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Prospects of Biotechnology in Grape Breeding

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ABSTRACT

Genetic improvement of grape cultivars to obtain high quality wine and table grape varieties by conventional breeding methods has been difficult and time consuming. The elite grape varieties developed by conventional breeding techniques have less resistance to fungal and bacterial diseases, drought, quality and yield per plant. Breeding programs of grapes are difficult due to lack of true bred from seed and few traits of importance. Though most grapes constitute large number of genes, they have less effect in tolerating biotic and abiotic stresses. Genetic improvement of grapevine (*Vitis vinifera* L.) through application of biotechnological techniques provide new strategies in grape breeding programs based on rapid selection or induction of desired traits by marker assisted breeding, genetic engineering and plant tissue culture. This review paper therefore, aims to discuss biotechnological techniques proposed for improvement of grape breeding.

Indexing terms/Keywords

Biotechnology; Grape breeding; Genetic engineering; Marker assisted breeding; Plant tissue culture.

Academic Discipline And Sub-Disciplines

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INTRODUCTION

Grapevine (*Vitis vinifera* L.) is a horticultural crop which is used worldwide for production of wine, table grapes, dried fruits and grape juice **(**Love and Paull, 2014).Genetic improvement of grape cultivars to obtain high quality wine and table grape varieties by conventional breeding methods is difficult and time consuming (Diab et al., 2011).The elite grapevine varieties developed by conventional breeding techniques with useful traits have been reported to be low in resistance to fungal and bacterial diseases, pest infections, drought, quality status and yield per plant (Diab et al., 2011). Breeding programs of grapes are difficult due to lack of true bred from the seed and few traits of significance. Most of grapes contain large number of genes with less effect in tolerating biotic and abiotic stresses (Vivier and Pretorius, 2000). Fungal diseases such as powdery mildew and down mildew affect grapes and are difficult to control even if farmers frequently apply fungicides. It is costy to afford and the fruits harvested after several application of fungicides contain high level of residuals in foodstuffs and destruct environment resulting in poor yield (Délye et al., 1997; Chen et al., 2007).In order to reduce effect on agricultural and health use, genetic improvement of grapevine (*Vitis vinifera* L.) is potential to improve grape production (Semagn et al*.,* 2006). The modern biotechnological techniques provide new strategies for grape breeding programs (Hock et al., 2003). They have offered many techniques for plant breeding based on rapid selection or inducing desired characteristics. By applying new techniques originating from biotechnology, plant breeding should be improved. New technology in grape breeding is imperative to improve quality, resistance to disease, taste, and yield (Maria et al., 2011).The technology should further be based on developing new grape varieties with early fruits bearing, high resistant to drought and cold (Kikkert and Reisch, 1996). During conventional breeding in grape, the possibility of self-fertilization depression and somatic mutations to occur is very high hence, affecting appropriate traits. On top of that, it has been reported that in appropriate genetic supplies and insufficient information about the grape genome proposed to be the important problems occurred during the grape improvement (Isci et al., 2010). Scientists and Researchers need to introduce new biotechnological tools to boost conventional breeding in grape sector (Kumpatla et al., 2012). Molecular markers played a major role in grape improvement for instance; they used to trace useful alleles in segregation population followed by mapping because even the simplest traits with large number of genes need to be manipulated (Guo et al., 2014). Some of the molecular markers that have been useful in grape breeding include; Simple Sequence Repeats (SSR), Inter Simple Sequence Repeat Amplified Fragment Length Polymorphism (AFLP) markers (Troggio et al., 2007; Jonah et al., 2011). It has been reported that, re-sequencing of genomes is useful for genome-wide discovery of markers with high-throughput genotyping platforms, for instance, SSRs and SNPs (Kumpatla et al., 2012). The new markers are useful for marker assisted selection, such as marker assisted backcross selection, breeding programs and new plan of grape breeding. According to suggestion by Gray et al., (2014), conventional breeding is not recommended practically for addition of desired disease resistance traits to elite cultivars of *Vitis* due to long lifecycle, severe inbreeding depression, as well as complex genetic control of oenological qualities. Most of the varieties cultivated worldwide are of many years ago, they have been maintained by stringent system of vegetative propagation (Kumpatla et al., 2012).This review aims to discuss the advanced biotechnological techniques for improvement of grape breeding based on genetic engineering, tissue culture and marker assisted breeding.

