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Review

Molecular Marker Applications in the Selection of Elite Genotypes for Plant Stress Tolerance and Genetic Fidelity

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Abstract

Molecular markers play a crucial role in accelerating crop production for sustainable agriculture by identifying resistant traits and enhancing genetic diversity. In this review, we examine the impact of the molecular markers on advancing our understanding of stress tolerance mechanisms in plants, addressing the pressing imperative to bolster global food production to meet the escalating demands of the growing population. Additionally, the application of molecular markers to evaluate the genetic accuracy of plants due to genetic changes caused by somaclonal variation during *in vitro* propagation is mentioned. Specifically, we highlight emerging technologies like MAS, MARS, MABC, GWAS, GS, DH production, speed breeding, and genome editing, which offer promising opportunities to enhance stress tolerance and genetic integrity in crop cultivars, aiding in addressing global food security challenges. The literature search focused on studies published in the last ten years. We utilized a combination of specific and broad keywords such as genetic stability, *in vitro* propagation, molecular markers, abiotic-biotic stress, and plant biotechnology. In conclusion, this review

analyzes the use of molecular markers in assessing the stress tolerance and genetic fidelity of *in vitro* grown plants.

Keywords
Abiotic-biotic stress; DNA-based markers; genetic stability; plant biotechnology

1. Introduction to Molecular Markers

Molecular markers linked to different traits can speed up the reproductive process. Having the ability to identify molecular markers that are connected to various aspects helps in the acceleration of production for sustainable and stable agricultural system-friendly products [1]. Over the years, scientists and breeders have used molecular markers to help identify resistant traits, capabilities of resisting drought, disease resistance, and high crop production. The accelerated production of stable and durable crops is essential in that durable crops can last longer, enabling the stable resistance of crops to varying environmental conditions. In plant breeding, molecular markers improve crop production, addressing critical global issues, including food shortage and global warming [2]. Genetic diversity among individuals and groups of plants, whether natural or human-induced, affects how they change and adapt over time. Different plant species and the differences in the species that exist within and between can assist in improving plant traits or plant performance. The differences in the degree of genetic diversity in a population consist of epigenetic profiles, DNA sequence, protein structures, and physiological characteristics. Gene flow, mutations, genetic recombination, and genetic drift are the causes of genetic diversity. Population genetics is central to defining plant diversity. Genetic diversity is imperative because of its potential to generate crop plants with more exceptional characteristics, substantially crucial for ensuring food security. For instance, molecular markers differ following genome-based discovery, and genetic and physical maps are created to assess genetic diversity. Creating a visual representation of the connections between markers helps connect genetic and physical distances. Molecular markers are crucial for measuring and preserving genetic diversity and surrogates for adaptation to environmental changes [3].

In addition, using molecular markers in plant genomics has led to significant advancements in our understanding of fundamental biological processes. Scientists utilize these markers to map and investigate gene function, uncovering the molecular foundation of plant traits and physiological reactions. This knowledge is crucial for deciphering complex characteristics of plants, such as when they flower, how they utilize nutrients, and their responses to stress. Studies involving molecular markers provide valuable insights that contribute to developing cutting-edge biotechnological methods. These methods aim to enhance crop productivity and address emerging challenges in agriculture, ultimately benefiting humankind [4].

1.1 Types of Molecular Markers

Numerous molecular markers serve as detection systems for analyzing genetic variations using genomic DNA. Hybridization-based markers are based on the ability of a DNA fragment (probe DNA) labeled in various ways to hybridize to similar or identical DNA in a DNA sample under investigation. This technique is widespread in the analysis of RFLP (restriction fragment length polymorphism).

