Preprints.org

Review

Not peer-reviewed version

Biotechnological approaches for plant protection and growth promotion

Vikas Kumar Singh , Simran Kotwal , Arshdeep Kaur , Sirisha Bora , Rahul Kumar , Venushree Sharma , Prashant Kaushik *

Posted Date: 28 July 2023

doi: 10.20944/preprints202307.2018.v1

Keywords: Biotechnology, Omics, MAS, Genome editing, RNAi

Preprints.org is a free multidiscipline platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This is an open access article distributed under the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 July 2023 doi:10.20944/preprints202307.2018.v1

Disclaimer/Publisher's Note: The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

Review

Biotechnological Approaches for Plant Protection and Growth Promotion

Vikas Kumar Singh [1], Simran Kotwal [2], Arshdeep Kaur [3], Sirisha Bora [4], Rahul Kumar [5], Venushree Sharma [6] and Prashant Kaushik [7,*]

[1] Department of Genetics and Plant Breeding, Chaudhary Charan Singh University, Meerut, 250004, Uttar Pradesh, India
[2] Department of Botany, Government Vidarbha Institute of Science and Humanities Amravati, Maharashtra, 444604, India
[3] Department of Botany, College of Basic Sciences and Humanities, Punjab Agricultural University, Ludhiana, 141004, Punjab, India
[4] Department of Genetics and Plant Breeding, Centurion university of technology and management, 761200, Odisha India
[5] Department of Genetics and Plant Breeding, Sardar Vallabh Bhai Patel University of Agriculture and Technology, Meerut 250110, Uttar Pradesh, India
[6] Department of Botany, Chaudhary Charan Singh University, Meerut, 250004, Uttar Pradesh, India
[7] Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València de València, 46022 Valencia, Spain
* Correspondence: prakau@doctor.upv.es

Abstract: For a significant period, conventional breeding and genetic modification were the key techniques that were effective in managing biotic and abiotic stresses in crops and adding desirable traits. However, the recent appearance of novel diseases and unexpected climatic changes that have substantial implications for agriculture worldwide have urged scientists to look for alternative methods to quickly manage seasonal crises. The primary emphasis of this chapter is on the obstacles and diverse biotechnological methods employed to enhance crop resilience against a range of biotic and abiotic stressors in plants. Furthermore, we consider gene transformation, omics techniques, genome editing, and other sophisticated biotechnological tools that utilize transcriptomic, proteomic, metabolomic, phenomics, RNA interference, and epigenome modifications for enhancing plant resilience. Lastly, we examine the potential of merging these eco-friendly and innovative methods with conventional breeding to promote modern agriculture and aid in enhancing tolerance to different biotic, abiotic stresses and growth promotion.

Keywords: biotechnology; omics; MAS; genome editing; RNAi

1. Introduction

Projections based on conservative estimates suggest that the human population will persist in its growth trajectory, with an expected increase of around 2 billion in the next 30 years. As a result, the population is projected to reach approximately 10 billion by 2050, up from the current 7.7 billion [1]. This presents significant challenges in feeding an exponentially expanding population. To tackle this issue, agriculture must assume a pivotal role in boosting food yields.

Throughout history, staple crops have been susceptible to both biotic and abiotic stresses, leading to food shortages and substantial economic losses. To address this issue, plant breeders have been developing resistant crop varieties for several years to minimize such losses. However, these stresses have eventually been able to overcome the resistance. To combat crop losses, numerous pesticides have been developed, resulting in heavy dependence on these compounds for plant disease control. Unfortunately, the use of pesticides has also incurred significant costs to both public health and the environment. The combined efforts of conventional breeding programs and plant

© 2023 by the author(s). Distributed under a Creative Commons CC BY license.

biotechnology, involving different techniques such as gene transformation, omics approaches, and plant transformation, have the potential to make substantial contributions to sustainable agriculture. Agricultural biotechnology has seen intensive research in the area of crop protection, with a focus on genetic engineering strategies to safeguard crops from different stresses that can impair productivity, such as biotics stresses; fungi, bacteria, viruses, insects, nematodes, abiotic stresses; drought, heat, nutrient deficiencies. In this chapter, the focus will be on the utilization of advanced biotechnological tools, including gene transformation, omics techniques, genome editing, and other sophisticated methods. These techniques make use of transcriptomic, proteomic, metabolomic, phenomics, RNA interference, and epigenome modifications to enhance plant resilience.

3. Biotechnological approaches for crop improvement

Biotechnological approaches for crop improvement refer to the application of modern biotechnology techniques to enhance the characteristics and performance of crops. These methods offer numerous benefits, including increased yield, improved resistance to pests and diseases, enhanced nutritional content, and adaptation to various environmental conditions [2]. Some of the key biotechnological approaches used for crop improvement include: genetic engineering, Marker-Assisted Selection (MAS), genomic selection, RNA interference (RNAi), genome editing, tissue culture and cloning, metabolic engineering, synthetic biology.

3.1. Gene Transformation

Gene transformation involving cry genes, fusion proteins, lectins, alpha-amylase inhibitor, and chitinase is an advanced biotechnological approach used to enhance crop resistance against pests and diseases. Each of these components plays a specific role in providing the plant with defense mechanisms, leading to improved protection against various threats.

3.1.1. Cry genes

Gene transformation, particularly the introduction of cry genes, is a widely used biotechnological approach for crop improvement. The protein which is known as crystal, also known as the cry toxin (cry from crystal). Crystal inclusions are produced during sporulation by the soil bacterium Bt. The inclusions are hazardous proteins expressed by Cry genes that have been demonstrated to be poisonous to several types of protozoa, nematodes, and insects are among them [3]. The harmful proteins (Bt) don't harm people or unintended organisms. Numerous studies have shown that Bt produces Cry toxins, which discontinues the formation of disease causing insect larvae. Some strains of Bt, such as *Bt israelensis*, can produce a poisonous crystal known as cytolytic protein, or Cyt toxin, in addition to the Cry toxins. The name "Cyt toxin" (or protein) originates from its ability to lyse various invertebrate and vertebrate cells *in-vitro*, where in dipteran, this Cyt toxin enhances the effectiveness of Bt [4].

The risk of insect pests developing resistance to broad-spectrum chemical insecticides has led to the engineering of various crop plants with different cry genes, providing resistance against major insect pests [5–7]. However, this development of Bt crops has also resulted in insects developing resistance to Bt toxins. There are a number of example available now where, Cry gene transformation used for crop improvement. For instance, *Cry1Ac* and *Cry2Ab* genes have been widely incorporated into cotton plants to confer resistance against bollworms (*Helicoverpa* spp.) and other lepidopteran pests. Bt cotton has been commercially grown in several countries, and its cultivation has shown significant reduction in insecticide usage and increased yields [8]. To guard maize against specific pests like the European corn borer (*Ostrinianubilalis*) and corn rootworm (*Diabrotica* spp.), the *Cry1Ab, Cry1F*, and *Cry3Bb1* genes have been inserted. Many nations, especially in North and South America, have widely embraced Bt maize [9]. Similarly, to increase resistance against lepidopteran pests including the soybean looper (*Chrysodeixis includens*) and velvet bean caterpillar (*Anticarsia gemmatalis*), the *Cry1Ac* and *Cry1F* genes have been put into soybean plants. The development and marketing of Bt soybeans are currently under consideration [10]. Furthermore, *Cry1Ab, Cry1Ac*, and

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 July 2023 doi:10.20944/preprints202307.2018.v1

Cry2A genes have been introduced into rice to protect against the rice stem borer (*Chilo suppressalis*) and other lepidopteran pests, without experiencing any decreased yields. It was discovered that the chimeric *B. thuringiensis* toxin *Cry2AX1* produced in rice was efficient against a number of significant lepidopteran insect pests [11–13]. In addition, *Cry3A* gene has been incorporated into potatoes to confer resistance against Colorado potato beetle (*Leptinotarsa decemlineata*), a major pest affecting potato crops. Bt potato varieties have been developed and are being evaluated for commercial cultivation [14].

3.1.2. Fusion proteins

A fusion protein is created by combining two or more functional protein domains into a single polypeptide chain. This approach allows for the introduction of multiple traits or functionalities into a crop plant through a single transformation event. Generation of fluorescent fusion proteins have found widespread application as powerful tools for directly visualizing protein localization and dynamics within cells. The novel method facilitates robust co-expression of chimeric fluorescent fusion proteins in plants, compatible with current fluorescent protein-based bio-imaging. It addresses conventional limitations by employing a single expression vector containing multiple semi-independent expressing cassettes with individual promoters, fluorescent tags, target proteins, and terminators [15]. The use of a single expression vector to produce chimeric fluorescent fusion proteins is completely compatible with various technologies such as CRISPR-Cas9, RNAi, and protein over-expression. These technologies have been employed to investigate the functions and interactions of multiple genes in plants, as indicated by studies [16–18]. Lectin has been utilized as a carrier protein to create several effective fusion proteins. The lectin domain has been identified as the factor responsible for the observed rise in insect mortality caused by fusion proteins, as it promotes the binding process and facilitates the entry of the toxin into the insect more effectively [19]. Here are recent examples of studies related to fusion proteins in crop gene transformations, for instance, disease resistance; fusion proteins have also been investigated for disease resistance in crops. The recent study focused on a fusion protein combining an antimicrobial peptide with a plant defensin. This fusion protein enhanced resistance against fungal pathogens in tomato plants [20]. Nutritional enhancement; the fusion protein combining a high-lysine protein with a storage protein. This fusion protein resulted in increased lysine, content in rice grains, addressing a nutritional deficiency in this staple crop.

3.1.3. Lectins

Lectins are proteins that occur naturally in various organisms, such as animals, plants, and microorganisms. They have the ability to bind specifically to carbohydrates, such as sugars and glycoproteins, and play various roles in biological processes. There are four primary categories of plant lectins that may be identified based on the overall domain architecture: merolectins, hololectins, chimerolectins, and superlectins. According to Peumans and Damme [21], lectins' main roles include acting as storage proteins, cell surface adhesion, and recognition molecules in the immune system, and defensive mechanisms for plants against infections and pests that invade their environments. In the field of genetic engineering and crop improvement, lectins have been explored for their potential applications in several ways, few of them are discussed in this chapter. For instance, antifungal activity; plants have a predominance of lectins that bind to chitin, a complex polymer found in the cell walls of fungi. CBL lectins attach to chitin and prevent fungal development. *Fusarium solani*, *Fusarium moniliforme*, *Fusarium oxysporum*, *Colletotrichum lindemuthianum*, and *Aspergillus niger* were all susceptible to the antifungal effects of a galctose binding lectin that was isolated from seeds of *Bauhinia ungulata* L. (*Caesalpinoideae*) [22]. Similarly, by introducing lectin genes into plants, they aim to create crops that can produce lectins toxic to specific insect, reducing the need for chemical insecticides. Extensive documentation exists regarding the insecticidal properties of plant lectins against a variety of hemipteran insects [23]. *Lepidoptera*, *Coleoptera*, and *Homoptera*, three key insect orders, have all been discovered to be noxious to lectins from a variety of sources. Larval weight loss, mortality, feeding inhibition, delays in total developmental time, adult emergence, and fecundity in

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 July 2023 doi:10.20944/preprints202307.2018.v1

the first and second generations is some of the negative impacts of lectins on biological parameters of insects [24].

3.1.4. Alpha-amylase inhibitors (AAIs)

It has been investigated as a tool in gene transformations for crop improvement. AAIs are proteins that inhibit the activity of alpha-amylase, an enzyme involved in the breakdown of starch. By introducing AAI genes into crops, researchers aim to enhance resistance against pests and improve various agronomic traits. Mehrabadi et al. [25] reported that numerous phytophagous insects, including those found in stored products, rely on alpha-amylases for their survival, as they consume diets that are rich in polysaccharides. The insects convert starch to maltose and subsequently to glucose using alpha-amylases. It has been observed that only alpha-amylases capable of breaking down starch or glycogen have been identified in insects. When alpha-amylases are inhibited by AAIs, it can lead to compromised nourishment and a lack of energy. This has led to the exploration of AAIs as a potential means of conferring pest resistance to crops. Insects that feed on crops require alpha-amylase to digest starch, and the inhibition of this enzyme can disrupt their digestion and reduce their fitness. To enhance resistance against pests that rely on starch as a primary energy source, such as certain beetles, weevils, or caterpillars, researchers have incorporated AAI genes into crops. According to research [26], the presence of wheat alpha-amylase inhibitor (WAAI) in transgenic tobacco plants has been shown to elevate the mortality rate of lepidopteran larvae by 30-40%. Similarly, transgenic pea and azuki plants expressing alpha-amylase inhibitor derived from common bean have been observed to exhibit absolute resistance to bruchid beetle and weevil infestations.