BIOTECHNOLOGICAL APPROACHES IN GRAPE BREEDING

Tissue culture in modern grape breeding

Tissue culture is defined as biotechnological technique which involves *in vitro* aseptic culture of cells, tissues, organs as well as a whole plant with constant nutritional and environmental conditions. It is useful in virus and other pathogens elimination in addition to identification of new improved grape varieties (Thorpe, 2007). Tissue culture technology lies in the production of high quality and uniform planting material which can be produced in a year-round basis with respect to disease-free conditions during any season (Gray et al., 2014). Tissue culture technology requires controlled temperature, photoperiod and relative humidity during propagation process. However, these conditions are dependent on the plant species. Micropropagation produces high quality, disease-free and uniform planting materials. However, it is costy and requires skilled manpower (Thorpe, 2007).Abido et al., (2013) revealed that micropropagation is a useful assay of rapid mass propagation and regeneration via organogenesis and embryogenesis of valuable alleles obtained by non-conventional methods, this finding is similar with the previous study by Alizadeh et al., (2010). The study by other researchers has reported positive results of *In vitro* propagation on grapes (Filtiz et al., 2004). However, other cultivars are endangered for instance,"Muscat of Alexandria" cultivar, tissue culture technique proposed the useful method for reserving the cultivar (Abido et al., 2013). Six phases of micro propagation have been documented in grapevine namely: selection of explants, establishment of the explants *in vitro,* shoot production from the established explants, shoots multiplication from the sub cultured shoots, rooting of the sub cultured shoots, and transfer of propagules to soil (Chee et al., 1984). Rapid proliferation in tissue culture is achieved from tiny stem cuttings, axillary buds, and to a limited extent from somatic embryos and cell clumps in suspension cultures. The cultured cells and tissue can take several pathways (Popescu et al., 2010). Plant tissue cultures are initiated explants, taken from any part of a plant, explant is removed surgically, surface sterilized and placed on a nutrient medium to initiate the culture, that is multiplied repeatedly by subculture (Thorpe, 2007).Tissue culture technique has been applied in virus elimination on grapes, it has been reported to detect virus and assess resistance of grapevine to virus through grafting technique however, it is time consuming (Lee and Wetzstein, 1990). The methods for plant regeneration or plant propagation proved to have high efficiency in assisting the use of tissue culture technology in grape breeding. Efficient regeneration methods have been reported to show success in the application of genetic transformation in grapes (Vitis vinifera L.)(Stamp et al., 1990). The regeneration and micro propagation of grapevine is achieved through shoot tip and axillary bud culture by using high efficient in vitro shoot micropropagation protocol. Such a protocol is useful for future grape breeding using genetic engineering (Diab et al., 2012). Proliferations of callus, somatic embryogenesis and adventitious roots have previously been applied in grapevine (Thorpe, 2007). Haploids and homozygous diploids of seedless-seedless hybridization have been reported to have high potential in grapevine