PCR-based markers are constructed using various primers and PCR to detect differences in the amplification of polymorphic regions in the DNA molecule. PCR is based on making multiple copies of a region whose nucleotide sequence is known using a synthesized pair of oligonucleotides (primers). In the PCR-based group, RAPD (random amplified polymorphic DNA), AFLP (amplified fragment length polymorphism), SSR (simple sequence repeat), ISSR (inter simple sequence repeat), STS (sequence-tagged site), EST-SSR (expressed sequence tags derived simple sequence repeat), SRAP (sequence-related amplified polymorphism), TRAP (target region amplified polymorphism), CAPS (cleaved amplified polymorphic sequence), SCAR (sequence characterize amplified region. In recent years, using SNP (single nucleotide polymorphism), a marker based on DNA array and DNA chip technology, has become increasingly common in genome scanning. SNPs are polymorphisms caused by point mutations in a base(s) at a genetic locus, creating different alleles. However, chip technology has accelerated SNP identification in many samples with the application of chip technology [5]. In order to reveal the SNP profile, DNA sequence analysis, SSCP (single-strand conformational polymorphism), HA (heteroduplex analysis), ASO (allele-specific oligonucleotide) analysis, and hundreds of SNP profiles are investigated simultaneously. Different approaches are used, such as the DNA microarray method. Particularly, DNA microarray systems enable the simultaneous scanning of hundreds of SNP profiles [6]. DArt (diversity array technology) is a "high throughput" genome analysis based on microarray technology and DNA polymorphism technology. This technology measures the presence or amount of a unique DNA fragment originating from a population or organism's genomic DNA. DArT reveals a solid surface and an open platform for DNA polymorphism [7]. DArT has potential applications in germplasm characterization, genetic mapping, gene capture, molecular marker-assisted breeding, genome methylation changes tracking, and the determination of quantitative trait loci (QTL). The importance of molecular markers stems from their ability to read the genetic code, provide information about gene inheritance patterns, and make it easier to identify specific characteristics or traits. These markers are essential for breeding, disease diagnosis, forensic analysis, and evolutionary studies. All over the world, breeding and genetic projects involving various crops successfully employ numerous DNA or molecular markers. Across diverse applications, no individual molecular marker demonstrates absolute superiority. The optimal choice of a molecular marker depends on several variables, such as the specific context of its application, the expected degree of polymorphism, the availability of necessary technical infrastructure and expertise, time constraints, and financial factors [8]. The principles of the commonly used DNA markers are listed in Table 1.

Table 1 A comparison of some key features of the primary molecular markers.

	RFLP	RAPD	AFLP	SSR	ISSR	SNP	DArT
Principle	DNA is cut with specific enzymes. The resulting fragments are separated via gel electrophoresis.	Short, arbitrary primers amplify random DNA regions. Distinctive banding patterns are produced on agarose gels.	DNA fragments are selectively amplified using restriction digestion and ligation. PCR amplification follows this process.	Short, repeated DNA sequences (microsatellites) are amplified using PCR. Polymorphisms are detected based on variations in repeat numbers.	PCR amplifies regions between microsatellite sequences. High levels of polymorphism are provided.	Variations occur at single nucleotide positions in DNA sequences. SNPs enable precise genetic mapping and association studies.	Genomic DNA is hybridized to a microarray with thousands of probes. This enables simultaneous analysis of numerous markers without prior.
Level of polymorphism	Low-Medium	Medium-High	High	High	High	Extremely High	High
Inheritance	Co-dominant	Dominant	Dominant	Co-dominant	Dominant	Co-dominant	Dominant
Cloning and/or sequencing	Yes	No	No	Yes	Yes	Yes	Yes
Reproducibility	High	Low	Medium	High	Medium	High	High
Type of probes/primers	Low-copy DNA or cDNA clones	10 bs random nucleotides	Specific sequence	Specific sequence	Motif-based	Allele-specific PCR primer	Sequenced based
Required DNA (ng)	10000	20	500-1000	50	50	50-100	25-50