3.1.5. Chitinase

Chitin, a crucial component of pathogenic fungal cell walls, can be targeted by chitinases, which can deactivate the fungi without causing harm to the plants. This can increase not only the plant's defense mechanisms against fungal infections but also stimulate plant growth and yield. The use of chitinases and recombinant technologies can be an effective approach to enhance plant resistance to fungal diseases. Chitinases have a high affinity for polymer chitin, which allows them to break it down into N-acetylglucosamine and low-molecular-weight COS (chitooligosaccharides). It has the potential to be a top contender in the near future for managing plant diseases due to its simplicity of use [27]. Here are some important examples of chitinases uses in gene transformations in different crops. For instance, a vector was designed to generate synthetic microRNA (amiR-24) that targets the chitinase gene of *H. armigera*, which is one of the most destructive polyphagous pests [28]. Through the use of host-induced RNA interference, tobacco and tomato plants having resistant against *Helicoverpa armigera* were developed [29,30]. The introduction of a chitinase gene from *Spodoptera littoralis* resulted in the production of transgenic maize plants that are resistant to insects [29]. In addition, chitinases have also been studied for their role in combating fungal diseases in crops. For instance, a gene for maize (*Zea mays*) chitinase "chitinase 2" effectively works against pathogen rot *F. graminearium* [31]. Nicotiana sp.'s osmotin gene (ap24) and *Oryza sativa's* chitinase gene (ch11) have been found to mitigate sheath blight disease caused by *R. solani* [32]. The chitinase I gene from the *Hordeum vulgare* cultivar Haider-93 inhibits the growth of the phytopathogenic fungi *A. solani*, *R. solani*, and *V. dahliae*. Additionally, *Hordeum vulgare's* class 11 endochitinase gene hinders *A. solani* from developing [33,34]. By expressing chitinase genes in crops, researchers aim to enhance the plants' defense against fungal infections and improve disease resistance.

3.1.6. Protease Inhibitors

Protease inhibitors (PIs) are predominantly protein molecules that obstruct the activity of proteases, which are enzymes that facilitate the disintegration of proteins, produced by pathogens. While the function of some individual PIs and their target enzymes has been extensively studied, it is uncertain whether this defensive mechanism occurs naturally in plants. In addition, several plants

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 July 2023 doi:10.20944/preprints202307.2018.v1

produce multiple types of PIs, and it was previously unclear whether these proteins work collaboratively to provide protection or if they serve additional purposes.

The categorization of PIs into families based on the specific reactive site present in their sequences was proposed by Laskowski and Kato [35]. The adoption of this nomenclature simplified the categorization of PIs into four major families, namely: (I) cysteine protease inhibitors, (II) metalloprotease inhibitors, (III) aspartic protease inhibitors, and (IV) serine protease inhibitors. Plant PIs are classified according to their functional and biochemical properties, such as cysteine protease inhibitors, cereal trypsin/α-amylase inhibitors, mustard trypsin inhibitors, metallo carboxypeptidase inhibitors, potato-type II protease inhibitors, potato type I inhibitors, serpins, soybean trypsin (Kunitz) inhibitors, Bowman-Birk serine protease inhibitors and squash inhibitors. Rawlings and Barrett [36] presented an updated classification system for PIs, in which they are grouped into families and clans, akin to the classification system for peptidases/proteases suggested by Laskowski and Kato [35]. Nevertheless, this system aims to mirror the evolutionary relationships among PIs and is structured hierarchically, comprising three main levels: inhibitors, families, and clans, with the clan being the highest level of evolutionary divergence [36].

Here we cite some successful examples to reveal the potential of these PIs (Table 1). Four distinct PIs that were effective against spider mite, insect, herbivore, and fungal attacks were identified in *Arabidopsis thaliana*. These PIs were AtkTI4, AtkTI5, AtSerpin1, AtWSCP, and UPI [37–41]. In *Solanum tuberosum*, six different PIs were also identified [42–50]. Plant-parasitic nematodes (PPNs) are a serious global problem to cereal production [51–55]. Different types of PIs, such as cowpea trypsin inhibitor (CpTI), cystatins and serine proteinase inhibitors [56], have been reported to act following attacks by PPNs. These proteinase inhibitors commence production and become effective against all classes of proteinases from nematodes [57]. Various genes have been employed to confer resistance to different PPNs. Examples include PIN2 from potato (*Solanum tuberosum* L.) introduced into durum wheat (*Triticum durum* Desf.) to protect against *H. avenae*, Mi gene from tomato to confer resistance against *M. incognita*, Hs1pro-1 from sugar beet (*Beta vulgaris*) to defend against *H. schachtii*, Gpa-2 from potato to provide protection against *Globodera pallida*, and Hero A from tomato to impart resistance against *G. rostochiensis* [56,58,59]. Likewise, the "cysteine proteinases," which are the primary digestive enzymes in many nematodes, have been targeted for the production of plants resistant to nematodes. Hartl et al. [60] also discovered four serine PIs from *Solanum nigrum* that provided protection against various natural herbivorous insects in both field and greenhouse experiments. To summarize, PIs are a potential approach to attain control over plant pests, as they have been shown to safeguard specific tissues, serve as storage proteins, and regulate the activity and release of proteases.

Table 1. List of plant protease inhibitors with application in plant protection.

PIs Name	Origen	Application	References
AtKTI4, AtKTI5	*Arabidopsis thaliana*	spider mite	[37]
AtSerpin1	*Arabidopsis thaliana*	Insect attack	[38,39]
AtWSCP	*Arabidopsis thaliana*	Herbivore attack	[38,40]
UPI	*Arabidopsis thaliana*	Fungal, insect attack	[41]
Potato type 1	*Solanum tuberosum*	Nematodes	[42]
PCI	*Solanum tuberosum*	Fungal, insect attack	[43,44]
mPI	*Zea mays*	Fungal, insect attack	[44]
SaPIN2b	*Solanum americanum*	Insect attack	[45]
StPin1A, NaPI	*Solanum tuberosum*	*Helicoverpa* spp.	[45]
PSPI-21, PSPI-22	*Solanum tuberosum*	Fungal attack	[46]
CDI	*Solanum tuberosum*	Recombinant proteins	[47–50]
PIN2	*Solanum tuberosum* L.	PPNs attack	[56–58]

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 July 2023 doi:10.20944/preprints202307.2018.v1

Mi	*Solanum tuberosum* L.		
Hs1pro-1	*Beta vulgaris*		
Gpa-2	*Solanum tuberosum* L.		
Hero A	*Solanum tuberosum* L.		
PI-I, PI-II	*Solanum nigrum*	Insect attack	[60]
BBt	*Oryza sativa*	Fungal attack	[61]
CmPS-1	*Cucurbita maxima*	Insect attack	[62,64]
CPTI	*Vigna unguiculata*	Insect attack	[64,65]
SKTI	*Glycine max*	Parasitic, insect attack	[66]
SbBBI	*Glycine max*	Aphid parasitoids	[67]
Poplar Kunitz trypsin	*Populustrichocarpa* x *Populusdeltoides*		[66]
PfKI	*Passiflora edulis* Sims		[68]
ApKTI	*Adenantherapavonina*	Insect attack	[22,69]
BvSTI	*Beta vulgaris*		[70]
BTI-CMe	*Hordeum vulgare*		[71]
BWI-1a	*Fagopyrumsculentum*	Insect, fungal, bacterial	[72]
BBt	*Viciafaba*	Fungal attack	[73]
BBt, C/s, A/s	*Hordeum vulgare*	Fungal attack	[74]
AtKPI-1	*Arabidopsis thaliana*	Fungal attack	[75]
BBI	*Glycine max*	Therapeutic proteins	[76]
Chymotrypsin and trypsin	*Nicotiana alata*	Recombinant proteins	[77]

3.2. Omics approaches

The regulation of molecular factors determines plant responses to various biotic and abiotic stressors. Therefore, an integrated omics approach can be employed to comprehend the biological interactions and molecular mechanisms that plants invoke in response to these stressors. Genome sequencing of plants has identified a range of biotic and abiotic stress-responsive genes and broadened the genomic resources available for investigating stress tolerance within their gene pool. There are multiple “omics” studies have been reported i.e., genomics, transcriptomics, proteomics, epigenomics and metabolomics.

3.2.1. Role of transcriptomics in plant protection

The transcriptome is the complete set of transcripts in a cell, during a particular stage of physiological developmental. The transcriptome issued to understand the functional and molecular constituents of the plants [78]. The aim of transcriptome study is to categorize all types of transcripts such as mRNA, large & small RNA and non-coding RNA [79]. From a few past decades, transcriptome (RNA-sequencing) has made robust advances for the development of different stress resistance plant. The RNA-Seq technique provides valuable knowledge about gene expression in various environments and aids in uncovering previously undiscovered genes [80–94]. This, in turn, contributes to a deeper understanding of metabolic and cellular processes. The key advantage of RNA-Seq is its capability to analyse and compare gene expression patterns across multiple samples. Before the rise of deep sequencing technology, microarray analysis served as the primary method for quantifying gene expression levels. Although hybridization techniques have been extensively employed, their limited sensitivity presents challenges in detecting low-abundance targets and subtle changes in the expression levels of the target gene. Consequently, RNA-Seq has been shown to be more accurate than microarray analysis. RNA-Seq can quantify the absolute amount of each molecule

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 July 2023 doi:10.20944/preprints202307.2018.v1

in a cell population and directly compare the results across experiments. Furthermore, RNA-Seq can assist in discovering novel genes since existing transcript annotations in databases may not be exhaustive. Additionally, RNA-Seq results can be self-assembled, bypassing the requirement for known genome annotations and enabling the identification of new genes [95].

3.2.2. Role of metabolomics in plant protection

Metabolomics, an emerging and captivating tool in the field of omics, has been widely employed in crop improvement. It plays a critical role in assessing the tolerance of crops to biotic and abiotic stress and in metabolic-assisted breeding. Thus far, noteworthy progress has been made in the creation of cutting-edge metabolomics tools aimed at improving crop yields [96]. There are two types of metabolites in the plant metabolome, namely primary and secondary metabolites. Analysing both primary and secondary metabolites provides a comprehensive understanding of the biochemical mechanisms underlying plant metabolism [97]. Complex metabolic pathways are closely interconnected with various primary and secondary metabolites present in plants. Metabolites such as terpenes, alkaloids, and phenolics are chemical adaptations that enable plants to cope with environmental stressors or provide defense against microorganisms, insects, predators, and even other plants (allelochemics) [98]. The deployment of advanced metabolomics techniques, such as liquid chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectrometry (GC-MS) and non-destructive nuclear magnetic resonance (NMR) spectroscopy, has enabled the efficient detection, identification, evaluation, and assessment of these metabolites [99]. Metabolomics has been extensively used in various studies aimed at enhancing the biotic and abiotic resistance of crop plants. For instance, Yadav et al. [100] utilized metabolic profiling based on GC-MS to explore the mechanism of drought tolerance in eight different wheat cultivars. They noted a rise in the levels of glutamine, serine, methionine, lysine, and asparagine. Similarly, Yang et al. [101] also performed metabolic profiling on maize exposed to drought stress, using RP/UPLC-MS. Their findings revealed an increase in lipid and carbohydrate metabolism, as well as an acceleration of the glutathione cycle. Nam et al. [102] used LC-MS and GC-MS-based metabolic profiling to confirm the variation in metabolite accumulation in soybean under drought stress. Similarly, for biotic stress such as *Zymoseptoria tritici* in wheat, Seybold et al. [103] utilized FT-ICR-MS to analyze the metabolome and uncover stress-responsive mechanisms. Cuperlovic-Culf et al. [104] performed metabolomic profiling on wheat exposed to *Fusarium graminearum* using NMR spectroscopy, and detected the presence of multiple disease-resistant biomarkers such as 3-hydroxybutyrate, trehalose, phenylalanine, myoinositol, asparagine and L-alanine.