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improvement and genetic studies (Popescu et al., 2010). Previous,Sim and Golino, (2010) reported thatheat treatment is one of the technique for virus diseases elimination in grapevines. However, recently, tissue culture therapy has been used for elimination of not only viral diseases but also fungal and bacterial diseases .The technology of macro shoot tissue culture has proved to eliminate Ag*robacteriumvitis*bacterium, which causes grape crown gall disease however, does not reliably eliminate virus infections (Sim and Golino, 2010). Although, Sim, (2006) has been reported micro shoot tissue culture to eliminate many diseases including virus infections for instance, Grapevine fanleaf virusin grapevines, however,it is time consuming. Micropropagation in *Vitis vinifera* can be done using fragmented shoot apical meristems, axillary-bud microcuttings or through adventitious bud formation (Heloir et al., 1997). However other studies have reported less success in *Vitis vininefera* (Mhatre et al., 2000). Regarding the effort made by scientists to improve new technology in grape production, the application of tissue culture techniques in the grape-growing industry has not been well modernized (Gray et al., 2005).The reason is due to high cost compared to conventional methods as well as somaclonal variation. Rapid multiplication of grapevine plantlets has shown positive results through nodal cuttings comprising a single axillary bud (Bigger, 2010 and Nas et al., 2005). This method prevent somaclonal variation accompanied by hyperhydricity (vitrification) caused in part by excessive cytokinins in the multiplication medium (Bigger, 2010). Various *Vitis vinifera* L. genotypes has been regenerated by somatic embryogenesis from explants however, genotypic variation still exist (Martinelli and Gribaudo, 2001). Somatic embryogenesis and regeneration of complete *V. vinifera* was first reported by Mullins and Srinivasan (1976). The regeneration of *V. vinifera* somatic embryos were reported from anthers, immature zygotic embryos, immature leaves, tendrils, immature ovaries, leaf discs as well as from filaments (Mauro et al., 1986; Stamp and Meredith, 1988a; Stamp and Meredith, 1988b; Salunkhe et al., 1997; Nakano et al., 2000; Das et al., 2002 and Nakajima and Matsuta, 2003). Even though, embryogenic potential of *V. vinifera* varied with genotype and explant type, better results have been reported to be obtained from regeneration protocols which involve the use of anthers as explants (Bouquet and Torregrosa, 2003). On the other hand, based on the results by Martinelli*et al*., (2001) reported that this system provides poor results with *V. vinifera* cultivars. Therefore, the use of new explants for regeneration of somatic embryos in *V. vinifera* needs to be adopted. Massive propagation in grapes has reported in Chile (Tapia et al., 2007). It is difficult to achieve plant regeneration from grape (*Vitis* spp.) through somatic choice of improved genetically modified lines (Li, et al., 2014).

Genetic engineering

The continuous breeding and genetic engineering of grapevine have been reported to increase new varieties of grapevine with high quality and resistance to mildew diseases which ultimately increase grape production (Kurth et al., 2012). Genetic modification of grapevine offers high possibility to be a key technology for later grape improvement. Nevertheless, more research and testing proposed to be done in order to isolate agronomically useful genes from grapevine prototypes with desired phenotype (Kurth et al., 2012). Due to long time of conventional breeding in grapevine, biotechnology proposed the development of gene transfer tools for a particular variety to avoid antibiotic, selections should be done properly for precision grape breeding. A biotechnological method is among the alternatives and very effective tool to be adopted by plant breeders (Hock et al., 2003).The advantage of biotechnology helps breeders to identify the targeted traits inside the plant and transfer to the intended plant species during breeding (Hock et al., 2003).Genetic transformation in grapevine is used to improve varieties with deficiencies such as resistance to disease, yield and quality thus, enhancing grape yield (Tsvetkov et al., 1997). The first significant progress of grapevine transformation was reported when embryonic cell lines used as target tissue for grapevine transformations, leading to formation of commercially transgenic grape cultivars (Vivier and Pretorius, 2002). Though, this report is contradicted with the report by Akkurt et al., (2012)who reported that the wine industry has entered the 21st century without a single transgenic grapevine variety being used on a commercial scale. This was first innovated in 1989; however, recently the researchers have gradually shifted from the development of grapevine transformation technology to the implementation of the technology in the generation of useful plant lines (Vivier and Pretorius, 2002).García-Gonzáleset al., (2010) reported *cv*. Chancellor as an example of transgenic wine plant developed by genetic transformation expressing bacterial *tfdA*gene which resist against herbicies reducing lifespan of plant. Gene transfer approach allows introduction of new useful grapevine varieties for high quality wine making for consumers. Gene transfer scenarios are imaginable for introduced cultivars for example; 'chardonnay' or 'pinot noir' where upgraded single trait of resistance does not change varietal name and thus, the product wine (Kikkert and Reisch, 1996). The generation of genetic transformation in grape is useful for introducing new traits which were not present previously in grape genome so as to enhance grape breeding programs. The requirements to achieve genetic transformation of disease resistant genes include: recipient cells which can grow into whole plants, method of transferring genes into the cells, appropriate expression of the genes by the transformed plant cells, method for selecting transformed cells from non-transformed cells, regeneration of whole plants, and evaluation of disease resistance (Kikkert and Reisch, 1996).*Agrobacterium*-mediated transformation is among the biotechnological technique for development of fungal diseases in grapevine. More than 2,500 transformed lines have been produced, with approximately 750 lines already released to a biosafety field. Traits that are resistance to*Erysiphenecator, Botrytis cinerea*and for agronomical traits of 600 lines based on powdery mildew, *Botrytis cinerea* infections and many agronomical parameters have been evaluated (Hvarleva et al., 2009). Gene insertion in grapevines can also be done by use of biolistic particle bombardment into regenerative cells, followed by plant recovery (Vidal et al., 2003; Li et al., 2008) as the useful ways of gene insertion in grapevine breeding. These methods have been optimized over years and nowadays used as sources of transgenic plants in grapevine. However, it has been reported that *Agrobacterium* approach tend to harbor low-gene copy number and defined gene insertion (Li et al., 2006; Dutt et al., 2008) which is not recommended because large number of transgenic are required for easy identification of lines with high level of gene expression and performance to enhance the traits improvement (Kaniewski and Thomas, 1999).Therefore, testing many plant lines with conventional breeding has been suggested to select lines with high performance. Other study showed that the use of foreign genetic materials in food crops for instance, grape is the pivot of social and ethical public debate (Rommens, 2004). In spite of the modification of