1.2 Molecular Markers and Stress Tolerance

Throughout their growth cycles, plants encounter a range of adverse climatic conditions. These include biotic stresses such as herbivore attacks and pathogen infections, as well as abiotic stresses like heat, cold, drought, nutrient deficiencies, high salt levels, and the presence of hazardous metals and metalloids such as aluminum, cadmium, and arsenic in the soil. Agriculture globally grapples with numerous abiotic stressors, including salinity, drought, extreme temperatures, oxidative stress, and chemical toxicity, all contributing to environmental degradation and significant declines in crop yields. These stresses often elicit morphological, biochemical, physiological, and molecular changes that hamper plant growth and productivity. For instance, salinity and drought disrupt cellular homeostasis and ion distribution, primarily causing osmotic stress. Meanwhile, high temperatures, salinity, or drought trigger oxidative stress, resulting in protein denaturation and cellular damage. Consequently, these environmental stresses activate similar cellular signaling pathways and responses, which include the production of stress proteins, upregulation of antioxidants, and accumulation of compatible solutes [9]. Crops' susceptibility to abiotic stresses significantly impacts crop production and productivity, with vulnerability varying among species and stress types [10]. Abiotic factors, including heat (20%), drought (9%), cold (7%), and other stressors, account for approximately 50% of crop losses [11], posing severe threats to food security and influencing the natural distribution of plants. Plant cells regulate the dynamic process of stress adaptation at physiological, cellular, and molecular levels. To address concerns over declining agricultural productivity, food insecurity, and malnutrition, particularly exacerbated by climate change, agricultural scientists have prioritized understanding stress tolerance and adaptation mechanisms in crops. Plant stress adaptation is a continuously evolving process, regulated across various levels, from physiological to molecular, within plant cells [12]. Highlighting the urgency of the matter, the World Summit on Food Security has emphasized the need to increase food production by at least 70% by 2050 to accommodate the expanding global population [13]. Integrating molecular markers has significantly advanced the understanding and enhancement of plant stress tolerance. Molecular markers, such as DNA sequences or genetic variations, provide valuable insights into the genetic basis of stress tolerance traits. By identifying specific genes or genomic regions associated with stress tolerance, researchers can develop molecular tools for MAS in breeding programs. MAS enables the rapid and precise selection of stress-tolerant genotypes, developing resilient crop varieties [2]. Several studies have showcased the efficacy of molecular markers in augmenting abiotic stress tolerance in various crops. Table 2 presents studies on molecular markers and abiotic stress tolerance in various crops.

Table 2 Studies on abiotic stress tolerance using molecular markers.

Crop	Abiotic Stress	Marker Type	References
Bean	Drought	DaRT	[14]
Rice	Salinity	KASP	[15]
Barley	Salinity	ISSR	[16]
Cotton	Salinity	QTL	[17]
Wheat	Heat	miRNA-based SSR	[18]
Wheat	Drought	Indel/CAPS	[19]
Wheat	Cold	KASP	[20]
Soybean	Flooding	SNPs	[21]
Safflower	Drought	AFLP	[22]
Tomato	Heat	QTL	[23]
Sesame	Drought	SNPs	[24]
Petunia	Salinity	RAPD	[25]
Perennial grass (Miscanthus)	Drought	SSR	[26]
Tomato	Heat	RAPD, SCAR	[27]
Cassava	Drought	EST–SSR	[28]
Chickpea	Cold, Drought	AFLP	[29]
Tomato	Salinity	SSR	[30]
Safflower	Drought	SSR, ISSR	[31]

Utilizing molecular markers is a cornerstone in unraveling the intricate mechanisms governing biotic stress resilience in plants. These markers, which include different genetic elements like DNA sequences and genetic variations, give us a lot of information about the genetic basis of traits that protect plants from pests, diseases, and other living things that can be harmful. Numerous investigations have highlighted the effectiveness of molecular markers in enhancing biotic stress tolerance across different crops [32]. Table 3 provides a compilation of studies on molecular markers and biotic stress tolerance in various crops.

Table 3 Studies on biotic stress tolerance using molecular markers.

Crop	Biotic Stress	Marker Type	References
Wheat	Leaf rust	KASP	[33]
Wheat	Fusarium head blight	KASP	[34]
Wheat	Stripe rust	KASP	[35]
Wheat	Stem rust	KASP/STARP	[36]
Potatoes	Late blight	SSR	[37]
Potato	Late blight	DArT	[38]
Chickpea	Fusarium wilt	SSR	[39]
Pea	Fusarium root rot	SSR/SNP	[40]
Wheat	Powdery mildew	STARP	[41]
Melon	Powdery mildew	CAPS	[42]
Chickpea	Ascochyta blight	AFLP	[29]
Rice	Brown planthopper	SNP	[43]
Pea	Fusarium wilt	CAPS/SSR	[44]
Pea	Powdery mildew	SSR, SNP	[45]