3.2.3. Role of epigenomics in plant protection

According to Wu and Morris [105], the term "epigenetics" was introduced by Conrad Waddington in the 1940s. Waddington defined epigenetics as the study of inheritable modifications in gene function that do not entail changes in DNA sequence, and can be passed on during cell division or sexual reproduction. The genetic code, or DNA sequence, is only partially responsible for determining a trait, as the epigenetic code, or chromatin state, also plays a significant role. According to Grant-Downton and Dickinson [106,107], the nucleosome is the fundamental building block of chromatin, consisting of eight histone proteins (two of each H2A, H2B, H3, and H4) around which approximately 150 base pairs of DNA are coiled. Typically, DNA that is loosely packed around histones represents euchromatin, which is actively transcribed, while DNA that is tightly packed around histones represents heterochromatin, which is transcriptionally inactive, as discussed by Donà and Mittelsten Scheid [108], and Probst and Scheid [109]. The stability of a particular chromatin state is not guaranteed, as it can be altered by various processes. DNA undergoes methylation or demethylation, while histones can be subject to various post-translational modifications, including methylation, acetylation, ubiquitination, phosphorylation, sumoylation and biotinylation [110–112]. Of all the histone modifications, lysine residues' methylation and acetylation have received the most extensive research. Lysine acetylation introduces a negative charge to the histone, leading to the repulsion of the negatively charged DNA. This results in the relaxation or opening up of the

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 July 2023 doi:10.20944/preprints202307.2018.v1

chromatin structure (euchromatin), enabling the process of transcription. In contrast to acetylation, methylation of lysine residues does not change the charge of the histone. Each site of modification can experience mono-, di-, or tri-methylation of lysine residues, resulting in added functional diversity at these sites. According to Feng and Jacobsen [113], euchromatin is typically characterized by H3K4me3 and H3ac/H4ac, while heterochromatin is associated with H3K27me3, H3K9me2, and general histone deacetylation. The intricate modifications to chromatin ensure its flexibility in responding to diverse developmental and environmental stimuli. Several studies have reported changes in cytosine DNA methylation in response to drought stress in wheat seedlings, roots, and leaves, with tissue and genotype-specific modifications observed in two different wheat cultivars with varying drought tolerance [114]. Additionally, genome-wide analyses of histone acetyltransferases and deacetylases have provided evidence of the involvement of histone acetyl transferases in the response to drought stress [115]. Similar research has been conducted on the epigenetic changes resulting from PPN infection in various crops, such as tomatoes, soybean, and rice [116]. These studies have revealed the causal impact of hypomethylation on immunity, as demonstrated by a significant reduction in plant susceptibility upon treatment with the DNA methylation inhibitor 5-azacytidine.

3.2.4. Role of phenomics in plant protection

Phenomics involves analysing phenotypes on a large scale. Through advanced phenotyping techniques, it has become possible to predict genotypes that are susceptible to abiotic stress [117]. The implementation of an automated greenhouse system has demonstrated success as a high-throughput approach for plant phenotyping. The system enables non-destructive screening of plants using image acquisition techniques over a specific period. Capturing and analysing multiple plant images with advanced algorithms allows the prediction of specific phenotypes [117]. Plants displaying tolerant phenotypes have proven to be valuable sources of genomic resources and are frequently selected for diverse molecular techniques, such as high-throughput sequencing, to identify relevant alleles of interest. Nevertheless, phenomics has certain limitations as it may not always yield precise correlations between values obtained from pot culture and those from field experiments.

3.3. Genome editing technologies

The rapid growth of the global population necessitates the development of novel crop improvement techniques to satisfy the escalating need for food and nutritional security. Traditional approaches are inadequate and labour-intensive. The precise, efficient, and targeted alteration of genomic loci is made possible through a set of sophisticated molecular biology techniques collectively known as genome editing, as described in studies [118,119]. RNAi and CRISPR/Cas9 are innovative RNA-based methods, with RNAi being particularly useful in identifying stress-responsive genes. RNAi is a versatile tool for crop improvement, providing benefits such as increased yield, stress resilience, and improved nutrient content. However, updated regulatory frameworks, risk identification, and user-friendly approaches are necessary to fully leverage these benefits [120].

The four primary mechanisms of site-specific genome editing, namely mega nucleases (MegNs), zinc finger nucleases (ZFNs), transcription activator-like effector nuclease (TALENs), and CRISPR/Cas-9 (CRISPR/Cas-associated protein 9), have created new possibilities for breakthroughs in both medicine and agriculture.

3.3.1. Zinc finger nuclease (ZFNs)

The creation of zinc finger nucleases (ZFNs), which are artificial restriction enzymes designed for site-specific editing, marked the beginning of genome editing technology. ZFNs comprise multiple zinc finger domains and a Fok-1 endonuclease domain, creating hybrid heterodimeric proteins. The construction of ZFNs involves the utilization of zinc fingers, which are transcription factors that recognize 3-4 base pairs each. According to research [121], the zinc finger domain in eukaryotic transcription factors identifies DNA, and the nuclease domain, often derived from the

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 July 2023 doi:10.20944/preprints202307.2018.v1

bacterial restriction enzyme FokI, creates double-strand breaks. By combining the DNA-binding and DNA-cleaving domains, it is possible to create a highly precise set of "genomic scissors". The targeting of any gene in any organism is feasible using appropriately designed pairs of ZFNs. According to studies, zinc finger recognition depends on DNA sequence matching, and DNA repair mechanisms, including HR and NHEJ, are common in almost all species. Incorporating more zinc fingers (4, 5, and 6 finger pairs) in ZFNs has been shown to enhance specificity and efficiency. Additionally, the modular assembly of pre-characterized zinc fingers using standard recombinant DNA technology can improve targeting. As an illustration, the insertion of PAT gene cassettes resulted in herbicide tolerance and alterations in inositol phosphate profiles in developing maize seeds by disrupting the endogenous maize gene ZmIPK1, according to a study [122]. In maize, this technique has also been employed to stack multiple advantageous traits, allowing for greater potential in crop improvement [123,124].

3.3.2. Mega nucleases (MegNs)

Endo deoxyribonucleases known as Mega nucleases (MegNs) are naturally present in microbial life, eukaryotic mitochondria, and chloroplasts. These nucleases are typically encoded by genes found within self-splicing elements. MegNs are enzymes that exhibit high specificity, as they cleave ds-DNA selectively at recognition sites that comprise 14-40 base pairs. These nucleases hold great potential for various applications, such as the creation of therapies for inherited diseases caused by frameshift mutations or nonsense codons. Natural MegNs are not without limitations, as their use necessitates the introduction of a recognized cleavage site into the targeted region of interest. Additionally, separating the DNA-binding and DNA-cleavage domains presents challenges. Designing sequence-specific enzymes for all possible sequences is time-consuming, costly, and locating an enzyme that targets a specific locus is difficult. Consequently, routine utilization of MegNs in genome editing faces technical constraints and limitations [125].

3.3.3. Transcription activator-like effector nucleases (TALENs)

TALENs offer a cost-effective, efficient, and safer alternative to other genome editing tools. They possess the capability to target particular regions of the genome with precision. Similar to ZFNs and MegNs, TALENs require re-engineering for each targeted DNA sequence. Both TALENs and ZFNs are modular and possess natural DNA-binding specificities. TALENs are hybrid proteins formed by combining a non-specific Fok I restriction endonuclease domain with a DNA-binding domain that can recognize any arbitrary base sequence. The DNA-binding domain contains well-conserved repeats from transcription activator-like effectors (TALEs). The TALE protein comprises three domains: an amino-terminal domain with a transport signal, a DNA-binding domain with repeating 34 amino acid sequences arranged in tandem, and a carboxyl-terminal domain with a nuclear localization signal and a transcription activation domain. TALENs have been effective in editing plant genomes and hold promise for generating genetically modified laboratory animals to study human diseases. However, the TALEN technique may prove challenging for those with limited familiarity with molecular biology, and the size of TALENs is greater than that of ZFNs. In comparison to ZFNs, TALENs possess an advantage because each domain recognizes a single nucleotide. This feature simplifies the design of TALENs and results in less complex interactions between the DNA binding domains derived from TALENs and their targeted nucleotides [126,127]. Examples of the use of TALENs in plant genome editing include the creation of powdery mildew-resistant wheat, maize mutants exhibiting the glossy phenotype, and the improvement of cell wall composition and saccharification efficiency in sugarcane. In addition, TALENs can alter the nutritional profile of crops, for example, producing soybeans with high oleic acid and low linoleic acid contents, which can improve the shelf life and heat stability of soybean oil. Knocking out the vacuolar invertase (*VInv*) gene in potato tubers led to the production of tubers with reducing sugar levels that were undetectable. Moreover, TALENs have the potential to accelerate plant breeding by generating haploid plants that inherit chromosomes from only one parent, as evidenced in maize and

Brassica oleracea. These instances highlight the diverse applications of TALENs in plant genome editing [128–135].

3.3.4. Clustered regularly inter spaced short palindromic repeats (CRISPR)

The CRISPR-associated (Cas) system is a genome-editing tool that is widely used in plants and other organisms to enhance our comprehension of gene function, diagnose diseases, and improve crop quality. Although Cas9 is frequently employed for DNA modifications, its use for RNA manipulation at the post-transcriptional level is limited. The discovery of CRISPR dates back to 1987 when Japanese researchers were investigating a crucial gene in *Escherichia coli* [136]. Later, they were characterized by Francisco Mojica from the University of Alicante, Spain in 1993 and found to be involved in regulating gene expression in various bacteria and archaea. Over a decade later, they were identified as a bacterial adaptive defense system that comprises of CRISPR, DNA-targeting spacers, and Cas operon [137–140]. The identification of the CRISPR/Cas system in prokaryotes has resulted in its utilization as a sophisticated technique for editing DNA and RNA in different organisms. CRISPR/Cas systems are categorized into two primary classes, depending on whether a multi-protein effector complex or a single protein is present to modify the target. Further divisions into types and sub-types are based on differences in Cas proteins within these two classes. Type VI CRISPR/Cas systems, particularly Cas13, enable accurate RNA manipulation without causing permanent alterations to the genome. As a result, Cas13 is effective in studying RNA-related phenomena such as viral interference, RNA knockdown, and RNA detection in diverse organisms. Cas systems belonging to Class 2, particularly types II and V, are predominantly utilized as tools for genome editing. Cas9, which was one of the first proteins to be extensively examined and employed for DNA editing in animals, plants, and bacteria, is among the most commonly used [141–147].

CRISPR/Cas9 technology has been employed in tomato crops to improve the yield potential and quality of the crops by modifying the cis-regulatory control of quantitative trait loci. By employing CRISPR/Cas9, researchers have induced mutations in the SlCLV3 promoters in tomato to systematically investigate the correlation between phenotypic traits and cis-regulatory regions. This approach may prove beneficial in the advancement of tomato breeding [148]. In addition, endogenous plant upstream open reading frames (uORFs) have been edited using CRISPR/Cas9 to regulate mRNA translation, leading to the development of a mutant lettuce variety with enhanced tolerance to oxidative stress and an increased ascorbate content [149]. The utilization of CRISPR/Cas9 in targeting genes associated with tomato morphology, flower and fruit production, and ascorbic acid synthesis has enabled the introduction of favourable traits into wild tomato accessions, thereby expediting the domestication of crops [145]. The two-line system is crucial to hybrid *O. sativa* breeding, with thermo-sensitive genetic male sterility (TGMS) being extensively employed to enhance the crop's yield potential. CRISPR/Cas system has been implemented in the development of new thermo-sensitive genetic male sterility (TGMS) lines that are free of transgenes. This approach has resulted in the production of 11 new TGMS cultivars within a year, underscoring the technology's potential to enhance the efficiency of hybrid *O. sativa* breeding [150]. The CRISPR/Cas system has been employed to induce mutations in genes (DEP1, Gna1, IPA1, and GS3) in *O. sativa*, leading to the development of T2 generation mutants that exhibit traits such as increased grain number, larger grain size, and denser erect panicles [151]. Moreover, it is utilized to impart resistance against viruses such as Tomato yellow leaf curl virus (TYLCV), beet severe curly top virus (BSCTV) and Potato virus Y (PVY) in plants like *N. benthamiana*, cucumber and potatoes, respectively [152,153]. It has also been employed to generate herbicide-resistant crops, including soybean, rice, potatoes, maize, and flax, as well as to confer drought-resistant characteristics in maize (refer to Table 2 for details) [154–161].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 July 2023 doi:10.20944/preprints202307.2018.v1

Table 2. List of crops improved using genome-editing techniques (CRISPR/Cas9).

