Review Article

CRISPR-CAS: A SAVIOR FOR CLIMATE-THREATENED RICE: A REVIEW

Comment [NSN1]: Corrected

ABSTRACT

Comment [NSN2]: Corrected

The productivity of agriculture has been greatly impacted by climate change, especially rice production,severalbiotic and abiotic stressors, including heat, salt, drought, heavy metals, rice blast, and bacterial blight, can severely reduce rice yields and jeopardize global food security. Numerous strategies have been employed in this regard to cultivate rice varieties that may be able to adapt to changing climate conditions. Nowadays, crop development has undergone a revolution thanks to gene editing (GE) technologies. As one of the most useful, dependable, affordable, and labor-efficient GE technologies available, the CRISPR/Cas (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein) system has gained favor among plant researchers, notably rice breeders and geneticists. Since 2013 (the year the technique was first deployed in rice), CRISPR/Cas-based GE technologies have been employed to develop a variety of trait-specific climate-resilient rice lines. Several studies that have already been released attest to the effective use of GE technologies for rice development. However, this review's primary objective is to provide a succinct, thoroughly researched summary of the most current studies (starting in 2020) on the application of GE tools—particularly CRISPR-based systems for the production of CRISPR rice—to address the serious global climate change dilemma.

Comment [NSN3]: Corrected

Keywords: Climate resilience, Oryza sativa L., gene editing, CRISPR-Cas system, and food security

1. INTRODUCTION

Comment [NSN4]: Number section added

There are a number of issues threatening food security, not the least of which is climate change. It is one of the greatest problems facing humanity, not just now but potentially for many generations to come if it isn't handled[1]. Two million people presently experience stunted growth due to vitamin inadequacies, rice (Oryza sativa L.), may be essential to maintaining global food security in these severe circumstances as well as a significant source of remedies[2]. However, Asia is where most of it is grown, and this region appears to be more susceptible to shifting climate conditions[3]. Although indica rice has a larger market share than japonica rice, both subspecies of rice are prominent in the rice agricultural system. On the other hand, climate changes are having a major impact on rice output and nutritional quality[4]. These include extreme drought, heat, salinity, cold, and deviations in precipitation patterns. They are also causing an increase in illnesses and insect pest attacks There is a prediction of an 8°C rise in temperature, an average drought index of 129 (now at 52.45), and a possible 2100 rise in sea level[5]. These predictions might potentially have a catastrophic impact on food supplies. Furthermore, each degree of temperature increase may increase average precipitation and humidity by 3% and 7%, respectively, which would encourage crop illnesses and insect attacks[5, 6]. As a result, there could be severe output losses, a compromised food production system, and eventually widespread food poverty. Since rice needs ideal irrigation and a certain temperature to grow well, and extremely vulnerable to climate change[7]. A 1℃ increase in temperature is said to reduce paddy production by as much as 3.44%. Therefore, it is concerning to learn that the temperature in the Malaysian granary

area may rise from 0.3°C to 0.5°C and that precipitation may increase from 133 mm to 200 mm. There is a possibility that the temperature and rainfall in Cambodia could rise by 2.5℃ and 8.3%, respectively[8]. This would result in humid conditions during the rainy season and reduced humidity during the dry season this is why traditional plant breeding has been essential to the advancement of rice breeding programs both historically and currently[9]. Furthermore, because of their sluggish pace and limited effectiveness, CPBTs are not as effective against current issues including soil degradation, pollution accumulation, rapid climate change, and changing rainfall patterns. Given the current environmental issues and the increased demand for food, NPBTs, particularly gene editing (GE), may be a useful substitute for CPBTs in the fight against hunger and to guarantee global food security[10, 11]. The acceptability of genetically modified organisms (GMOs) produced by transgenic breeding is a topic of much discussion. A dependable, efficient, and reasonably priced method of altering crop plants' genetic composition without introducing foreign DNA is provided by the CRISPR-Cas system, one of the several GE systems[12]. Additionally, it is now simpler to alter a specific gene or genes in order to produce novel, eye-catching crop varieties because to the ability to access genome sequences and data on a wide variety of plant species[12].