2. Genetic Fidelity of *In Vitro* Derived Plants

Plant tissue culture, a biotechnological technique, has proven to be extremely beneficial in both plant breeding and the mass production of plants. By harnessing the ability of plant cells to differentiate into any cell, *in vitro* propagation is a crucial technique in plant biotechnology. Replicating plants *in vivo* can present difficulties, incur high costs, and may not consistently provide desired outcomes. Tissue culture technologies provide an alternative method for asexual plant propagation. Tissue culture, known as micropropagation, effectively achieves clonal propagation within a limited time and physical space [46]. Vegetative propagation aims to produce progeny plants that exhibit genetic similarity to a solitary parent plant. Cloning is a biological process that creates a group of plants known as a clone. The importance of clones in horticulture and other agricultural fields cannot be overstated. The potential of *in vitro* culture-based micropropagation has increased significance in producing economically important plants with medicinal, horticultural, agricultural, and pharmaceutical value [47]. In vitro, culture-based methods also propagate and conserve some plant species classified as vulnerable, threatened, and endangered. There is a controlled environment for *in vitro* propagation that helps plantlets grow and develop quickly and effectively, which is faster and easier than what happens in nature or living things [48]. Tissue culture serves as an alternate means of efficiently multiplying plants *in vitro*, in addition to its role in *in vitro* propagation and conservation. This approach can be employed to produce phytochemicals of medical importance, bioactive phytochemicals that are therapeutically beneficial, and secondary metabolites with antioxidant activity [49, 50]. Given the growing demands in the horticulture and herbal pharmaceutical sectors, it's crucial to scrutinize the advancements in biotechnological methods to ensure a consistent supply of high-quality and efficient components. Plant tissue culture is an invaluable technique for rapidly propagating economically important plants. Optimization studies carried out in plant tissue culture applications aim to get a high plant yield. When conducting studies on plant growth in a controlled setting, the main objective is to maximize the efficiency of

plant regeneration for large-scale production. Equally important is the need to maintain the genetic integrity and uniformity of the plants regenerated *in vitro*, ensuring that they possess the same genetic traits as the original donor plants [51]. Studies have shown that tissue culture conditions can cause plant stress, resulting in changes in the genetic stability of the cloned genotype and the appearance of genetic variations in regenerated plants [52]. Somaclonal variations can occur at any stage of the plantlet's development, especially during the multiplication phase, when conducting tissue culture for the mass production of commercially important plants. The observed variations are caused by stress induced by atypical *in vitro* conditions, frequent sub-culturing, the specific explant used, the type of culture medium employed, and the use of plant growth regulators in high concentrations combined with multiple subcultures. When cells are under a lot of stress, genetic or epigenetic changes occur during the stages of *in vitro* cultivation, such as callus formation and somatic embryogenesis in plants [52-54]. Plants derived from axillary branching typically do not exhibit variations, whereas cultures that undergo a callus phase are believed to promote a higher rate of mutation [52]. These factors lead to heritable DNA damage, impeding the precise clonal character of the offspring. Somaclonal variation, a new word for changes in both genes and epigenetics, is seen during *in vitro* propagation and can affect phenotypes [55]. In order to clone and preserve superior genotypes, it is necessary to ensure a high level of genetic homogeneity among the regenerated plants. Ensuring the genetic homogeneity of *in vitro*-produced plants early is extremely important. Hence, it is crucial to verify the genetic consistency of the propagated plants with respect to the parent plants in order to validate their suitability for specific purposes [56]. Although regenerated plantlets may display comparable physical traits to the donor plants, this does not necessarily imply their genetic resemblance to the mother plants [57]. Several methods are used to check the genetic stability of plantlets grown in a lab. These include changes in their shape, cytogenetic analysis to find changes in the number and structure of chromosomes, and biochemical and molecular DNA markers [58, 59]. Maintaining a high degree of genetic homogeneity among the regenerated plants is crucial to ensure the exact reproduction and retention of the best genotypes selected for their exceptional characteristics [60]. Researchers have employed several strategies to assess genetic stability, relying on morphophysiological, biochemical, and cytological methods. Generally, these methods concentrate on traits susceptible to *in vitro* treatment, environmental conditions, and the type of explants utilized. DNA-based molecular markers effectively control the genetic stability and confirm genotypes that exhibit what was expected under *in vitro* growth conditions [61]. Molecular markers are more accurate and reliable than phenotypical, biochemical, and physiological markers in genetic fidelity studies [62]. Identifying these changes early on with molecular tools makes it possible to enhance the micropropagation process and eliminate genetically unstable plants. Molecular markers are employed to confirm the regenerated plantlets' genetic integrity and the devised process's dependability. When assessing the genetic accuracy of micropropagated plants, it is preferable to employ multiple molecular markers that target distinct regions of the genome rather than relying on a single marker [61]. Different DNA markers that can be used to check the genetic fidelity of plants are listed in Table 4.