Crop	Target gene	Target trait	Reference
Tomato	SP5G, SP, CLV3, GGP1, WUS	Domestication	[145]
Rice	ALS	Herbicide	[155]
Soybean	ALS	Herbicide	[156]
Maize	ALS	Herbicide	[157]
Potato	ALS	Herbicide	[158]
Flax	EPSPS	Herbicide	[159]
Cassava	EPSPS	Herbicide	[160]
Maize	ARGOS8	Drought	[161]

3.4. RNA interference

RNA interference (RNAi) is a group of molecular processes that utilize a small RNA fragment to selectively target specific nucleic acid sequences and control gene expression [143]. Noncoding RNA-based gene regulation is an ancient process that may have existed prior to the emergence of cellular life [143,162,163]. It was first proposed by Jacob and Monod [164], but it took several decades before it could be experimentally verified. Since then, the topic has gained significant attention and has been thoroughly investigated, particularly during the 1980s [165].

RNAi employs small RNA molecules to regulate gene expression at the transcriptional and post-transcriptional levels. Insights into the molecular architecture and functional modules of RNAi have been gleaned from recent structural studies, providing valuable knowledge. Structural studies have examined proteins and nucleic acid complexes involved in RNA biogenesis, including Argonaut, RNase III, Dicer, PIWI, Drosha, and DGCR8. These studies have elucidated the mechanisms underlying nucleic acid recognition and cleavage [166]. Despite its intricacy, the RNAi phenomenon is mediated by only three principal proteins: Ago-Piwi, Dicer-like protein (which typically comprises RNAseIII and helicase domains), and RNA-dependent RNA polymerase (RdRP), along with several auxiliary proteins [167]. At least some members of four out of the five eukaryotic supergroups possess these three components. However, excavates either lack homologs of Dicer or possess Dicer-like proteins that lack either the helicase component or the tandem RNase III portion. As a result, the existence of RNAi in most excavates is uncertain [168]. However, it is believed that a functional RNAi system containing these three proteins may have been present before the most recent common ancestor of existing eukaryotes, based on the "starphylogeny" of the five supergroups [169,170]. In addition, the dispersed distribution of the ancient paralogous pair Ago-Piwi across four eukaryotic supergroups suggests that this duplication occurred prior to the divergence of the supergroups [167,171]. RNAi is a mechanism that suppresses gene expression at the transcription or translation stage of specific genes and is composed of two primary pathways: small interfering (si) RNA-mediated gene silencing and miRNA-based pathways. These pathways defend against viruses and transposable elements and regulate gene expression in eukaryotes, respectively [172].

3.5. Marker-assisted selection

Marker-assisted selection (MAS) is a technique that entails identifying genes associated with particular traits in crops, such as quality traits, disease resistance, and tolerance to different stresses, for the purpose of selection [173–176]. In addition to identifying cultivars, assessing genetic diversity and purity, selecting parents, and studying heterosis, MAS is also a valuable tool for enhancing crop yield and nutritional quality. This could aid in narrowing the gap between the demand and supply of food needed to sustain the ever-increasing population [177]. The selection of plants carrying genomic regions that control the expression of desirable traits through the use of molecular markers is a key aspect of the MAS process [178]. MAS involves examining markers to identify DNA segments that are genetically associated with and impart resistance to specific diseases. Using DNA markers,

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 July 2023 doi:10.20944/preprints202307.2018.v1

it has proven to be a successful approach in developing disease-resistant cultivars, as demonstrated by several studies. An example of the successful integration of genes/QTLs, including Pi2, Pi9, GM1, and GM4, through marker-assisted selection to confer resistance against blast and gall midge in a rice variety (CRMAS2621-7-1) was reported by Das and Rao [179]. Jamaloddin et al. [180] employed gene-specific markers (xa13prom (xa13), pTA248 (Xa21), RM224 (Pi1), and Pi54MAS (Pi54)) to create two rice lines, TH-625-491, and TH-625-159 which harbour four genes and exhibit a strong resistance to bacterial blight and blast diseases. Hanson et al. [181] also used MAS to create F7 multiple disease-resistant tomato lines that exhibited resistance to yellow late blight, leaf curl disease, grey leaf spot, bacterial wilt, and tobacco mosaic virus. Similarly, Calayugan et al. [182] utilized marker-assisted selection to develop a nutritionally valuable rice variety.

3.6. Anther culture

Plant tissue culture is a laboratory technique that allows for the cultivation of plant cells, tissues in a sterile and controlled environment. In the field of plant breeding, haploid culture is a tissue culture method that employs plant reproductive organs as explants. By using haploid culture, recessive genes associated with tolerance to environmental stressors like drought, low temperature, and nutrient deficiency in self-pollinating plants can be identified. Afterwards, these haploid plants can be duplicated to generate double haploid (DH) lines, leading to the production of homozygous plants that possess the desired traits [183].

The anther is a male reproductive structure found in flowers, typically consisting of two lobes and a sac-like shape. Within the anther, microsporangia produce pollen grains through meiotic cell division. Androgenesis is a procedure that entails aseptically isolating the anther from the bud of flower and cultivating it on nutrient media to generate haploid plantlets [184]. The effectiveness and replicability of DH production are closely tied to the management of crucial elements in the process. An example of the importance of optimizing the composition of culture media can be seen in androgenesis, where it plays a crucial role in determining the fate of microspores [185]. Some microspores follow a direct embryogenesis pathway, while others undergo an indirect organogenesis pathway. In the direct embryogenesis method, the anther behaves like a zygote and forms embryoids. These embryoids are then transferred to a favourable growth medium that promotes the development of the radical and plumule, leading to the eventual formation of haploid plantlets. In the indirect organogenesis method, the anther undergoes repeated cell divisions, resulting in the formation of callus tissue. After hormonal treatment, the callus tissue differentiates into shoot and root tissues, eventually giving rise to haploid plantlets. Through anther culture, various rice varieties like PSBRc50 'Bicol' [186]; Risabell, Jankas, Abel [187]; CR Dhan 801 (CRAC2224-1041, IET18720); hybrid 'CRHR 32' [188] were released. Among wheat varieties, AC Andrew [189], Huapei 8 [190], Kharoba [191] and GK Déva [192] were developed through anther culture.

3.7. Embryo culture

It refers to the *in-vitro* cultivation of isolated immature or mature embryos, which include extraction of mature embryo before drying from completely formed seeds and put in a culture medium for *in-vitro* growth. Culturing embryos as explants offer several advantages, as outlined in Table 3.

Table 3. Enumeration of different types of embryo culture based on the explants used.

Explants	**Objectives**
Non-viable embryos	To derive the F_1 plants.
Mature and intact seed embryo	To study germination, dormancy, and embryonic growth
Immature embryo	Differentiation pattern of embryos to plantlets

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 July 2023 doi:10.20944/preprints202307.2018.v1

Surgically dissected embryo	Dedifferentiation and redifferentiation capacity of embryo
Adventitious embryos	Facilitation of clonal propagation
Undifferentiated seed embryo	Improve seed-plant turnover

Although zygotic or seed embryos are often utilized as explants to initiate callus cultures and development is facilitated by nourishing endosperm tissue, when two distantly related species are crossed, the endosperm tissue degenerates, impeding embryo development and preventing the formation of a viable plant. In such cases, embryo rescue techniques are employed to recover the hybrid embryo. Embryo rescue, performed through in vitro culturing of the embryo by various approaches such as embryo culture, ovule culture, and ovary culture [193].

3.8. Protoplast fusion

The term protoplast refers to living plant cells that have lost their cell walls due to enzymatic digestion. Their totipotent nature emphasizes their potential as a potent biotechnological tool for in vitro manipulation and crop improvement, bypassing sexual reproduction [194]. To isolate protoplasts, there are two commonly used methods: mechanical isolation (physical disruption) and enzymatic isolation (cellulose and pectinase enzymes). Protoplasts are delicate and have fully exposed outer plasma membranes, necessitating the removal of the cell wall without causing damage.

Somatic hybridization, achieved through protoplast fusion, is a valuable technique for producing hybrids between different species or genera. It involves merging protoplasts from two distinct genomes, identifying somatic hybrid cells, and subsequently regenerating hybrid plants [195]. It was first successfully demonstrated 50 years ago using *Nicotiana glauca* Graham and *Nicotiana langsdorffii* Weinm, enables gene transfer between species and facilitates the integration of parental nuclear and cytoplasmic genomes. Table 4 illustrates several examples of somatic hybridization in plants involving interspecific, intergeneric, and intertribal crosses. Protoplast fusion techniques can be categorized into chemical fusogen and electric fusion methods. The optimal chemical fusion of protoplasts is achieved by combining PEG with calcium [196]. To perform electric fusion, protoplasts are inserted into a culture vessel with electrodes and a potential difference is applied. This method is more effective and less harmful to the protoplasts, but it demands costly equipment [197].

Protoplast fusion can lead to the formation of cybrids in variable frequencies. Generating cybrids at high frequencies can be achieved through two methods: irradiating one parent protoplast before fusion or another approach is to generate enucleate protoplasts of one species and then fusing them with normal protoplasts of another species [198]. Protoplasts can also be used as an alternative to *Agrobacterium* for direct gene transfer. *Agrobacterium's* capacity to manipulate cells stems from its plasmids, and the first successful recovery of a transgenic plant was achieved through *Agrobacterium*-mediated transformation of tobacco protoplasts. Protoplast-mediated gene transfer has provided a breakthrough in genetic engineering [199].

Table 4. List of somatic hybridization in various plants.

Interspecific hybrids	
Brassica	*Nicotiana*
B. campestris + B. oleracea;	*N. tabacum + N. alata; N. tabacum+N. glauca;*
B. oleracea+ B. napus	*N. tabacum + N. rustica; N. tabacum+N. octophora*
B. nigra + B. napus	*N. mesophila + N. tabacum*
B. carinata + B. napus	*N. glutinosa + N. tabacum*
Intergeneric hybrids	
Nicotiana × Lycopersicon;	*N. tabacum + L. sculentum;*
Nicotiana × Petunia	*N. tabacum + P. inflorata*

Brassica × Eruca	*B. napus + E. sativa*
Atropa × Datura	*A. belladonna + D. inoxia*
Raphanus× Brassica	*R. sativus + B. oleracea*
Solanum × Lycopersicon	*S. tuberosum + L. sculentum*
Moricandia× Brassica	*M. arvensis + B. oleracea*
Intertribal hybrids	
Brassica × Arabidopsis	*B. Campestris + A. thaliana*
Thlaspi × Brassica	*T. perfoliatum+ B. napus*

3.9. Somaclonal variation

Somaclonal variation is the spectrum of differences observed in plants regenerated through somatic cell culture in vitro. It is a dynamic and innovative approach that enhances genetic diversity, expands the genetic foundation, and thereby enriches the genetic pool for significant and progressive advancements in crop improvement [200]. Somaclonal variation encompasses genetic or epigenetic changes in the DNA that result in discernible phenotypic differences compared to the original parent plant. These variations can arise from factors such as gene mutations, chromosomal abnormalities, genetic rearrangements, methylation changes, the presence of transposable elements, availability of phytohormones, extended periods and composition of in vitro culture and other mechanical aspects during the culturing process [201]. Moreover, the applicability of somaclonal variation is reduced by genotype dependency and genetic fidelity issues. Culture-induced genetic variations are heritable, whereas epigenetic variations are typically unstable, non-heritable, and tend to disappear in sexually reproducing plants. Molecular markers and reversed-phase HPLC (RP-HPLC) are useful tools for evaluating these genetic and epigenetic variations. Somaclonal variation can be categorized as androclonal, protoclonal, gynoclonal and calliclonal, and gametoclonal based on the tissue cultures used, including gametic, anther, ovary, protoplast, and callus. In vitro techniques, such as plant tissue culture, have been employed to induce various types of variation to expand and enhance the genetic diversity (Table 5).

Table 5. List of somaclonal variation in different plants.

