., S.A. Mohammadi and M. Labra. (2013). Genetic structure and diversity analysis in *Vitis vinifera* L. cultivars from Iran using SSR markers. Sci Hort. 160: 29–36.

Edge-Garza, D.A., J.J. Luby and C. Peace. (2015). Decision support for cost-efficient and logistically feasible marker-assisted seedling selection in fruit breeding. Mol. Breed. 35: 223.

Eibach, R., E. Zyprian, L. Welter and R. Töpfer. (2007). "The use of molecular markers for pyramiding resistance genes in grapevine breeding," Vitis. 46: 120–124.

Emanuelli, F., S. Lorenzi, L. Grzeskowiak, V. Catalano, M. Stefanini, M. Troggio and M.S. Grando. (2013). Genetic diversity and population structure assessed by SSR and SNP markers in a large germplasm collection of grape. BMC Plant Biol. 13:39. doi:10.1186/1471-2229-13-39.

FAO. (2014). Genebank Standards for Plant Genetic Resources for Food and Agriculture. Rev. ed. Rome

Fennell, A. (2014). Genomics and functional genomics of winter low temperature tolerance in temperate fruit crops. Crit Rev Plant Sci. 33(2–3): 125–140.

FAOSTAT. (2017). FAO statistical databases, production statistics. http://faostat.fao.org/.

Gascuel, Q., G. Diretto, A.J. Monforte, A.M. Fortes and A. Granell. (2017). Use of natural diversity and biotechnology to increase the quality and nutritional content of tomato and grape. Front Plant Sci. 8: 652.

Grosser, J.W. Gmitter Jr, F.G. (2011). Methods of crop breeding achieved by interspecific and intragenic hybrid. Plant Cell Tiss Organ Cult. 104: 343–357.

Guo, X., W. Chen, Y. Guo, Z. Liu, H. Lin, J. Tang, K. Li and Y. Zhao. (2015). Influencing factors of embryo rescue in seedless grape. Pak J Bot. 47(2): 669-673.

Hansen, M.K. (2000). "Genetic Engineering is not an Extension of Conventional Plant Breeding; How genetic engineering differs from conventional breeding, hybridization, wide crosses and horizontal gene transfer," *Consumer Policy Institute/Consumers Union* January, 2000.

Hvarleva, T., A. Bakalova, K. Rusanov, G. Diakova, I. Ilieva, A. Atanassov and I. Atanassov. 2009. Toward marker assisted selection for fungal disease resistance in grapevine. Biotechnol. Biotechnol. Equip. 23(4): 1431–1435.

Hensz, R.A. (1981). Bud mutations in citrus cultivars in Texas. International Citrus Congress (4th : 1981 : Tokyo, Japan) International Society of Citriculture. 1**:** 89-91.

Holme, I.B., T. Wendt and P.B. Holm. (2013). Intragenesis and cisgenesis as alternatives to transgenic crop development. Plant Biotechnol J. 11(4): 395–407.

Karaagac, E., A.M. Vargas, M.T. Andrés, I. Carreño, J. Ibáñez, J. Carreño and J.A. Cabezas. (2012). Marker assisted selection for seedlessness in table grape breeding. Tree Genet Genomes. 8(5): 1003–1015.

Khawale, R.N., V. Yerramilli and S.K. Singh. (2007). Molecular marker-assisted selection of in vitro chemical mutagen-induced grapevine mutants. Curr Sci. 92(8): 1056-1060.

Kicherer, A., K. Herzog, N. Bendel, H. Klück, A. Backhaus, M. Wieland, J.C.L. Rose, L. Klingbeil, T. Läbe, C. Hohl, W. Petry, H. Kuhlmann, Seiffert and U. Töpfer. (2017). "Phenoliner: A New Field Phenotyping Platform for Grapevine Research," *Sensors*. 17(7).

Kim, S.H., I. Shin, K.H. Cho, D.H. Kim, H.R. Kim, J.H. Kim and H.S. Hwang. (2013). Plant regeneration and transformation of grape (*Vitis labrusca* L.) via direct regeneration method. Plant Biotechnol J. 40(4): 210–216.

Knowles, D.G., M. Röder, A. Merkel and R. Guigó. (2013). Grape RNA-seq analysis pipeline environment. Bioinformatics. 29(5): 614–621.

Laimer, M. (2007). Transgenic grapevines. Transgenic Res. 1(1): 219-227.

Leão, P.C., C.D. Cruz and S.Y. Motoike. (2011). Genetic diversity of table grape based on morphoagronomic traits. Sci Agric. 68(1): 42–49.

Leão, P.C.S., S. Riaz, R. Graziani, G.S. Dangl, S.Y. Motoike and M.A. Walker. (2009). Characterization of a Brazilian grape germplasm collection using microsatellite markers. Am J Enol Vitic. 60(4): 517–524.

Li, J., X.Wang and Y. Wang. (2015). Embryo rescue technique and its applications for seedless breeding in grape. Plant Cell Tiss Organ Cult. 120: 861–880.