Table 4 Studies on genetic fidelity conformity using molecular markers.

Plant Species	Genetic Fidelity Conformity	References
Camelina sativa L.	RAPD	[63]
Hedychium coronarium L.	RAPD, ISSR	[64]
Rubus fruticosus L.	SRAP	[65]
Menthol piperita L.	ISSR	[66]
Musa spp. L.	SSR	[67]
Chlorophytum comosum L.	RAPD	[68]
Dendrobium transparens L.	RAPD, ISSR	[69]
Hedychium longicornutum L.	RAPD	[70]
Liquidambar orientalis L.	ISSR	[71]
Hancornia speciosa L.	SSR, ISSR	[72]
Dendrobium crepidatum L.	RAPD	[73]
Artocarpus heterophyllus L.	RAPD	[74]
Oryza sativa L.	SSR	[75]
Andrographis alata L.	RAPD, ISSR	[76]
Dioscorea deltoidei L.	ISSR	[77]
Prunus salicina L.	RAPD, ISSR	[78]
Dendrobium fimbriatum L.	RAPD, ISSR	[79]
Annona reticulata L.:	ISSR	[80]
Rheum rhabarbarum L.	SRAP	[81]
Rubus fruticosus L.	RAPD, SRAP	[82]
Artemisia vulgaris L.	ISSR	[83]
Pavetta indica L.	RAPD, ISSR	[84]
Andrographis echioides L.	ISSR	[85]

3. Emerging Technologies and Future Prospects

In 2020, the global population of individuals experiencing hunger surpassed 800 million, a number expected to rise alongside the expansion of the world's populace. This trend exacerbates the effects of climate change and raises concerns about heightened conflict. Dependence on outdated breeding techniques, which typically require 7–10 years to develop high-yielding, stable crop varieties, is deemed unsustainable. However, integrating traditional breeding methods with state-of-the-art molecular marker technologies presents promising avenues to tackle these issues. Various molecular marker applications, including MAS (marker-assisted selection), MARS (marker-assisted recurrent selection), MABC (marker-assisted backcrossing), GWAS (genome-wide association studies), GS (genomic selection), DH (doubled haploid) production, speed breeding, and genome editing, are transforming the field of plant breeding. These technologies facilitate the rapid identification and selection of superior genotypes with enhanced stress tolerance and genetic fidelity.

In MARS, the F_2 population is improved using phenotypic data and marker scores in the first marker-based selection cycle. Subsequently, three further marker-based selection cycles are conducted, only relying on marker scores. In biparental populations, QTL mapping involves the

contribution of beneficial alleles from both parents [86]. MARS is frequently preferred for enhancing intricate characteristics such as resistance to abiotic and biotic factors and increasing the production of grains by selectively breeding native genes in a progressive way [87, 88]. Recent breakthroughs in scientific exploration, especially within genetics, genomics, and crop physiology, have unveiled novel avenues for mitigating the effects of various stresses, a feat previously deemed challenging, if not unattainable, just a few decades ago.

MABC is one of the best methods because it uses molecular markers to find and choose the genes that protect plants from these stressors. MABC streamlines the transfer of stress-tolerant traits from donor parents to elite genotypes [89]. The study used MABC to improve the GS-23 sorghum variety's stay-green traits by combining stg3A and stg3B QTLs. SNP and SSR markers facilitated accurate hybrid identification, enhancing sorghum breeding precision. Field tests showed that the stay-green QTLs were successfully introduced, proving that breeding has progressed [90]. Various crops such as chickpeas [91], corn [92], and rice [93] have demonstrated MABC.

Researchers utilize GWAS to uncover correlations between genetic variations and specific traits. GWAS provides valuable insights into the genetic underpinnings of stress tolerance, empowering breeders to pinpoint genomic regions linked to desirable traits [94]. Using an MLM (mixed linear model) to examine the first MAGIC indica rice population subset, researchers identified significant markers within a specific chromosomal region, notably in proximity to previously reported QTLs associated with salt sensitivity and the Saltol QTL [95]. This study found a lot of new candidate genes, mainly transcription factors linked to salt-related traits. These findings will help scientists improve rice in the future [96]. Additionally, a separate GWAS investigation focused on potassium transport-related genes in potatoes under salinity stress [97]. Sahito et al. [98] highlighted the pivotal role of GWAS in identifying genomic loci and allelic variants governing resistance to diseases and pests, stress response, and signal transduction genes in maize.