Crop	Invitro technique	Trait	Reference
Hordeum vulgare	Immature zygotic embryo culture	Induced variation	[202]
	Mature embryo supported by endosperm	Somaclonal variation	[203]
	Culture of mature embryo	Somaclonal variation	[204]
Triticum aestivum	Microspore culture	Genetic variation	[205]
Zea mays	Immature embryo culture	Genetic variation	[206]

4. Conclusions

The decline in agricultural production due to different biotic and abiotic stresses is a significant concern. In response to these issues, farmers often resort to the use of chemical pesticides due to their ability to provide a quick solution. The adverse impacts of indiscriminate pesticide usage on human health as well as the environment have prompted the investigation of alternative pest management approaches. Host plant resistance, which is an environmentally friendly strategy, has become a crucial element of Integrated Pest Management (IPM) programs. Disease-resistant crop varieties provide a stable and eco-friendly approach to pest control. Although advances have been made in

identifying sources of resistance to biotic and abiotic stress in crops, conventional techniques for developing insect-resistant varieties are slow and difficult due to the intricate nature of quantitative traits at multiple loci. However, newer biotechnological tools offer promising opportunities for developing sustainable, multi-mechanistic resistance to biotic and abiotic stresses. Biotechnological methods are being employed to create new plant resistance traits that offer effective protection against crop pests in various crops. This involves using novel molecules, insecticidal genes, and modifying the expression of genes. Biotechnological advancements, including genome editing, genetic transformation, and marker-assisted breeding, among others, are projected to accelerate the creation of disease-resistant crops presently and in the future. RNA interference and genome editing using CRISPR/Cas9 offer new methods for producing disease-resistant crops. Biotechnology has become a valuable tool for addressing the global pest problem, leading to the development of cost-effective, pesticide-resistant, and eco-friendly insect-resistant crops. With careful and ethical use, biotechnology has the potential to provide significant benefits.

References

1. Zsögön A, Peres LE, Xiao Y, Yan J, Fernie AR. Enhancing crop diversity for food security in the face of climate uncertainty. The Plant Journal. 2022;109(2):402-414.
2. Malhi GS, Kaur M, Kaushik P. Impact of climate change on agriculture and its mitigation strategies: A review. Sustainability 2021:27;13(3):1318.
3. Abo-Bakr A, Fahmy EM, Badawy F, Abd El-Latif AO, Moussa S. Isolation and characterization of the local entomopathogenic bacterium, *Bacillus thuringiensis* isolates from different Egyptian soils. Egypt J Biol Pest Control 2020;30:54–63
4. Höfte H, and Whiteley H. Insecticidal crystal proteins of *Bacillus thuringiensis*. Microbiological reviews. 1998;53(2):242-255.
5. Tabashnik BE et al. Efficacy of genetically modified Bt toxins alone and in combinations against pink bollworm resistant to Cry1Ac and Cry2Ab. PLoS ONE 2013;8(11):e80496, https://doi.org/10.1371/journal.pone.0080496
6. Nicolia A, Manzo A, Veronesi F, and Rosellini D. An overview of the last 10 years of genetically engineered crop safety research. Crit Rev Biotechnol 2013;34:77–88. https://doi.org/10.3109/07388551.2013.823595
7. Pardo-Lopez L, Soberon M, Bravo A. Bacillus thuringiensis insecticidal three-domain Cry toxins: Mode of action, insect resistance and consequences for crop protection. FEMS Microbiol. 2013;37:3–22. https://doi.org/10.1111/j.1574-6976.2012.00341.x
8. Tian JC, Wang XP, Chen Y, Romeis J, Naranjo SE, Hellmich RL, Shelton AM. Bt cotton producing Cry1Ac and Cry2Ab does not harm two parasitoids, *Cotesia marginiventris* and *Copidosoma floridanum*. Scientific reports 2018;8(1):307.
9. Domingo JL, Bordonaba JG. A literature review on the safety assessment of genetically modified plants. Environment International 2011;37(4):734-742. https://doi.org/10.1016/j.envint.2011.01.003
10. Bengyella L, Yekwa EL, Iftikhar S, Nawaz K, Jose RC, Fonmboh DJ, Roy P. Global challenges faced by engineered Bacillus thuringiensis Cry genes in soybean (*Glycine max* L.) in the twenty-first century. Biotech 2018;3(8):1-15.
11. Fujimoto H, Itoh K, Yamamoto M, Kyozuka J, Shimamoto K. Insect resistant rice generated by introduction of a modified δ-endotoxin gene of *Bacillus thuringiensis*. Biotechnol 1993;11:1151–1155.
12. Chakraborty M, Reddy PS, Mustafa G, Rajesh G, Narasu VL, Udayasuriyan V, Rana D. Transgenic rice expressing the cry 2AX1 gene confers resistance to multiple lepidopteran pests. Transgenic research 2016; 25:665-678.
13. Nayak P, Basu D, Das S, Basu A, Ghosh D, Ramakrishnan N A, Sen SK. Transgenic elite indica rice plants expressing CryIAcꝺ-endotoxin of *Bacillus thuringiensis* are resistant against yellow stem borer (*Scirpophaga incertulas*). Proceedings of the National Academy of Sciences. 1997;94(6):2111-2116. https://doi.org/10.1073/pnas.94.6.2111
14. Mi X, Ji X, Yang J, Liang L, Si H, Wu J, Wang D. Transgenic potato plants expressing cry3A gene confer resistance to Colorado potato beetle. Comptes Rendus Biologies 2015;338(7):443-450. https://doi.org/10.1016/j.crvi.2015.04.005
15. Peng, X, Zhong G, Wang, H. Co-expression of Multiple Chimeric Fluorescent Fusion Proteins in an Efficient Way in Plants. Journal of Visualized Experiments. 2018;(137), e57354.
16. Li XT, Xie YY, Zhu QL, Liu YG. Targeted genome editing in genes and cis-regulatory regions improves qualitative and quantitative traits in crops. Molecular Plant 2017;10 (11):1368-1370

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 July 2023 doi:10.20944/preprints202307.2018.v1

17. Zhu Q, Yu S, Zeng D, Liu H, Wang H, Yang Z, Liu, YG. Development of "purple endosperm rice" by engineering anthocyanin biosynthesis in the endosperm with a high-efficiency transgene stacking system. Molecular plant 2017;10(7),918-929.
18. Tang HW, et al. Multi-step formation, evolution, and functionalization of new cytoplasmic male sterility genes in the plant mitochondrial genomes. Cell Research 2017;27(1):130-146
19. Yang YY, Mei F, Zhang W, Shen Z, Fang J. Creation of Bt rice expressing a fusion protein of Cry1Ac and Cry1I-like using a green tissue-specific promoter. J Econ Entomol 2014;107:1674–1679. https://doi.org/10.1603/EC13497
20. Stotz HU, Spence B, Wang Y. A defensin from tomato with dual function in defense and development. Plant molecular biology 2009;71:131-143.
21. Peumans WJ, Van Damme EJ. Lectins as plant defense proteins. Plant physiology 1995;109(2):347.
22. SILVA JL, CAVALCANTI MA. Cornicularиella brasiliensis, a new species of coelomycetes in the rhizosphere of *Caesalpinia echinata* (Fabaceae, Caesalpinioideae) in Brazil. Phytotaxa 2014;178(3):197-204.
23. Macedo MLR, Oliveira CFR, Oliveira CT. Insecticidal activity of plant lectins and potential application in crop protection. Molecules. 2015;20:2014–2033. https://doi.org/10.3390/molecules20022014
24. Habibi J, Backus EA, Czapla TH. Plant lectins affect survival of the potato leafhopper (*Homoptera: Cicadellidae*). Journal of Economic Entomology 1993;86(3):945-951. https://doi.org/10.1093/jee/86.3.945
25. Mehrabadi M, Bandani AR, Saadati F, Mahmudvand M. Alpha-Amylase activity of stored products insects and its inhibition by medicinal plant extracts. Journal of Agricultural Science and Technology 2011;13:1173-1182
26. Morton RL, Schroeder HE, Bateman KS, Chrispeels MJ, Armstrong E, Higgins TJV. Bean alpha-amylase inhibitor 1 in transgenic peas (*Pisum sativum*) provides complete protection from pea weevil (*Bruchus pisorum*) under field conditions. Proceedings of the National Academy of Sciences USA 2000;97(8):3820-3825.https://doi.org/10.1073/pnas.070054597
27. Dean R, Van Kan JA, Pretorius ZA, Hammond-Kosack KE, Di Pietro A, Spanu PD, Rudd JJ, Dickman M, Kahmann R, Ellis J. The top 10 fungal pathogens in molecular plant pathology. Mol Plant Pathol 2012;13:414–430. https://doi.org/10.1111/j.1364-3703.2011.00783.x
28. Osman GH, Assem SK, Alreedy RM, El-Ghareeb DK, Basry MA, Rastogi A, Kalaji HM. Development of insect resistant maize plants expressing a chitinase gene from the cotton leaf worm, Spodoptera littoralis. Sci Rep 2015;5:18067.
29. Reddy K, Rajam M. Targeting chitinase gene of *Helicoverpa armigera* by host-induced RNA interference confers insect resistance in tobacco and tomato. Plant Mol Biol 2016;90:281–292.
30. Arakane Y, Zhu Q, Matsumiya M, Muthukrishnan S, Kramer KJ, Properties of catalytic, linker and chitin-binding domains of insect chitinase. Insect Biochem Mol Biol 2003;33:631–648. doi: 10.1016/s0965-1748(03)00049-3
31. Dowd PF, Naumann TA, Price NP. Johnson, E.T. Identification of a maize (*Zea mays*) chitinase allele sequence suitable for a role in ear rot fungal resistance. Agri Gene 2018;7;15–22. https://doi.org/10.1016/j.aggene.2017.10.001
32. Sripriya R, Parameswari C, Veluthambi K. Enhancement of sheath blight tolerance in transgenic rice by combined expression of tobacco osmotin (ap24) and rice chitinase (chi11) genes. In Vitro Cell Dev Biol Plant 2017;53:12–21.
33. Toufiq N, Tabassum B, Bhatti MU, Khan A, Tariq M, Shahid N, Nasir IA, Husnain T. Improved antifungal activity of barley derived chitinase i gene that overexpress a 32 kDa recombinant chitinase in *Escherichia coli* host. Braz J Microbiol 2017;42:414–421
34. Khan A, Nasir IA, Tabassum B, Aaliya K, Tariq M, Rao AQ. Expression studies of chitinase gene in transgenic potato against *Alternaria solani*. Plant Cell Tissue Organ Cult 2017;128:563–576.
35. Laskowski Jr M, Kato I. Protein inhibitors of proteinases. Ann Rev Biochem 1980;49(1):593-626.
36. Rawlings ND, Barrett AJ. Peptidases, families, and clans. Encyclopedia of Genetics, Genomics, Proteomics and Bioinformatics 2004. https://doi.org/10.1002/047001153X.g306216
37. Arnaiz A, Talavera-Mateo L, Gonzalez-Melendi P, Martinez M, Diaz I, Santamaria ME. *Arabidopsis kunitz* trypsin inhibitors in defense against spider mites. Front Plant Sci 2018 10;9:986.
38. Rustgi S, Boex-Fontvieille E, Reinbothe C, von Wettstein D, Reinbothe S. Serpin1 and WSCP differentially regulate the activity of the cysteine protease RD21 during plant development in *Arabidopsis thaliana*. Proc Natl Acad Sci 2017;114(9):2212-7.
39. Stuiver MH, Custers JH. Engineering disease resistance in plants. Nat 2001;411(6839):865-8.
40. Roberts TH, Hejgaard J. Serpins in plants and green algae. Funct Integr Genom 2008;8:1-27.
41. Laluk K, Mengiste T. The Arabidopsis extracellular unusual serine protease inhibitor functions in resistance to necrotrophic fungi and insect herbivory. Plant J 2011;68(3):480-94.
42. Turra D, Bellin D, Lorito M, Gebhardt C. Genotype-dependent expression of specific members of potato protease inhibitor gene families in different tissues and in response to wounding and nematode infection. J Plant Physiol 2009;166(7):762-74.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 July 2023 doi:10.20944/preprints202307.2018.v1