Despite the fact that a number of reviews on genetic engineering in rice have already been published, however, given rice's high value and major contribution to food security, an updated progress of rice genetic engineering was required[13]. Therefore, the main goal of this spatiotemporal brief review is to provide an overview of the latest advancements and success stories of CRISPR-Cas-based GE systems, particularly CRISPR-Cas9, in the last three years for the development of climate-smart rice lines[14]. In-depth discussions are also had regarding future directions and regulatory issues

2. CAN CRISPR BE USED TO BREED CLIMATE-RESILIENT VARIETY?

Comment [NSN5]: Number section added

2.1 Varitese of rice without transgenes

Comment [NSN6]: Corrected

These days, the robustness, precision, and applicability of CRISPR-Cas-based GE in agricultural plants—particularly rice—make it highly valued. When compared to other GE methods, CRISPR-Cas alone might be quite important in the Creation of climate-resistant rice lines through the execution of several procedures, such as knock-in, knock-out, epigenetic modifications, and transcriptional control of distinct genes governing diverse features , the regulation of genetically modified organisms (GMOs) indels, base pair alterations, and targeted sequence modification achieved using homologous recombination that is indistinguishable from spontaneous mutation[15]. Thus, in order to create novel rice varieties that may be commercialized, Rice lines free of foreign DNA altered using CRISPR will be employed in rice breeding projects. As an example, this spatiotemporal analysis reports that, since 2020, 126 research initiatives focusing on the development of climate change have used CRISPR-Cas based GE systems[16].

2.1 Rice genome rewiring using crispr to promote abiotic stress tolerance

Comment [NSN7]: Corrected

Abiotic stressors, which harm rice's growth and developmental processes, include drought, heat, cold, salt, herbicide, and salinity. These factors collectively produce significant production losses in rice[17]. The development of rice cultivars that can endure quickly changing abiotic environmental circumstances is something that is urgently needed. In this context, CRISPR-Cas systems have shown to be a potential approach. Therefore, its current uses against abiotic stressors in rice are covered in this section[18].

2.2*Drought*

Drought stress is one of the main risks to agricultural productivity that might significantly impact food security. By the end of 2050, it is predicted that over half of the world's arable land will experience acute water scarcity [19]. It is indisputable that crop types resistant to drought, particularly rice, must be produced in order to mitigate any potential negative effects. In order to solve this, researchers have created new rice lines that are drought-resistant by removing the undesirable sensitivity (Se)-producing gene or genes using CRISPR-Cas-based GE systems[20]. According to Santosh 366 bp deletion made with CRISPR-Cas9 in the drought and salinity resistant 1 gene enhanced water retention against dehydration stress and resulted in increased leaf yield. The phenotypic of the CRISPR-edited mutant plants demonstrated resilience to drought stress. Similarly, the pyrabactin resistance-like

(OsPYL9) gene null mutation based on CRISPR-Cas9 led to drought tolerance, decreased transpiration rate[21], and increased cuticular buildup of leaf wax and higher grain weight. 66 drought-induced microRNAs were identified and it was discovered that OsmiR535 and OsmiR818b were among the drought-responsive microRNAs. In drought-responsive OsmiR535, a 5 bp homozygous deletion enhanced tolerance to PEG, dehydration, NaCl, and abscisic acid (ABA) stressors. Similarly, the CRISPR-Cas9 system-generated loss of function of the OsABA8ox2 gene, which encodes the enzyme ABA 8, -hydroxylase, resulted in a long, narrow rooting system that is useful for obtaining water during dry spells. Furthermore, stomatal density has a significant impact on water transpiration, which makes it a crucial factor in drought stress. [22]Therefore, the disruption caused by CRISPR-Cas9 brought about premature protein truncation in circumstances where water was scarce. leading to an increase in grain production. Furthermore, in comparison to the wild, the CRISPR-. Similar to this, under drought conditions. When a plant is stressed by drought, its production of cuticular wax slows down water loss and may protect it from severe damage. This enhanced the plants' tolerance for drought[23].

2.3 Heat

Comment [NSN8]: Number subsection added

The average yearly temperature changes worldwide in 2022 was 1.4 °C, according to temperature statistics that the FAO recently released in May 2023. This temperature increase could have a substantial impact on crop yield. By the middle of the twenty-first century, the average global temperature might reach 3°C. As a result, the news that a 1°C rise in mean temperature may have an impact on agricultural plants' grain yield of 6-7% is concerning[24]. Scientists have used a CRISPR-based genetic engineering approach on several occasions to create heat-resistant rice lines in response to the negative consequences of prolonged heat stress [25].