Luscombe, N.M., D. Greenbaum and M. Gerstein. (2001). What is bioinformatics? An introduction and overview. Year book of Medical Informatics, pp 83–99.

Martínez, L.E., P.F. Cavagnaro, R.W. Masuelli and M. Zúñiga. (2006). SSR-based assessment of genetic diversity in South American *Vitis vinifera* varieties. Plant Sci. 170(6): 1036–1044.

Mcclure, K.A., J. Sawler, K.M. Gardner, D. Money and S. Myles. (2014). Genomics: a potential panacea for the perennial problem. Am J Bot. 101(10): 1780–1790.

Mezzetti, B., T. Pandolfini, O. Navacchi and L. Landi. (2002). Genetic transformation of *Vitis vinifera* via organogenesis. BMC Biotechnol. 2:18.

Migicovsky, Z. and S. Myles. (2017). Exploiting wild relatives for genomics-assisted breeding of perennial crops. Front Plant Sci. 8.

Migicovsky, Z., J. Sawler, K.M. Gardner, K.M. Aradhya, H. Bernard, B.H. Prins, H.R. Schwaninger, C.D. Bustamante, E.S. Buckler, G. Zhong, P.J. Brown and S. Myles. (2017). Patterns of genomic and phenomic diversity in wine and table grapes. Horticulture Research. 4:17035.

Mullins, M.G., F.C.A. Tang and D. Facciotti. (1990). Agrobacterium-mediated genetic transformation of grapevines: transgenic plants of *vitis rupestris* scheele and buds of *Vitis vinifera* L. Nat Biotechnol. 8(11): 1041–1045.

Mulwa, R.M.S., M.A. Norton, S.K. Farrand and R.M. Skirvin. (2007). Agrobacterium -mediated transformation and regeneration of transgenic "chancellor" wine grape plants expressing the tfdA gene.Vitis. 46 (3): 110–115

Mwamahonje, A., D. Kilambo and T. Feyissa. (2015). Molecular genetic diversity of grapevines to produce powdery mildew resistant genotypes. AJRC. 3(8): 10–33.

Myles, S., A.R. Boyko, C.L. Owens, P.J. Brown, F. Grassi, M.K. Aradhya and E.S. Buckler. (2011). Genetic structure and domestication history of the grape. Proc Natl Acad Sci USA. 108(9): 3530–3535.

Ola´h R., A. Zok, A. Pedryc, S. Howard and L.G. Kovács. (2009). Somatic embryogenesis in a broad spectrum of grape genotypes. Sci Hort. 120: 134–137.

Pathirana, R. (2011). Plant mutation breeding in agriculture. CABI. 6(32): 1–20.

Perl, A. and Y. Eshdat. (1998). DNA transfer and gene expression in transgenic grapes. Biotechnol Genet Eng Rev. 15(1): 365–386.

Pierozzi, N.I., M.F. Moura, N.I. Pierozzi and M.F. Moura. (2016). Karyotype analysis in grapevines. Rev Bras Frutic. 38(1): 213–221.

Prado, M.J., M.P. Grueiro, M.V. González, P.S. Testillano, C. Domínguez, M. López and M. Rey. (2010). Efficient plant regeneration through somatic embryogenesis from anthers and ovaries of six autochthonous grapevine

cultivars from Galicia (Spain). Sci Hort. 125: 342–352.

Riaz, S., J.M. Boursiquot, G.S. Dangl, T. Lacombe, V. Laucou, A.C. Tenscher and M.A. Walker. (2013) Identification of mildew resistance in wild and cultivated Central Asian grape germplasm. BMC Plant Biol. 13:149.

Riaz, S., A.C. Tenscher, R. Graziani, A.F. Krivanek and M.A. Walker. (2009). Using marker assisted selection to breed for pierce's disease resistance in grapevine. Am J Enol Vitic. 6(2): 200–207.

Riaz, S., A.C. Tenscher, D.W. Ramming and M.A. Walker. (2011). Using a limited mapping strategy to identify major QTLs for resistance to grapevine powdery mildew (*Erysiphe necator*) and their use in marker-assisted breeding. Theor Appl Genet. 122(6): 1059–1073.

Roby, J.P., C. Van Leeuwen, E. Gonçalves, A. Graça and A. Martins. (2014). The preservation of genetic resources of the vine requires cohabitation between institutional clonal selection, mass selection and private clonal selection. BIO Web of Conferences. 3.

Sabir, A., H. Ikten, N. Mutlu and D. Sari. (2018). Genetic Identification and Conservation of Local Turkish Grapevine (Vitis vinifera L.) Genotypes on the Edge of Extinction. Erwerbs-Obstbau. **60:** 31–38

Schnell, R.J., D.N. Kuhn, J.S. Brown, C.T. Olano, W. Phillips-Mora, F.M. Amores and J.C. Motamayor. (2007). Development of a marker assisted selection program for cacao. Phytopathology. 97(12): 1664–1669.