43. Quilis J, Meynard D, Vila L, Avilés FX, Guiderdoni E, San Segundo B. A potato carboxypeptidase inhibitor gene provides pathogen resistance in transgenic rice. Plant Biotechnol J 2007;5(4):537-53.
44. Quilis J, López-García B, Meynard D, Guiderdoni E, San Segundo B. Inducible expression of a fusion gene encoding two proteinase inhibitors leads to insect and pathogen resistance in transgenic rice. Plant Biotech J 2014;12(3):367-77.
45. Dunse KM, Stevens JA, Lay FT, Gaspar YM, Heath RL, Anderson MA. Coexpression of potato type I and II proteinase inhibitors gives cotton plants protection against insect damage in the field. Proc Natl Acad Sci 2010;107(34):15011-5.
46. Valueva TA, Mosolov VV. Role of inhibitors of proteolytic enzymes in plant defense against phytopathogenic microorganisms. Biochem (Moscow) 2004;69:1305-9.
47. Castilho A, Windwarder M, Gattinger P, Mach L, Strasser R, Altmann F, Steinkellner H. Proteolytic and N-glycan processing of human α 1-antitrypsin expressed in *Nicotiana benthamiana*. Plant Physiol 2014;166(4):1839-51.
48. Goulet C, Khalf M, Sainsbury F, D'Aoust MA, Michaud D. A protease activity–depleted environment for heterologous proteins migrating towards the leaf cell apoplast. Plant Biotech J 2012;10(1):83-94.
49. Robert S, Khalf M, Goulet MC, D'Aoust MA, Sainsbury F, Michaud D. Protection of recombinant mammalian antibodies from development-dependent proteolysis in leaves of *Nicotiana benthamiana*. PLoS One 2013;8(7):e70203.
50. Grosse-Holz F, Madeira L, Zahid MA, Songer M, Kourelis J, Fesenko M, Ninck S, Kaschani F, Kaiser M, van der Hoorn RA. Three unrelated protease inhibitors enhance accumulation of pharmaceutical recombinant proteins in *Nicotiana benthamiana*. Plant Biotechnol J 2018;16(10):1797-810.
51. Singh VK, Chaturvedi D, Pundir S, Kumar D, Sharma R, Kumar S, Sharma S, Sharma S. GWAS scans of cereal cyst nematode (*Heterodera avenae*) resistance in Indian wheat germplasm. Molecular Genetics and Genomics 2023;298(3):579-601.
52. Kumar D, Sharma S, Sharma R, Pundir S, Singh VK, Chaturvedi D, Singh B, Kumar S, Sharma S. Genome-wide association study in hexaploid wheat identifies novel genomic regions associated with resistance to root lesion nematode (*Pratylenchus thornei*). Scientific reports 2021;11(1):3572.
53. Chaturvedi D, Pundir S, Singh VK, Kumar D, Sharma R, Röder MS, Sharma S, Sharma S. Identification of genomic regions associated with cereal cyst nematode (*Heterodera avenae* Woll.) resistance in spring and winter wheat. Scientific Reports 2023;13(1):5916.
54. Pundir S, Sharma R, Kumar D, Singh VK, Chaturvedi D, Kanwar RS, Röder MS, Börner A, Ganal MW, Gupta PK, Sharma S. QTL mapping for resistance against cereal cyst nematode (*Heterodera avenae* Woll.) in wheat (*Triticum aestivum* L.). Scientific Reports 2022;12(1):9586.
55. Pundir S, Singh VK, Kumar S, Chaturvedi D, Kumar D, Kanwar RS, Kumar A, Börner A, Sharma S, Sharma S. Validation of resistance to cereal cyst nematode (*Heterodera avenae*) and yield performance study in doubled haploid lines of wheat (*Triticum aestivum* L.). Genetic Resources and Crop Evolution 2023;70(1):107-13.
56. Lilley CJ, Devlin P, Urwin PE, Atkinson HJ. Parasitic nematodes, proteinases and transgenic plants. Parasitology Today 1999;15(10):414-7.
57. Ali MA, Azeem F, Abbas A, Joyia FA, Li H, Dababat AA. Transgenic strategies for enhancement of nematode resistance in plants. Front Plant Sci 2017;8:750.
58. Fuller VL, Lilley CJ, Urwin PE. Nematode resistance. New Phytol 2008;180(1):27-44.
59. Vishnudasan D, Tripathi MN, Rao U, Khurana P. Assessment of nematode resistance in wheat transgenic plants expressing potato proteinase inhibitor (PIN2) gene. Transgenic Res 2005;14:665-75.
60. Hartl M, Giri AP, Kaur H, Baldwin IT. The multiple functions of plant serine protease inhibitors: defense against herbivores and beyond. Plant Signal Behav 2011;6(7):1009-11.
61. Qu LJ, Chen J, Liu M, Pan N, Okamoto H, Lin Z, Li C, Li D, Wang J, Zhu G, et al. Molecular cloning and functional analysis of a novel type of Bowman-Birk inhibitor gene family in rice. Plant Physiol 2003;133:560–570.
62. Yoo BC, Aoki K, Xiang Y, Campbell LR, Hull RJ, Xoconostle-Cázares B, Monzer J, Lee JY, Ullman DE, Lucas WJ. Characterization of *cucurbita maxima* phloem serpin-1 (CmPS-1). A developmentally regulated elastase inhibitor. J. Biol. Chem 2000;275:35122–35128.
63. La Cour Petersen M, Hejgaard J, Thompson GA, Schulz A. Cucurbit phloem serpins are graft-transmissible and appear to be resistant to turnover in the sieve element-companion cell complex. J Exp Bot 2005;56:3111–3120.
64. Lingling L, Lei J, Song M, Li L, Cao B. Study on transformation of cowpea trypsin inhibitor gene into cauli flower (*Brassica oleracea* L. var. botrytis). Afr J Biotechnol 2005;4:45–49.
65. Pujol M, Hernandez CA, Armas R, Coll Y, Alfonso-Rubi J, Perez M, Ayra C, González A. Inhibition of *Heliothis virescens* larvae growth in transgenic tobacco plants expressing cowpea trypsin inhibitor. Biotechnol 2005;22:27–130.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 July 2023 doi:10.20944/preprints202307.2018.v1

66. Major IT, Constabel CP. Functional analysis of the Kunitz trypsin inhibitor family in poplar reveals biochemical diversity and multiplicity in defense against herbivores. Plant Physiol 2008;146:888–903.
67. Azzouz H, Cherqui A, Campan ED, Rahbé Y, Duport G, Jouanin L, Kaiser L, Giordanengo P. Effects of plant protease inhibitors, oryzacystatin I and soybean Bowman-Birk inhibitor, on the aphid *Macrosiphum* euphorbiae (*Homoptera, Aphididae*) and its parasitoid *Aphelinus abdominalis* (*Hymenoptera, Aphelinidae*). J Insect Physiol 2005;51:75–86.
68. Botelho-Junior S, Machado OL, Fernandes KV, Lemos FJ, Perdizio VA, Oliveira AE, Monteiro LR, Filho ML, Jacinto T. Defense response in non-genomic model species: Methyl jasmonate exposure reveals the passion fruit leaves' ability to assemble a cocktail of functionally diversified Kunitz-type trypsin inhibitors and recruit two of them against papain. Planta 2014;240:345–356.
69. Migliolo L, de Oliveira AS, Santos EA, Franco OL, de Sales MP. Structural and mechanistic insights into a novel non-competitive Kunitz trypsin inhibitor from *Adenanthera pavonina* L. seeds with double activity toward serine- and cysteine-proteinases. J Mol Graph Model 2010;29:148–156.
70. Smigocki AC, Ivic-Haymes S, Li H, Savić J. Pest protection conferred by a Beta vulgaris serine proteinase inhibitor gene. PLoS ONE 2013;8:e57303.
71. Hamza R, Pérez-Hedo M, Urbaneja A, Rambla JL, Granell A, Gaddour K, Beltrán JP, Cañas LA. Expression of two barley proteinase inhibitors in tomato promotes endogenous defensive response and enhances resistance to Tuta absoluta. BMC Plant Biol. 2018;18:24.
72. Cheng Z, Li JF, Niu Y, Zhang XC, Woody OZ, Xiong Y, Djonović S, Millet Y, Bush J, McConkey BJ, et al. Pathogen-secreted proteases activate a novel plant immune pathway. Nature 2015;521:213–216.
73. Ye XY, Ng TB, Rao PF. A Bowman-Birk-type trypsin-chymotrypsin inhibitor from broad beans. Biochem. Biophys. Res. Commun. 2001;289:91–96.
74. Pekkarinen AI, Longstaff C, Jones BL. Kinetics of the inhibition of fusarium serine proteinases by barley (*Hordeum vulgare* L.) inhibitors. J Agric Food Chem 2007;55:2736–2742.
75. Pariani S, Contreras M, Rossi FR, Sander V, Corigliano MG, Simón F, Busi MV, Gomez-Casati DF, Pieckenstain FL, Duschak VG. Characterization of a novel Kazal-type serine proteinase inhibitor of Arabidopsis thaliana. Biochimie 2016;123:85–94.
76. Komarnytsky S, Borisjuk N, Yakoby N, Garvey A, Raskin I. Cosecretion of protease inhibitor stabilizes antibodies produced by plant roots. Plant Physiol 2006;141:1185–1193.
77. Kim TG, Kim HM, Lee HJ, Shin YJ, Kwon TH, Lee NJ, Jang YS, Yang MS. Reduced protease activity in transformed rice cell suspension cultures expressing a proteinase inhibitor. Protein Expr Purif 2007;53:270–274.
78. Wang Z, Gerstein M, Snyder M. RNA-Seq: a revolutionary tool for transcriptomics. Nature reviews genetics. 2009;10(1):57-63.
79. Martin JA, Wang Z. Next-generation transcriptome assembly. Nature Reviews Genetics. 2011;12(10):671-82.
80. Clamp M, Fry B, Kamal M, Xie X, Cuff J, Lin MF, Kellis M, Lindblad-Toh K, Lander ES. Distinguishing protein-coding and noncoding genes in the human genome. Proc Natl Acad Sci 2007;104(49):19428-33.
81. Mortazavi A, Williams BA, McCue K. Mapping and quantifying mammalian transcriptomes by RNA-seq. Nat Methods 2008;5:621–628.
82. Shendure J. The beginning of the end for microarrays? Nat Methods 2008;5(7):585-7.
83. Pickrell JK, Marioni JC, Pai AA, Degner JF, Engelhardt BE, Nkadori E, Pritchard JK. Understanding mechanisms underlying human gene expression variation with RNA sequencing. Nat 2010;464(7289):768-72.
84. Tuch BB, Laborde RR, Xu X, Gu J, Chung CB, Monighetti CK, Stanley SJ, Olsen KD, Kasperbauer JL, Moore EJ, Broomer AJ. Tumor transcriptome sequencing reveals allelic expression imbalances associated with copy number alterations. PloS One 2010;5(2):e9317.
85. Filichkin SA, Priest HD, Givan SA, Shen R, Bryant DW, Fox SE, Wong WK, Mockler TC. Genome-wide mapping of alternative splicing in Arabidopsis thaliana. Genome Res 2010;20(1):45-58.
86. Kong LA, Wu DQ, Huang WK, Peng H, Wang GF, Cui JK, Liu SM, Li ZG, Yang J, Peng DL. Large-scale identification of wheat genes resistant to cereal cyst nematode Heterodera avenae using comparative transcriptomic analysis. BMC Genom 2015;16:1-8.
87. Cao K, Li H, Wang Q, Zhao P, Zhu G, Fang W, Chen C, Wang X, Wang L. Comparative transcriptome analysis of genes involved in the response of resistant and susceptible peach cultivars to nematode infection. Sci Hortic 2017;215:20-27.
88. Chen C, Cui L, Chen Y, Zhang H, Liu P, Wu P, Qiu D, Zou J, Yang D, Yang L, Liu H. Transcriptional responses of wheat and the cereal cyst nematode Heterodera avenae during their early contact stage. Sci Rep 2017;7(1):14471.
89. Kumar M, Gantasala NP, Roychowdhury T, Thakur PK, Banakar P, Shukla RN, Jones MG, Rao U. De novo transcriptome sequencing and analysis of the cereal cyst nematode, Heterodera avenae. PloS One 2014;9(5):e96311.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 July 2023 doi:10.20944/preprints202307.2018.v1