For instance, the following factors control OsNAC006 TF: indole acetic acid, gibberellin, H2O2, ABA, heat, and cold, induced by CRISPR-Cas9 have shown thermotolerance in rice plants, which is accompanied by increased H2O2 and O2-levels. Similarly, ntl3-1 and ntl3-2 mutants were created by CRISPR-Cas9-oriented disruption of NTL TF at two locations[26]. While the ntl3-2 mutant displayed both a shortened protein and a novel C-terminus, the ntl3-1 mutant only produced a truncated protein. Remarkably, both mutants displayed thermotolerance at 45°C for five days with a reduced survival rate, regardless of the post-GE protein structure[27]. However, other genes that are favorably linked to heat tolerance also need to be expressed in order to guarantee heat tolerance. For instance, a single bp mutation based on CRISPR-Cas9. For ten days, lrk1 mutants were exposed to 35°C, which decreased dark respiration and slowed their morphological growth. The semi-rolled leaf 10 (SRL10) gene's first exon contains 1-, 2-, and 4-bp deletions. These mutations resulted in three separate mutant lines with the same semi-rolled leaf phenotype and reduced thermosensitivity[28].

2.4 Acidity

Comment [NSN9]: Number subsection added

Osmotic, oxidative, and ion toxicity stressors are brought about by salinity stress, which has a significant impact on rice plant growth during the seedling and reproductive phases. Interestingly, rice that can withstand salt is the best grain crop to grow on saline-alkaline and coastal tidal areas because of its high potential for use[29]. Using CRISPR-Cas mediated GE, numerous investigations have been conducted to identify and define the gene(s) associated with salinity[29].

For example, the gene that controls the shallow root growth angle. A single base pair alteration in the third exon of the OsqSOR1 gene resulted in premature truncation and the production of soil surface roots, which increase salinity tolerance. In addition, CRISPR-Cas9[30] . Two oriented mutations in OsRR22, with -20 bp in the M16 exon and −1 bp in the M18 exon, produced more fresh leaves and increased root and shoot weight. Plant responses to stress are regulated by heterotrimeric G proteins. G protein producing genes (gs3, dep1) underwent null mutations based on CRISPR-Cas9 to develop salinity tolerance that deletion of the first and second exons of the paraquat tolerance 3 (OsPQT3) gene resulted in enhanced germination and increased salt tolerance (150mM NaCl). Moreover, this gene may function as an off-switch to disable the stress mechanism[31]. For both salinity sensitivity and tolerance, the balance between Na+ and other salts is necessary. Oust (open stomata 2) mutants treated to 150 mM NaCl exhibited increased morphological growth and decreased K+ concentration. In the OsbHLH024 TF, a single base pair deletion improved resistance to oxidative stress by maintaining the proper ratio of Ca2+, Zn2+, and Mg2+ minerals. According to [32]TFs, like genes, may also induce Na+ and K+ compartmentation to produce salt tolerance.

Comment [NSN10]: Corrected

However, due to their complex function, steroid hormones also have a role in stress tolerance, making it difficult to use them to enhance plants. According to, SERK2 is a gene associated with steroids that improved salt tolerance[33]. The deletion of the violaxanthin de-epoxidase (OsVDE) gene using CRISPR-Cas9 has also been shown to bring about salinity tolerance, as evidenced by increased ABA levels, greater survival rates, and stomatal closure. Similarly, ABA biosynthesis also plays certain functions in salinity tolerance. The induction of stress tolerance is mostly determined by genotypic background, even though gene disruption may play a role [33, 34]. Surprisingly, only WT_HD lines exhibited salinity tolerance due to a mutation in the OsBadh2 gene, demonstrating the important role that genetic background plays in the acquisition of stress tolerance[35].

On the other hand, it has also been documented that positive regulators of salinity tolerance are disrupted, resulting in the introduction of traits that cause rice lines to become sensitive to salt[36]. for example, the loss of the Ca2+sensor, calmodu-lin (OsCaM1) gene, resulted in a smaller primary root as well as shortened lateral root length and reduced root density, showing its vulnerability to salt stress. Similarly, methylglyoxal and glyoxalase I activity were enhanced when CRISPR-Cas9-based mutation in the first exon of the glyoxalase (OsGLYi3) gene produced saline hypersensitivity (.Crop plant salt tolerance is significantly impacted by the Na+ and K+ balance in the root zones. Additionally, mitochondrial phosphate knock-out lines[37].