cell culture and gene insertion techniques, there is need of new innovative technology for enhancing precision breeding (Gray et al., 2014). New genome sequence of *V. vinifera*variety 'Pinot Noir' and availability of its computational analysis has been established (Valasco et al., 2007).This has contributed in grapevine genes identification based on their associated genetic elements, isolate them from sexually-compatible disease-resistant relatives, and insert them into elite cultivars. The report by Gray et al., (2014) based on application of precision breeding modification in grapevine had shown good results. In addition, positive result in the field trials has been documented (Grumet, 2002). The application of biotechnological techniques enhances precision breeding in grapevine which ultimately contributes to agricultural production (Holme et al., 2013).In addition, studies suggest the use of transgenic lines and genetic transformation platform for testing genes derived from functional genomics experiments from *Vitis* or other species by crossing the crop species with traits from linkage mapping as well as quantitative trait loci (QTL) identification and mapping of genes using SNPs markers (Hvarleva et al., 2009).

Transgene silencing in grapevine

Gene silencing refers to the inactivation of the genes. For instance, RNA silencing is among the important mechanism in plant for defending against viral spread. In this technology, RNA silencing is composed of small RNAs such as micro RNAs (miRNAs) and small interfering RNAs (siRNAs) originated from double-stranded RNA (dsRNA) (Vaucheret, 2006). The host-encoded RNA dependent RNA polymerases speed up viral infection using viral genome for production of dsRNA (Xie et al., 2001). In grapevine, distinct RNA silencing pathways repress gene expression at the transcriptional or post-transcriptional level and in both cases silencing is mainly associated with siRNAs and/or DNA methylation (Baulcombe, 2004). Grapevine fanleaf virus (GFLV) is the causative agent of grapevine fanleaf viral disease in grapevines (Gambino et al., 2009).The study by Vigne et al., (2004) has revealed few lines of transgenic rootstock expressing the coat protein gene which prove the resistance to disease. Regarding grapevines transformed with the coat protein of GFLV (GFLV-CP) obtained previously (Gambino et al., 2005) strict correlation between number of Transfer-DNA insertion and messenger RNA accumulation levels has not been documented. However, in these lines multicopies transgene insertions with repeated sequences and integrated binary vector sequences in complex arrangements have been reported (Kurth et al., 2012). The study by Gambino et al., (2009), reported that insertion of foreign DNA into a grapevine genome may change its structure, which may affect transgene expression. Transgene silencing was reported in the transgenic grapevines analysed*in vitro* culture condition conducted for six years. Maghuly et al., (2007) has reported high transgenic expression in young rather than mature leaves in grapevine. Transgene technology provides a powerful tool for developing traits which are difficult to accomplish through conventional breeding (Zhong, 2001). For instance, the report by Burger et al., (2003) has documented the possibility of developing an RNA virus-based vector to introduce desired traits into grapevine without adding new traits in the genome allowed the vector to express recombinant proteins in the phloem tissue which is used for sugar transport in plant from root to berries (Kurth et al., 2012). Vector produces useful RNA interference (RNAi) which has ability to control expression of endogenous genes through virus-induced gene-silencing technology (Gambino et al., 2009). Vector ensures effective genetic capacity, stability of accomplishing the technology. It is useful in functional genomics of grapevine and disease control by RNAi- which enabled vaccination against pathogens (Burger et al., 2003).