CRISPR-Cas9 technology and other genome editing methods are promising for making crop plants resistant to different stresses by simultaneously targeting multiple stress-sensitive genes in a high-performing cultivar [99]. Hossain et al. [100] provided an updated overview of CRISPR-Cas genome editing technology's concept, application, and mechanism for improving crop plants' resilience to abiotic stress. Nascimento et al. [101] examined the utilization of CRISPR/Cas as a supplementary tool in crop breeding programs aimed at developing modified cultivars resilient to various abiotic stressors. GS is suggested as a substitute for MAS. It involves using DNA markers covering the complete genome to monitor complicated characteristics such as yield. This method enables the quick identification of a wide range of parents, resulting in higher breeding value in future generations and thereby accelerating genetic progress within a very short period. However, obstacles such as the process of determining the order of DNA sequences, the methods used for determining an organism's genetic makeup, and the ability to achieve desired results at a reasonable cost still pose substantial challenges to the widespread use of genomic selection for speeding the process of plant breeding [102].

GS made choosing the best plants much faster and more efficient than the traditional method for most plants in Arabidopsis, maize, and barley [103]. In their study, Shikha et al. [104] discovered several SNPs consistently present in different locations and characteristics. This finding is significant because it provides essential information for selecting better genotypes and candidate genes for breeding drought-tolerant maize hybrids. Rutkoski et al. [105] performed tests to evaluate the efficacy of several GS models in predicting the impact of drought and heat stress on wheat.

Introducing multiple traits through traditional breeding methods is time-consuming and requires several generations of backcross breeding. Therefore, DH technology has become a valuable complement to conventional breeding practices. This approach enables the generation of fully homozygous lines in a single generation from heterozygous parents, whereas traditional breeding methods require multiple generations of selfing to achieve near homozygosity [106]. The DH method is a promising way to solve the problems that come with hybrid rice because it makes high-yielding doubled haploids with stable grain quality [107, 108].

Breeders can quickly evaluate how well a plant responds to stress and identify the best genetic traits using speed breeding methods. Speed breeding is a technique that uses artificial conditions to improve plant growth and accelerate the breeding process. The method allows for the rapid and consistent creation of genetically identical offspring, accelerating the development and release of new plant varieties [109]. Although speed breeding methods can be expensive and demand specific expertise and resources, they can potentially speed up traditional breeding programs greatly and result in stable and identical genetic traits in a shorter period [110]. What distinguishes speed breeding is its versatility across various germplasms, eliminating the requirement for *in vitro* culturing tools or the need to traverse different regions to find suitable climates, as is necessary for double haploid and shuttle breeding approaches [111]. Speed breeding has demonstrated effectiveness in many crops, such as wheat [112], rice [113], peas [114], and chickpeas [115]. Using molecular markers in plant breeding has a vast potential to speed up the creation of better genotypes that can handle stress better and stay true to their genes. These emerging technologies pave the way for the sustainable production of resilient crop varieties, which are vital for addressing global food security challenges amidst climate change and population growth.

4. Conclusion

This review underscores the critical factor of genetic fidelity in plant breeding, especially tissue culture-based micropropagation technologies. The need to produce original plants to maintain the genetic uniformity of regenerated plants to conserve superior genotypes is defined. Somaclonal variation generated via tissue culture processes and its genetic stability evaluation challenges are detailed. In addition, the review considers novel molecular marker technologies and innovative breeding strategies, including marker-assisted selection, genome editing, and speed breeding, which present new, exciting opportunities for boosting stress tolerance and improving genetic integrity in various crop cultivars. They can be applied with other genomic tools to accelerate the process of creating robust cultivars generally used to counteract food security issues around the globe in light of climate changes and increasing populations.

Author Contributions

Ezgi Cabuk Sahin: Writing-original draft. Yildiz Aydin: Writing-Review and editing. Ahu Altinkut Uncuoglu: Conceptualization, writing-review and editing. All authors have read and approved the published version of the manuscript.

Competing Interests

The authors have declared that no competing interests exist.

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