The transporters (OsMPT3;1 and OsMPT3;2) genes inhibited impaired morphological growth and lowered Na+efflux, K+, and Ca+influx as well. Similar to genes, TF may also positively control one's ability to tolerate salinity. Unbalanced K+ and Na+ influx was the result of salt sensitivity phenotypes caused by CRISPR-Cas9-oriented disruption of a trihelix (OsGTγ-2) TF[38]. Similarly, salt-sensitive phenotypes, such as shorter plants than controls, were produced by CRISPR-Cas9-mediated editing of the third exon of BEAR1, a bHLH[39].TF Interestingly, salt-sensitive lines were also produced by bHLH044 TF knockout mutants. This sensitivity was caused by increased amounts of reactive oxygen species, which were then followed by higher levels of lipid peroxidation and H2O2[40].

2.5 Chill

Comment [NSN11]: Number subsection added

Since rice is susceptible to chilling stress, cultivating it in northern regions with much lower yearly temperatures will require inducing chilling tolerance [41]. As a result, numerous initiatives have been undertaken to either study the genes implicated in chilling tolerance or to generate chilling tolerance. Numerous genes that because tolerance have been identified in the past. For example, the disruption of the genes for grain size (GS3), panicle length (OsPIN5b), and cold tolerance (OsMYB30) by CRISPR-Cas9 resulted in increased yield and cold tolerance. Furthermore, reported that two triple mutants, consisting of ospin5b/gs3/osmyb30-25 and ospin5b/gs3/osmyb30-4, showed better grain yield and improved cold tolerance[42]. Similarly, reduced temperature exposure of CRISPR-Cas9-based osatp4 and ostcd3 mutants resulted in increased cold tolerance and decreased chlorophyll contents. Although altering positive regulators is not necessary, the CRISPR-Cas9 system's use assisted in clarifying their crucial role in cold stress. Thus, it demonstrates that the CRISPR-Cas9 system plays a crucial role in creating new germplasm that is resistant to cold stress in addition to helping to grow it.in functional examination of the recently discovered and cloned genes or genes[43].

2.6 Herbicide

Comment [NSN12]: Number subsection added

Weeds pose a serious threat to rice cultivation and severely reduce yield. Herbicide-resistant Acetolectase Synthase (ALS) gene catalyzes the first step of branched amino acid biosynthesis, which is the primary target of bispyribac sodium (BS) and other herbicides. Recently, basmati rice's ability to withstand BSE was enhanced by the conversion of tryptophan to leucine at the ALS gene's 548th position[47].Furthermore, auxin hormones are involved in several processes in plants, such as vascular differentiation, tissue elongation, and embryogenesis. Auxinic herbicides are used to deal with dicot weeds. Auxin signaling f-box (OsAFB4) auxin receptor-directed disruption by CRISPR-Cas9 enhanced tolerance against picloram and 2,4-dichlrophenoxyacetic acid[48]. Furthermore, it has been demonstrated that single bp substitution is essential for creating rice lines resistant to herbicides. They were able to identify 16 different mutations in OsACC1 that correspond to herbicide resistance. Furthermore, triple mutants of OsPUT knock-out lines based on CRISPR-Cas9 have also obtained paraquat herbicide resistance[49]. In addition to BS, glyphosate is a common herbicide that also has an impact on the primary crop. Herbicide tolerance was produced by

a single base pair substitution in the glyphosate-resistant gene (EPSPS) from 96G to A. Therefore, in this instance, it can also be said that CRISPR-based base editors have demonstrated their ability to produce mutants that may resemble naturally occurring ones in addition to improving rice tolerance to herbicides. In the previous three years, there have been no reports of herbicide tolerance[35].