Cisgenesis and intragenesis in grapevine breeding

Cisgenesis is the production of genetically modified plants using donor DNA fragment from the species itself or from a cross compatible species (Devi et al., 2013). The newly introduced gene does not change and includes its own introns and regulatory sequences and is free of vector DNA, except T- DNA border sequences that flank the cisgene(Dhekney et al., 2011).The resultant phenotype of the cisgenic plant can be achieved through conventional breeding however, it takes long time (Devi et al., 2013). It introduces only the desired gene, thus avoiding linkage drag that can be resulted from conventional cross breeding. To produce cisgenic plants genes must be isolated, cloned or synthesized and transferred back into a recipient where stably integrated and expressed crossable species. (Dhekney et al., 2011).In intragenesis, an artificially synthesized novel combination of DNA fragments, but from the species itself or from a cross.(Dhekney et al., 2011).The genomic sequence of *Vitis vinifera* has been completed, making available new sources of information to improve grape traits by genetic manipulation **(**Espinoza et al., 2013**)**. For instance, the grape is one among the most important crops worldwide, with a significant economic impact for many countries; to date, no cisgenic or intragenic grapevine plants have been described **(**Espinoza et al., 2013**)**. Grapes are especially sensitive to transgenesis due to the concerns of winemakers about the introduction of foreign genes into elite grape varieties and their potential effects on enological characteristics and wine attributes **(**Espinoza et al., 2013**)**. Thus knowledge about native genes suitable for genetic manipulation helps to improve grape traits by transgenic technologies. VvMYBA1, which belongs to the R2R3MYB family, has reported to be essential for anthocyanin synthesis (Cutanada et al., 2009). VvMYBA1 and VvTMT2 promoter genes are expressed during grape ripening; these promoters regulate movement to the cell of sugar content in grapes (Williams et al., 2000; Agasse et al., 2009).These promoters express genes at specific stages during berry development. Pathogen resistance can also be induced at certain berry ripening stages. It has been documented that commercial grape cultivars are susceptible to different disease causing pathogens such as, Powdery mildew *(Erysiphenecator)* (Gadoury et al., 2012), downy mildew *(Plasmoparaviticola)* (Gessler et al., 2011) and grey mold *(Botrytis cinerea)* (Dean et al., 2012). These pathogens cause severe loss of grape yield especially during maturity stage as well as post-harvest life. Therefore there is need of synthesizing antifungal compounds at specific stages which could provide pathogen resistance strategies without detrimental effects on fruit quality. The cisgenic gene VVTL-1 has been developed and shown fungal disease resistance in grape.