90. Shukla N, Yadav R, Kaur P, Rasmussen S, Goel S, Agarwal M, Jagannath A, Gupta R, Kumar A. Transcriptome analysis of root-knot nematode (*Meloidogyne incognita*)-infected tomato (*Solanum lycopersicum*) roots reveals complex gene expression profiles and metabolic networks of both host and nematode during susceptible and resistance responses. Mol Plant Pathol 2018;19(3):615-33.
91. Jain KK, Kumar A, Shankar A, Pandey D, Chaudhary B, Sharma KK. De novo transcriptome assembly and protein profiling of copper-induced lignocellulolytic fungus *Ganoderma lucidum* MDU-7 reveals genes involved in lignocellulose degradation and terpenoid biosynthetic pathways. Genom 2020;112(1):184-98.
92. Shankar BA, Kaushik P, Alyemeni MN, Alansi S, Yousuf PY. Transcriptional alterations associated with overexpression of a chlorogenic acid pathway gene in eggplant fruit. Journal of King Saud University-Science 2023;*35*(3):102577.
93. Kaushik P, Kumar S. Data of de novo assembly of the leaf transcriptome in Aegle marmelos. Data in brief. 2018 Aug 1;19:700-3.
94. Kaushik P, Kumar S. Transcriptome analysis of bael (Aegle marmelos (L.) corr.) a member of family Rutaceae. Forests. 2018 Jul 26;9(8):450.
95. Zhao W, Pollack JL, Blagev DP, Zaitlen N, McManus MT, Erle DJ. Massively parallel functional annotation of 3′ untranslated regions. Nature biotechnology. 2014;32(4):387-91.
96. Shulaev V, Cortes D, Miller G, Mittler R. Metabolomics for plant stress response. Physiol Plant 2008;132(2):199-208.
97. Sung J, Lee S, Lee Y, Ha S, Song B, Kim T, Waters BM, Krishnan HB. Metabolomic profiling from leaves and roots of tomato (*Solanum lycopersicum* L.) plants grown under nitrogen, phosphorus or potassium-deficient condition. Plant Sci 2015;241:55-64.
98. Palomares-Rius JE, Kikuchi T. Omics fields of study related to plant-parasitic nematodes. J Int OMICS 2013;3(1):1-0.
99. Che-Othman MH, Jacoby RP, Millar AH, Taylor NL. Wheat mitochondrial respiration shifts from the tricarboxylic acid cycle to the GABA shunt under salt stress. New Phytol 2020;225(3):1166-80.
100. Yadav AK, Carroll AJ, Estavillo GM, Rebetzke GJ, Pogson BJ. Wheat drought tolerance in the field is predicted by amino acid responses to glasshouse-imposed drought. J Exp Bot 2019;70(18):4931-48.
101. Yang Han Y, xiu Li A, Li F, rong Zhao M, Wang W. Characterization of a wheat (*Triticum aestivum* L.) expansin gene, TaEXPB23, involved in the abiotic stress response and phytohormone regulation. Plant Physiol Biochem 2012;54:49-58.
102. Nam KH, Kim DY, Kim HJ, Pack IS, Kim HJ, Chung YS, Kim SY, Kim CG. Global metabolite profiling based on GC–MS and LC–MS/MS analyses in ABF3-overexpressing soybean with enhanced drought tolerance. Appl Biol Chem 2019;62(1):1-9.
103. Seybold H, Demetrowitsch T, Hassani MA, Szymczak S, Reim E, Haueisen J, Rühlemann M, Franke A, Schwarz K, Stukenbrock EH. Hemibiotrophic fungal pathogen induces systemic susceptibility and systemic shifts in wheat metabolome and microbiome composition. BioRxiv 2019:702373.
104. Cuperlovic-Culf M, Wang L, Forseille L, Boyle K, Merkley N, Burton I, Fobert PR. Metabolic biomarker panels of response to fusarium head blight infection in different wheat varieties. PLoS One 2016;11(4):e0153642.
105. Wu CT, Morris JR. Genes, genetics, and epigenetics: a correspondence. Sci 2001;293(5532):1103-5.
106. Grant-Downton R, Dickinson HG. Epigenetics and its implications for plant biology. 1. The epigenetic network in plants. Ann Bot 2005;96(7):1143-64.
107. Grant-Downton RT, Dickinson HG. Epigenetics and its implications for plant biology 2. The ‘epigenetic epiphany’: epigenetics, evolution and beyond. Ann Bot 2006;97(1):11-27.
108. Donà M, Mittelsten Scheid O. DNA damage repair in the context of plant chromatin. Plant Physiol 2015;168(4):1206-18.
109. Probst AV, Scheid OM. Stress-induced structural changes in plant chromatin. Current opinion in plant biology. 2015 Oct 1;27:8-16.
110. Henderson IR, Jacobsen SE. Epigenetic inheritance in plants. Nature. 2007 May 24;447(7143):418-24.
111. Mirouze M, Paszkowski J. Epigenetic contribution to stress adaptation in plants. Curr Opin Plant Biol 2011;14(3):267-74.
112. Jiang D, Berger F. DNA replication–coupled histone modification maintains Polycomb gene silencing in plants. Sci 2017;357(6356):1146-9.
113. Feng S, Jacobsen SE. Epigenetic modifications in plants: an evolutionary perspective. Curr Opin Plant Biol 2011;14(2):179-86.
114. Duan H, Li J, Zhu Y, Jia W, Wang H, Jiang L, Zhou Y. Responsive changes of DNA methylation in wheat (*Triticum aestivum*) under water deficit. Sci Rep 2020;10(1):7938.
115. Li S, He X, Gao Y, Zhou C, Chiang VL, Li W. Histone acetylation changes in plant response to drought stress. Genes 2021;12(9):1409.
116. Leonetti P, Molinari S. Epigenetic and metabolic changes in root-knot nematode-plant interactions. Int J Mol Sci 2020;21(20):7759.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 July 2023 doi:10.20944/preprints202307.2018.v1

117. Shahzad R, Iqbal MM, Jamil S, Afza N, Ahmad S, Nisar A, Kanwal S, Yousaf MI, Abbas G, Akhter S. Harnessing the potential of modern omics approaches to study plant biotic and abiotic stresses. In Plant Perspectives to Global Climate Changes 2022;101-122.
118. Chen K, Gao C. Targeted genome modification technologies and their applications in crop improvements. Plant Cell Rep. 2013;33:575–83.
119. Gao C. Genome editing in crops: from bench to field. Natl Sci Rev. 2015;2:135.
120. Kaur R, Choudhury A, Chauhan S, Ghosh A, Tiwari R, Rajam MV, RNA interference and crop protection against biotic stresses. Physiol Mol Biol Plants 2021;27(10):2357–2377.
121. Bibikova M, Carroll D, Segal DJ, Trautman JK, Smith J, Kim YG . Stimulation of homologous recombination through targeted cleavage by chimeric nucleases. Mol Cell Biol (2001) ;21:289–297
122. Shukla VK, Doyon Y, Miller JC, DeKelver RC, Moehle EA, Worden SE, Precise genome modification in the crop species Zea mays using zinc-finger nucleases. Nature. 2009;459:437–41.
123. Ainley WM, Sastry-Dent L, Welter ME, Murray MG, Zeitler B, Amora R, Trait stacking via targeted genome editing. Plant Biotechnol J. 2013;11:1126–34.
124. Cantos C, Francisco P, Trijatmiko KR, Slamet-Loedin I, Chadha-Mohanty PK, Identification of "safe harbor" loci in indica rice genome by harnessing the property of zinc-finger nucleases to induce DNA damage and repair. Front Plant Sci. 2014;5:302
125. Gaj T, Sirk SK, S-l S, Liu J ,Genome-editing technologies: principles and applications. Cold Spring Harb Perspect Biol 2016; 8:a0237
126. Huo Z, Tu J, Xu A, Li Y, Wang D, Liu M ,Generation of a heterozygous p53 R249S mutant human embryonic stem cell line by TALEN-mediated genome editing. Stem Cell Res 2019; 34:1013
127. Khan SH ,Genome-editing technologies: concept, pros, and cons of various genome-editing techniques and bioethical concerns for clinical application. Mol Ther Nucleic Acids 2019;16:326–334
128. Wang Y, Cheng X, Shan Q, Zhang Y, Liu J, Gao C, Qiu JL. Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nat Biotechnol. 2014;32:947–51
129. Char SN, Unger-Wallace E, Frame B, Briggs SA, Main M, Spalding MH, et al. Heritable site-specific mutagenesis using TALENs in maize. Plant Biotechnol J. 2015;13:1002–10.
130. Jung JH, Altpeter F. TALEN mediated targeted mutagenesis of the caffeic acid O-methyltransferase in highly polyploid sugarcane improves cell wall composition for production of bioethanol. Plant Mol Biol. 2016;92:131–42.
131. Kannan B, Jung JH, Moxley GW, Lee SM, Altpeter F. TALEN-mediated targeted mutagenesis of more than 100 COMT copies/alleles in highly polyploid sugarcane improves saccharification efficiency without compromising biomass yield. Plant Biotechnol J. 2018;16:856–66.
132. Haun W, Coffman A, Clasen BM, Demorest ZL, Lowy A, Ray E, et al. Improved soybean oil quality by targeted mutagenesis of the fatty acid desaturase 2 gene family. Plant Biotechnol J. 2014;12:934–40.
133. Demorest ZL, Coffman A, Baltes NJ, Stoddard TJ, Clasen BM, Luo S, et al. Direct stacking of sequence-specific nuclease-induced mutations to produce high oleic and low linolenic soybean oil. BMC Plant Biol. 2016;16:225.
134. Kelliher T, Starr D, Richbourg L, Chintamanani S, Delzer B, Nuccio ML, et al. MATRILINEAL, a sperm-specific phospholipase, triggers maize haploid induction. Nature. 2017;542:105–9.
135. Sun Z, Li N, Huang G, Xu J, Pan Y, Wang Z, et al. Site-specific gene targeting using transcription activator-like effector (TALE)-based nuclease in Brassica oleracea. J Integr Plant Biol. 2013;55:1092–103.
136. Ishino Y, Shinagawa H, Makino K, Amemura M, and Nakatura A. Nucleotide sequence of the iap gene, responsible for alkaline phosphataseisoenzyme conversion in Escherichia coli, and identification of the gene product.J. Bacteriol. 1987;169, 5429–5433.
137. Mojica FJM, and Rodriguez-Valera F. The discovery of CRISPR in archaea and bacteria. FEBS J. 2016;283, 3162–3169.
138. Mojica F J M, Díez-Villaseñor C, Soria E, Juez G, Francisco J, Mojica M. Biological significance of a family of regularly spaced repeats in the genomes of Archaea, Bacteria and mitochondria. Mol. Microbiol. 2002; 36, 244–246.
139. Bolotin A, Quinquis B, Sorokin A, and Ehrlich S D. Clustered regularly interspaced short palindrome repeats (CRISPRs) have spacers of extrachromosomal origin. Microbiology 2005;1066, 2551–2561.
140. Barrangou R, Fremaux C, Deveau H, Richards M, Boyaval P, Moineau S. CRISPR provides acquired resistance against viruses in prokaryotes. Science .2007 ;315, 1709–1712.
141. Makarova KS, Wolf YI, Alkhnbashi OS, Costa F, Shah SA, Saunders SJ, Barrangou R, Brouns SJ, Charpentier E, Haft DH. An updated evolutionary classification of CRISPR–Cas systems. Nat. Rev. Microbiol. 2015, 13, 722–736.
142. Makarova KS, Wolf YI, Koonin EV. Classification and nomenclature of CRISPR-Cas systems: Where from here? CRISPR J. 2018, 1, 325–336.
143. Koonin EV, Makarova KS, Zhang F. Diversity, classification and evolution of CRISPR-Cas systems. Curr. Opin. Microbiol. 2017,37, 67–78.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 July 2023 doi:10.20944/preprints202307.2018.v1

144. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 2012, 337, 816–821.
145. Li T, Yang X, Yu Y, Si X, Zhai X, Zhang H, et al. Domestication of wild tomato is accelerated by genome editing. 2018; Nat Biotechnol.
146. Zhou C, Zhou H, Ma X, Yang H, Wang P, Wang G, Zheng L, Zhang Y, Liu X. Genome-wide identification and characterization of main histone modifications in Sorghum decipher regulatory mechanisms involved by mRNA and long noncoding RNA genes. J. Agric. Food Chem. 2021, 69, 2337–2347.
147. Kavuri NR, Ramasamy M, QiY and Mandadi K. Applications of CRISPR/Cas13-Based RNA Editing in Plants. Cells 2022, 11, 2665.
148. Rodriguez-Leal D, Lemmon ZH, Man J, Bartlett ME, Lippman ZB. Engineering quantitative trait variation for crop improvement by genome editing. Cell.2017;171:470–80.
149. Zhang H, Si X, Ji X, Fan R, Liu J, Chen K, et al. Genome editing of upstream open reading frames enables translational control in plants. Nat Biotechnol. 2018;36:894–8
150. Zhou H, He M, Li J, Chen L, Huang Z, Zheng S, Zhu L, Ni E, Jiang D, Zhao B. Development of commercial thermo-sensitive genic male sterile rice accelerates hybrid rice breeding using the CRISPR/Cas9-mediated TMS5 editing system. Sci. Rep. 2016, 6, 1–12.
151. Li M, Li X, Zhou Z, Wu P, Fang M, Pan X, Lin Q, Luo W, Wu G, Li H. Reassessment of the four yield-related genes Gn1a, DEP1, GS3, and IPA1 in rice using a CRISPR/Cas9 system. Front. Plant Sci. 2016, 7, 377.
152. Ali Z, Abulfaraj A, Idris A, Ali S, Tashkandi M, Mahfouz MM. CRISPR/Cas9-mediated viral interference in plants. Genome Biol. 2015, 16, 1–11.
153. Ji X, Zhang H, Zhang Y, Wang Y, Gao C. Establishing a CRISPR–Cas-like immune system conferring DNA virus resistance in plants. Nat. Plants 2015, 1, 1–4.
154. Zhan X, Zhang F, Zhong Z, Chen R, Wang Y, Chang L, Bock R, Nie B, Zhang J. Generation of virus resistant potato plants by RNA genome targeting. Plant Biotechnol. J. 2019, 17, 1814–1822.
155. Endo M, Mikami M, Toki S. Biallelic gene targeting in rice. Plant Physiol. 2016;170:667–77.
156. Li Z, Liu ZB, Xing A, Moon BP, Koellhoffer JP, Huang L, et al. Cas9-guide RNA directed genome editing in soybean. Plant Physiol. 2015;169:960–7
157. Svitashev S, Young JK, Schwartz C, Gao H, Falco SC, Cigan AM. Targeted mutagenesis, precise gene editing, and site-specific gene insertion in maize using Cas9 and guide RNA. Plant Physiol. 2015;169:931–45.
158. Butler NM, Baltes NJ, Voytas DF, Douches DS. Geminivirus-mediated genome editing in potato (*Solanum tuberosum* L.) using sequence-specific nucleases. Front Plant Sci. 2016;7:1045.
159. Sauer NJ, Narváez-Vásquez J, Mozoruk J, Miller RB, Warburg ZJ, Woodward MJ, et al. Oligonucleotide-mediated genome editing provides precision and function to engineered nucleases and antibiotics in plants. Plant Physiol. 2016;170:1917–28.
160. Hummel AW, Chauhan RD, Cermak T, Mutka AM, Vijayaraghavan A, Boyher A, et al. Allele exchange at the EPSPS locus confers glyphosate tolerance in cassava. Plant Biotechnol J. 2018;16:1275–82.
161. Shi H, Tschudi C, Ullu E. An unusual Dicer-like1 protein fuels the RNA interference pathway in Trypanosoma brucei Rna 2006;12(12):2063-72.
162. Jeffares DC, Poole AM, Penny D. Relics from the RNA world. J Mol Evol. 1998; 46(1):18–36.
163. Engelhart AE, Adamala KP, Szostak JW. A simple physical mechanism enables homeostasis in primi-tive cells. Nat Chem. 2016;8(5):448–453.
164. Jacob F, Monod J. Genetic regulatory mechanisms in the synthesis of proteins. J Mol Biol. 1961; 3(3):318–356.
165. Wagner EGH, Simons RW. ANTISENSE RNA CONTROL IN BACTERIA, PHAGES, AND PLAS-MIDS. Ann Rev Microbiol. 1994;48(1):713–42.
166. Nowotny M, Yang W. Structural and functional modules in RNA interference. Current opinion in structural biology. 2009;19(3):286-93.
167. Cerutti H, Casas-Mollano JA. On the origin and functions of RNA-mediated silencing: from protists to man. Curr Genet. 2006; 50:81–99.
168. Macrae IJ, et al. Structural basis for double-stranded RNA processing by Dicer. Science. 2006;311:195–198.
169. Keeling PJ, et al. The tree of eukaryotes. Trends Ecol Evol. 2005; 20:670–676.
170. Makarova KS, et al. Ancestral paralogs and pseudoparalogs and their role in the emergence of the eukaryotic cell. Nucleic Acids Res. 2005; 33:4626–4638.
171. Hutvagner G, Simard MJ. Argonaute proteins: key players in RNA silencing. Nat Rev Mol CellBiol. 2008; 9:22–32.
172. Nowotny M and Yang W et al. Structural and functional modules in RNA interference. Curr Opin Struct Biol. 2009 June ; 19(3): 286–293.
173. Prabhu AS, Filippi MC, Silva GB, Silva Lobo VL, Morais OP. An unprecedented outbreak of rice blast on a newly released cultivar BRS Colosso in Brazil. In Advances in genetics, genomics and control of rice blast disease 2009;257-266. Springer Netherlands.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 July 2023 doi:10.20944/preprints202307.2018.v1

174. Saini DK, Devi P, Kaushik P. Advances in genomic interventions for wheat biofortification: a review. Agronomy 2020:2;10(1):62.
175. Kumar A, Longmei N, Kumar P, Kaushik P. Molecular Marker Analysis of Genetic Diversity in Maize: A Review. OBM Genetics 2022;6(1):1-9.
176. Chugh V, Kaur D, Purwar S, Kaushik P, Sharma V, Kumar H, Rai A, Singh CM, Kamaluddin, Dubey RB. Applications of Molecular Markers for Developing Abiotic-Stress-Resilient Oilseed Crops Life 2022;13(1):88.
177. Gouda G, Gupta MK, Donde R, Mohapatra T, Vadde R, Behera L. Marker-assisted selection for grain number and yield-related traits of rice (*Oryza sativa* L.). Physiol Mol Biol Plants 2020;26:885-98.
178. Das G, Patra JK, Baek KH. Insight into MAS: a molecular tool for development of stress resistant and quality of rice through gene stacking. Front Plant Sci 2017;8:985.
179. Das G, Rao GJ. Molecular marker assisted gene stacking for biotic and abiotic stress resistance genes in an elite rice cultivar. Front Plant Sci 2015;6:698.
180. Jamaloddin M, Durga Rani CV, Swathi G, Anuradha C, Vanisri S, Rajan CP, K Das G, Rao GJ. Molecular marker assisted gene stacking for biotic and abiotic stress resistance genes in an elite rice cultivar. Front Plant Sci 2015;6:698.
181. Hanson P, Lu SF, Wang JF, Chen W, Kenyon L, Tan CW, Tee KL, Wang YY, Hsu YC, Schafleitner R, Ledesma D. Conventional and molecular marker-assisted selection and pyramiding of genes for multiple disease resistance in tomato. Sci Hortic 2016;201:346-54.
182. Calayugan MI, Formantes AK, Amparado A, Descalsota-Empleo GI, Nha CT, Inabangan-Asilo MA, Swe ZM, Hernandez JE, Borromeo TH, Lalusin AG, Mendioro MS. Genetic analysis of agronomic traits and grain iron and zinc concentrations in a doubled haploid population of rice (Oryza sativa L.). Sci Rep 2020;10(1):2283.
183. Eliby S, Bekkuzhina S, Kishchenko O, Iskakova G, Kylyshbayeva G, Jatayev S, Soole K, Langridge P, Borisjuk N, Shavrukov Y. Developments and prospects for doubled haploid wheat. Biotechnol Adv 2022;60:108007.
184. Tripathy SK. Anther culture for double haploid breeding in rice - A way forward. Rice Genomics Genet 2018;9(1):1-6.
185. Wijerathna-Yapa A, Ramtekey V, Ranawaka B, Basnet BR. Applications of in vitro tissue culture technologies in breeding and genetic improvement of wheat. Plants. 2022;11(17):2273.
186. Senadhira D, Zapata-Arias FJ, Gregorio GB, Alejar MS, De La Cruz HC, Padolina TF, Galvez AM. Development of the first salt-tolerant rice cultivar through indica/indica anther culture. Field Crops Res 2002;76(2-3):103-10.
187. Pauk J, Jancso M, Simon-Kiss I. Rice doubled haploids and breeding. Advances in haploid production in higher plants. 2009;189-97.
188. Rout P, Naik N, Ngangkham U, Verma RL, Katara JL, Singh ON, Samantaray S. Doubled haploids generated through anther culture from an elite long duration rice hybrid, CRHR32: Method optimization and molecular characterization. Plant Biotech. 2016;33(3):177-86.
189. Sadasivaiah RS, Perkovic SM, Pearson DC, Postman B, Beres BL. Registration of'AC Andrew'wheat. Crop Sci 2004;44(2):696-8.
190. Ming-hui K, Yan H, Bing-yan H, Yong-ying Z, Shi-jie W, Li-juan M, Xin-you Z. Breeding of newly licensed wheat variety Huapei 8 and improved breeding strategy by anther culture. Afr J Biotechnol 2011;10(85):19701-6.
191. Elhaddoury J, Lhaloui S, Udupa SM, Moatassim B, Taiq R, Rabeh M, Kamlaoui M, Hammadi M. Registration of ‘Kharoba’: A bread wheat cultivar developed through doubled haploid breeding. J Plant Regist 2012;6(2):169-73.
192. Pauk J, Lantos C, Cseuz L, Papp M, Óvári J, Beke B, PUGRIS T. GK Déva’dihaploid módszer segítségével el állított új szi búzafajta (‘GK Déva’, new released winter wheat variety using dihaploid method). XXVI. Növénynemesítési Tudományos Napok, Szeged, Hungary. 2020:04-5.
193. Mondal B, Chaturvedi SK, Das A, Kumar Y, Yadav A, Sewak S, Singh NP. Embryo rescue and chromosomal manipulations. In Chickpea: Crop wild relatives for enhancing genetic gains 2020;95-130. Academic Press.
194. Avila-Peltroche J, Won BY, Cho TO. An improved protocol for protoplast production, culture, and whole plant regeneration of the commercial brown seaweed *Undaria pinnatifida.* Algal Res 2022;67:102851.
195. Tomiczak K, Adamus A, Cegielska-Taras T, Kiełkowska A, Smyda-Dajmund P, Sosnowska K, Szała L. Tissue culture techniques for the production of interspecific hybrids in poland: history and achievements. Acta Societatis Botanicorum Poloniae. 2022;3;91.
196. Ranaware AS, Kunchge NS, Lele SS, Ochatt SJ. Protoplast technology and somatic hybridisation in the family Apiaceae. Plants 2023;12(5):1060.
197. Gieniec M, Siwek J, Oleszkiewicz T, Maćkowska K, Klimek-Chodacka M, Grzebelus E, Baranski R. Real-time detection of somatic hybrid cells during electrofusion of carrot protoplasts with stably labelled mitochondria. Sci Rep 2020;10(1):18811.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 July 2023 doi:10.20944/preprints202307.2018.v1

198. Bruznican S, Eeckhaut T, Van Huylenbroeck J, De Keyser E, De Clercq H, Geelen D. An asymmetric protoplast fusion and screening method for generating celeriac cybrids. Sci Rep 2021;11(1):4553.
199. Hsu CT, Lee WC, Cheng YJ, Yuan YH, Wu FH, Lin CS. Genome editing and protoplast regeneration to study plant–pathogen interactions in the model plant *Nicotiana benthamiana*. Front Genome Ed 2021;2:627803.
200. Tefera AA. Review on application of plant tissue culture in plant breeding. J Natural Sci Res. 2019;9(3):20-5.
201. Ranghoo-Sanmukhiya VM. Somaclonal variation and methods used for its detection. In Propagation and Genetic Manipulation of Plants; Siddique I Ed. Springer: Singapore, 2021:1-8.
202. Orłowska R. Barley somatic embryogenesis-an attempt to modify variation induced in tissue culture. J Biol Res 2021;28:1-2.
203. Aydin M, Arslan E, Taspinar MS, Karadayi G, Agar G. Analyses of somaclonal variation in endosperm-supported mature embryo culture of rye (*Secale cereale* L.). Biotechnol Biotechnol Equip 2016;30(6):1082-9.
204. Rashad SE, Abdel-Tawab FM, Fahmy EM, Sakr MM. Somaclonal Variation from Mature Embryo Ex-Plants of Some Egyptian Barley Genotypes. Egypt J Genet Cytol 2020;49(1).
205. Lantos C, Bóna L, Nagy É, Békés F, Pauk J. Induction of in vitro androgenesis in anther and isolated microspore culture of different spelt wheat (*Triticum spelta* L.) genotypes. Plant Cell,Tissue Organ Culture 2018;133:385-93.
206. Wang K, Shi L, Liang X, Zhao P, Wang W, Liu J, Chang Y, Hiei Y, Yanagihara C, Du L, Ishida Y. The gene TaWOX5 overcomes genotype dependency in wheat genetic transformation. Nat Plants 2022;8(2):110-7.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.