2.7 Thick metal

Comment [NSN13]: Number subsection added

Heavy metal exposure is harmful to both people and plants because, even at low quantities, the metals can harm organs. Eating heavy metals puts human health at risk since they are persistent, non-biodegradable, and stable. A growing amount of cadmium (Cd) has been found contaminated agricultural soil, making it one of the heavy metals most dangerous to life[50]. Cadmium affects rice quality and yield, but it also interacts with metabolic pathways and may be linked to a number of cancers and bone diseases in humans. Cadmium is a severe health danger. Regretfully, only a small number of genes implicated in the metabolism of mercury have been found to yet, despite extensive study being done to uncover the genes connected to mercury poisoning[50]. For instance, shoots of null mutants of the low cadmium (oslcd) gene accumulated less Cd when exposed to soil enriched with Cd. Similarly, brown rice mutants lacking the mir535 gene deposited 35% less Cd than the control under controlled stress conditions of 2 µmol/L Cd. Furthermore, a similar Cd tolerance with little manganese buildup has been produced by deleting the gene associated with the natural resistance associated macrophage protein (OsNramp5) using CRISPR-Cas9. Copper oxide nanoparticles, or CuO NPs, are detrimental to rice plants in addition to lead. Oscerk1 mutants showed resistance to CuO NPs, and this was accompanied by an increase in H2O2 buildup and antioxidant system modulation, respectively[51]. In addition to heavy metal contamination, pesticide residues can also have an impact on fertilized soils. The primary factor in the catabolization of oxyfluorfen pesticide residues in soil is the acetyltransferase (OsACE2) gene. However, the expected morphological growth and enhanced oxyfluorfen accumulation were achieved by downregulating this gene utilizing CRISPR-Cas9-based manufacturing, suggesting a positive association with oxyfluorfen catabolization[52].

2.8 Other

Comment [NSN14]: Number section added

Rice genome rewiring using the CRISPR system to build resistance to biotic stresses. In rice, biotic stressors such as insects and illnesses can result in yield losses of up to 40%, and occasionally 100%. Numerous pathogens, such as bacterial, fungal, and viral infections, target rice, resulting in a variety of diseases that eventually impede or cease the crop's ability to grow and develop [53]. Therefore, creating rice cultivars resistant to pathogens and insects is crucial for ensuring global food security. In this sense, the development of rice lines resistant to biotic stress is being greatly aided by the CRISPR-Cas system. Thus, in this section, the contributions of the CRISPR-Cas system are emphasized[54].

3. BLAST OF RICE

Comment [NSN15]: Number section added

A hemi biotrophic fungus called Magnaporthe oryzae is the source of the fungal disease known as rice blast. Because of the fatal harm it causes to rice, it has been thoroughly researched. Additionally, researchers have created a rice M. oryzae pathosystem that is utilized as a main model for researching plant-microbe interactions[54]. In the last three years, researchers have used this model to carry out a number of investigations in which they produced rice blast-resistant lines using CRISPR-Cas systems. In contrast, the pathogen invasion was delayed by single FLR1 mutant lines and single FLR2 receptor deletion lines, respectively[55]. Variations in the expression of other genes may lead to variations in resistance or susceptibility. Similarly, OsDjA2 and ethylene-responsive factor 104 (ERF104) have been disrupted resulting in blast resistance, which is typified by decreased disease symptoms. It has also been noted that salicylic acid (SA) has an indirect correlation with blast resistance and may play a significant function in the plant immune system[56]. A UDP-glucosyltransferase gene called UGT74J1 was down-regulated, which increased SA buildup and enhanced blast resistance in addition to inducing many PR-related genes. Further research is necessary to confirm whether blast resistance results from increased SA accumulation or from the overexpression of PR-related genes[57]. Moreover, it has been demonstrated that reducing the pathogen's avirulence activity can effectively induce blast resistance. For instance, ubiquitin-conjugating enzyme 26 (osubc26) mutants based on CRISPR-Cas9 shown reduced avirulence activity through compromising proteasome function via means of AvrPiz-t cell disintegration. In addition to gene disruption, rice lines have also been engineered to develop

blast resistance through the use of CRISPR-Cas-based gene knock-in techniques[58]. By inserting exon #2 of the Pi-ta gene (blast resistant gene) into the Pi-ta gene (susceptible gene) has created rice blast resistance through blight resistance induction. However, in the last three years, other similar genes and TFs have also been discovered, and their expression is necessary to guarantee blast resistance. In order to guarantee blast resistance, certain genes may ultimately express themselves[59].

Comment [NSN16]: Number section added

4. SPORADIC BLIGHT

Rice bacterial blight, which is brought on by Xanthomonas oryzae pv. Oryzae (Xoo), is a damaging rice disease that can cause a 75% reduction in grain output. Xoo might activate the gene responsible for the host's susceptibility, and then use its endogenous transcription activator-like effectors (TALEs) to take control of the host's machinery[60]. Additionally, TALEs may attach to effector binding elements (EBE) in the upstream regions of SWEET genes or other genes that are vulnerable to plants, hence causing bacterial illnesses. Targeted mutation with enhanced resistance against Xoo was found in the OsSWEET14 gene promoter, which is characterized by the AvrXa7 deletion[61]. Have consistently gained resistance against the Asian and African races of Xoo (AXO1947). Apart from the knockout of genes [62].