Marker assisted breeding in grapevine

Molecular markers are identifiable DNA sequence, found at specific locations of the genome and associated with the inheritance of a trait or linked gene (Karaagac et al, 2012). Molecular markers are useful tools for genetic resources management of *Vitis vinifera*. They have efficiency over morphological markers, since they can be analysed giving new dimension to breeding with respect to time required for improved crop varieties (Jonah et al., 2011). Simple sequence repeats (SSR) markers have reported to be useful and available in the Internet accessible databases for public use (Isçi et al., 2010). In order to improve resistance to fungi, insects, bacteria, viruses and nematodes in grapevine, molecular markers play a key role for identification of useful traits hence improve grape breeding (Isçi et al., 2010). Molecular markers have been useful in screening for resistance gene to diseases for instance, Regent cultivar which has been reported by Eibach et al., (2007) carrying *Run1*-gene for resistance to powdery and *Rpv1*-gene for resistance to down mildew respectively, these genes have been reported to be originated from *Muscadinia rotundifolia*(Bouquet et al.,2000; Wiedemann-Merdinogluet al.,2006) in German since 1996 for commercial purpose. It was obtained after screening with several molecular markers (Zyprianet al., 2002, Salakhutdinovet al., 2003; Akkurt, 2004; Fischer et al.,2004). Gene coding in the *Vitis* gene pool can be used for breeding programs using molecular markers which contribute to the identification of agronomically useful genes for widening maker assisted selection (Hvarleva et al., 2009). Moreover, DNA sequence-based markers have been generated which, in turn, made the construction of genetic linkage maps possible (Doligez et al., 2006; Di Gaspero et al., 2007). The availability of linkage maps and molecular markers makes the mapping of agronomically favorable genes increasingly straightforward. This study is similar with current study by Guo et al., (2014) which has been reported that, improvement of grapevine varieties using marker assisted selection is useful due to complicated genetic background, large plant body and long life cycle leading to difficulties in grape breeding. Marker assisted selection is among the most anticipated and several cited benefits of molecular markers as selection tools in breeding programs (Eibach et al., 2007). Some of the researchers have established and used molecular marker-techniques to prove useful in marker assisted selection for targeted trait identification (Jonah et al., 2011). It has been reported that molecular markers are helpful for analysis of viticulture traits, for instance, disease resistances during early breeding stage (Guo et al., 2014). Marker assisted selection helps breeders to identify the recommended DNA of grapevine so as to come up with new variety composed of potential traits based on the target of the researcher for instance yield, taste, disease resistance, drought resistance, nutrition status (Semagn et al., 2006). It is useful for precise identification of seedlings which have inherited the desired gene shortly after germination or even before expression of the trait appeared in the progeny for instance, Guo et al., (2014) crossed female cultivar with early maturing strong flavour against male producing large berries maturing late so as to produce new varieties with combined traits. Karaagac et al., (2012) reveal that, untargeted progeny can be removed and size of hybrid population can be reduced early during breeding process. Furthermore, marker assisted backcross of single gene has been reported the most effective powerful approach (Jonah et al., 2011**.** Molecular genetic map used for identifying has been reported the best linked genes from both parents to enhance breeding in grape. Furthermore, marker assisted backcross of single gene has reported to be the most effective powerful approach which uses DNA markers (Semagn et al., 2006)**.** Molecular genetic map is used for identifying the best linkage of genes from both parents to enhance breeding in grape. Molecular marker has reported to shorten time for introduction of new seedless varieties in table grapes (Karaagac et al., 2012). The molecular markers which are closely linked to major loci useful for traits in table grape breeding have been reported currently to be available. Therefore, it is critical to functionally test each genetic entity to find their contribution toward trait attributes (Gray et al., 2014). On the other view, the introgression of useful genes/traits from one grape variety to another by conventional breeding has been documented to be complicated for instance, the transfer of seedless trait from *V. vinifera* to *V. rotundifolia* to produce commercially useful seedless muscadine grape cultivars has not been successful (Gray, 1991). However, it has been possible to introgress single trait from one variety to another without changing agronomic performance therefore, preventing genetic constraints for *V.vitis* improvement (Gray, 2011). Many traits such as powdery mildew resistance and seedlessness have reported to be genetically controlled and are useful in marker-assisted breeding in grapevines. Some of the reported markers for marker assisted breeding in *Vitis vinifera* are listed in the Table 1 below.

Table 1. List of reported markers for marker assisted breeding in *Vitis vinifera*

Trait/Allele	Symbol	Chromosome	Origin	Selected references
seedlessness	SdI	18	*V. vinifera*	(Akkurt*et al*., 2012)
hermaphroditism	Sex	2	*V. vinifera*	(Karaagac*et al*., 2012)
berry size (berry weight)	Be size	18	*V. vinifera*	(Akkurt*et al*., 2012); (Karaagac*et al*., 2012)
Powdery mildew	Ren3	15	*V. hybrid*	(Wiedemann-Merdinoglu*et al*., 2006)

CONCLUSION

In this review, biotechnological approaches of grape breeding have been discussed and new techniques of grape breeding have been proposed to improve grape production. Grape breeders have been emphasized to integrate both useful conventional and biotechnological techniques to produce new useful grape varieties. This goal should be well implemented by introducing well equipped laboratories and there should be collaboration among breeders worldwide to

widen experience and exchanging new innovations based on grape breeding. This will help to come up with new improved varieties constituted with useful traits which are resistant against diseases, drought, pests and short maturity therefore, contributing increase in agricultural production. In addition, new innovative molecular markers should be applied in identification of potential traits. Genetic transformation, micro propagation, marker assisted selection and genetic engineering technologies should be prioritized and conducted without error. This will help to obtain correct information which will be useful for introducing new grape varieties in grape breeding program.

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