Sánchez-Mora, F.D., L. Saifert, J. Zanghelini, W.T. Assumpção, C.A. Guginski-piva, R. Giacometti, E.I. Novak, G.H. Klabunde, R. Eibach, L. Dal Vesco, R.O. Nodari and L.J. Welter. (2017). Behavior of grape breeding lines with distinct resistance alleles to downy mildew (*Plasmopara viticola*). CBAB. 17: 141-149.

Sefc, K.M., F. Lefort, M.S. Grando, M.R. Thomas and F. Lefort. (2001). Microsatellite markers for grapevine. In: Roubelakis-angelakis KA (ed) a state of the art. Molecular biology and biotechnology of the grapevine. Kluwer Academic Publishers, Dordrecht, p 433-466.

Sharma, D.R., R. Kaur and K. Kumar. (1996). Embryo rescue in plants: a review. Euphytica 89: 325–337.

Sinski, I., D.D. Bosco, N.I. Pierozzi, J.D.G. Maia, P.S. Ritschel and V. Quecini. (2014). Improving in vitro induction of autopolyploidy in grapevine seedless cultivars. Euphytica. 196(2): 299–311.

Sousa, E.C., A.M.A. Uchôa-Thomaz, J.O.B. Carioca, S.M. Morais, A. De Lima, C.G. Martins and L.L. Rodrigues. (2014). Chemical composition and bioactive compounds of grape pomace (*Vitis vinifera* L.), Benitaka variety, grown in the semiarid region of Northeast Brazil. Food Sci Technol Res. 34(1): 135–142.

Sweetman, C., D.C. Wong, C.M. Ford and D.P. Drew. (2012). Transcriptome analysis at four developmental stages of grape berry (*Vitis vinifera* cv. Shiraz) provides insights into regulated and coordinated gene expression. BMC Genomics. 13: 691.

Tehrim, S. and V. Saji. (2011). In Vitro Establishment, Conservation and its Implications for Grape Germplasm Biodiversity. Romanian Biotechnological Letters. 16(6): 6781-6789.

This, P., T. Lacombe and M.R. Thomas. (2006). Historical origins and genetic diversity of wine grapes. Trends in Genetics. 22(9): 511-519.

Töpfer, R., L. Hausmann, M. Harst, E. Maul, E. Zyprian and R. Eibach. (2011). New horizons for grapevine breeding. Fruit Veg Cereal Sci Biotech. 5(1): 79-100.

Ulukapi, K. and A.G. Nasircilar. (2015). Developments of gamma ray application on mutation breeding studies in recent years. International Conference on Advances in Agricultural, Biological & Environmental Sciences. July 22-23, 2015 London(UK).

Valdez, J.G. (2005). Immature embryo rescue of grapevine (*Vitis vinifera* L.) after an extended period of seed trace culture. Vitis. 44(1): 17–23.

Vardi, A., I. Levin and N. Carmi. (2008). Induction of seedlessness in citrus: From classical techniques to emerging biotechnological approaches. J Am Soc Hortic Sci. 133(1): 117–126.

Viana, A.P., M.D.V. Resende, S. Riaz, M.A. Alker, A.P. Viana, M.D.V. Resende and M.A. Dewalker. (2016). Genome selection in fruit breeding: application to table grapes. Sci Agric. 73(2): 142–149.

Villano, C. (2015). Discovery and genotyping of DN sequence variations in grape. PhD Thesis. University of Naples Federico II, Italy.

Vivier, M.A. and I.S. Pretorius. (2000). Genetic improvement of grapevine: tailoring grape varieties for the third millennium. S Afr Jenol Vitic. 21: 5-26.

Vouillamo, J.F., P.E. Mcgover, A. Ergul, G. So¨Ylemezog˘lu, G. Tevzadze, C.P. Meredith and M.S. Grando. (2006). Genetic characterization and relationships of traditional grape cultivars from Transcaucasia and Anatolia. Plant Genet Resour. 4(2): 144–158.

Yang, D., W. Li, S. Li, X. Yang, J. Wu and Z. Cao. (2007). In vitro embryo rescue culture of F_1 progenies from crosses between diploid and

tetraploid grape varieties. Plant Growth Regul. 51: 63–71.

Yilmaz, Y. and R.T. Toledo. (2004). "Health aspects of functional grape seed constituents," Trends Food Sci.Technol. 15: 422–433.

Zhang, X.X., M. Liu, M.Y. Wang, C.Q. Shi and X.Y. Cheng**.** (2013). Developmental and morphological study of the coleorhizae in hemerocallis (Liliaceae). Pak J Bot. 45(5): 1673-1676.

Zhao, S.J. and Z.J. Guo. (2004). Advances in research of breeding seedless triploid grapes. J Fruit Sci. 21(4): 360–364.

Zhou, Y., M. Massonnet, J. Sanjak, D. Cantu, B.S. Gaut. (2017). Evolutionary genomics of grape (*Vitis vinifera* ssp. *vinifera*) domestication. Proc Natl Acad Sci U S A. 114(44): 11715-11720.