Other SWEET genes have also been altered by scientists; added indels using CRISPR-Cas9 to the susceptibility gene's promoter (OsSWEET13). Phenotypic assessment hasn't been done yet, though. In addition to SWEET genes, GE has been used to investigate the effect of pathogen-induced disease-inducing genes, including as Xa13, Xa1, and Xa23, in rice blight resistance[62]. It is important to note that these two genes may also be involved in broad spectrum resistance (BSR). Moreover, blight resistance was also brought about by changing the EBEs of the Xa13 gene's promoters. In addition to EBEs, UPT boxes are crucial for developing blight resistance. Site-specific mutations in the Xa13 gene's UPT box caused by CRISPR-Cas12a resulted in blight-resistant phenotypes and disrupted TALE binding sites[63]. Therefore, CRISPR-based GE technologies may be some of the most promising methods for fighting bacterial blight and lowering production losses brought on by it[64].

Blight on Sheaths Rhizoctonia solani, the cause of sheath blight, causes the entire plant to wither and lodge, which can lower grain output by up to 50%. To instill disease resistance in crop plants, susceptibility genes must be identified[65]. Over the past three years, a great deal of work has been done to discover and modify the genes that are susceptible to disease using the CRISPR-Cas system. According to other research, sheath blight resistance is favorably correlated with the majority of genes. The Protein Phosphate (OsPP2A1) gene, for instance, produced five mutants when the CRISPR-Cas9 system was used to edit the gene. pp2a-1-1 had one or two bp deletions in the first exon, while pp2a-2 had a single bp deletion in the eleventh exon. Furthermore, pp2a-5 had a 1 pb loss in the first exon in addition to the 1 bp insertion in pp2a-3 and pp2a-4's first and second exons, respectively[66]. Remarkably, in comparison to controls, all of these mutants showed hypersensitivity to the sheath blight disease. NH4+ uptake is positively correlated with sheath blight disease, as demonstrated by the hypersensitivity of osamt1 lines (an ammonium transporter) to isolates of R. solani.[67]. Furthermore, resistance to different diseases or disorders has been established by some genes. In light of this, each of these applications highlights the immense potential of CRISPR-based GE technology to produce rice varieties that are resilient to climate change and will enhance global food security[68].

5. POSSIBILITIESE, OBSTACLES AND THE FUTURE

Comment [NSN17]: Corrected

Transposases are a few GE technologies that have been utilized for a long time to improve rice. Since the first use of CRISPR-Cas systems in rice in 2013, a number of advancements in rice DNA and RNA editing have been noted. Globally, a multitude of CRISPR-Cas system variations, including CRISPR-Cas9/-Cas12/-Cas13, DNA/RNA base editors, and prime editors, have been effectively employed to produce unique and desirable rice lines. Apart from their immense promise, CRISPR-Cas systems present many constraints and difficulties. Other noteworthy outcomes include accuracy and efficiency in targeting the desired DNA/RNA fragment, as well as off-target effects. Numerous attempts have been made, and countless more are required, to overcome these obstacles.

For instance, several alternative PAM sites (such NAG, NGA, NNGG, NNG, NAA, etc.) have been discovered to increase system efficiency. The wild-type Cas9 protein may be able to identify the NGG and NAG PAM sites in

rice. Moreover, claim that the use of NGA alone or in conjunction with NAG may improve editing efficiency. Nevertheless, despite the fact that SpyCas9 first recognized NAG PAM, it has been discovered that NGG has exhibited a significant affinity for the SpyCas9 protein. If this is not the case, CRISPR-Cas systems may be able to find the target gene or targets in the genome that can be disrupted (PAM independent). Furthermore, finding more Cas9 nuclease variants can help overcome the issue of introducing larger SpyCas9 nuclease into plants. One recent example of a reduced Cas9 nuclease that can be used therapeutically is the one that was produced.Off-target repercussions are only one more growing problem that requires attention. Off-targets can be classified as either (1) Cas protein dependent or (2) Cas protein independent, especially in the case of base editors. As a result, both types are significant and call for a conclusive response. Numerous in silico, in vitro, in vitro in cellulo, and in cellulo based approaches are already available to identify genome-wide CRISPR-Cas off-target regions. The current advancements in genomics and next-generation sequencing (NGS) will surely help discover CRISPR-edited plants without off-target mutations and reduce off-target mutations. Furthermore, CRISPR-based GE technology is now more reliable due to the restricted off-target activities of prime editing, base editing, and CRISPR-Cpf1 type systems. When everything is taken into account, it is anticipated that off-target effects will be much less, potentially even nonexistent.One of the most important procedures in introducing CRISPR/Cas system reagents into the target plant, aside from off-targets, is tissue culture. One of the main obstacles to improving employing CRISPR-Cas based GE technology is the optimization of tissue culture for each tar-geted crop and varied economically important tree species (which are difficult to propagate and/or close their extinction). There have been a lot of developments and accomplishments in this area in recent times. Fortunately, grafting and mobile CRISPR have recently been combined by researchers to avoid the time-consuming and laborious tissue culture method. The CRISPR/Cas9 reagents are delivered to the distal parts of the unmodified grafted scion as RNA from transgenic roots (rootstock), where they are translated into proteins to induce heritable mutagenesis at desired loci. have thoroughly studied this technique, and it is shown to that it has a huge potential to develop genetically stable and transgene-free plants for field and vegetable crops, as well as for fruits and other useful trees. It has been shown that CDB is an effective method for achieving the heritable transformation of several plant families' species. Furthermore, there have been multiple attempts to conduct tissue culture-free GE by in planta transformation .

Genetically engineered plants with detectable amounts of foreign DNA are prohibited from being grown for commercial purposes in a number of nations. In a similar vein, the question of whether CRISPR-edited plants should be regarded as genetically modified organisms (GMOs) and go through the GMO regulatory process or not, and proceed directly to the next stage of field trials and commercialization, is still being debated in many nations, including the European Union (UN). In this context, a few countries have made decisions about genetically modified crops and have finished or are nearly finished developing their legal frameworks. Given that the mutations or genetic alterations of the CRISPR-edited plants are comparable to those that can arise in natural populations or through conventional breeding, the UK, Argentina, Australia, Brazil, Canada, Chile, China, Colombia, India, Israel, Japan, and the USA recently approved the use of GE tools for agricultural purposes and exempted the plants from laws pertaining to genetically modified organisms (GMOs). On the other hand, in the EU and New Zealand, GE crops are regulated as genetically modified organisms. However, the purpose of the inaugural meeting of EU agricultural ministers was to deliberate on the EU's latest proposal about innovative genomic techniques (NGT), which encompasses genetic engineering. According to the studies, a few EU countries approved of the recommendations made on GE technology, while a few others voiced worries about potential consequences. Similarly, various nations across the globe.

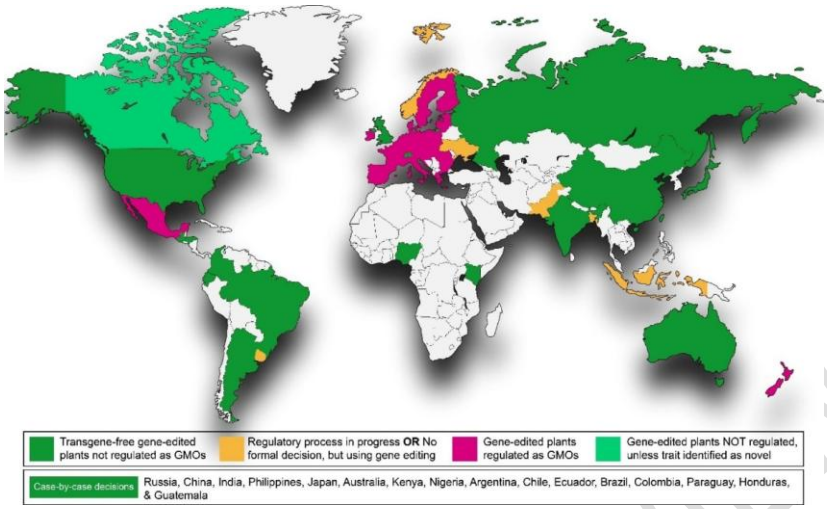

Fig.1. Current global regulations pertaining to gene editing and plants that have undergone genetic editing

Comment [NSN18]: Corrected

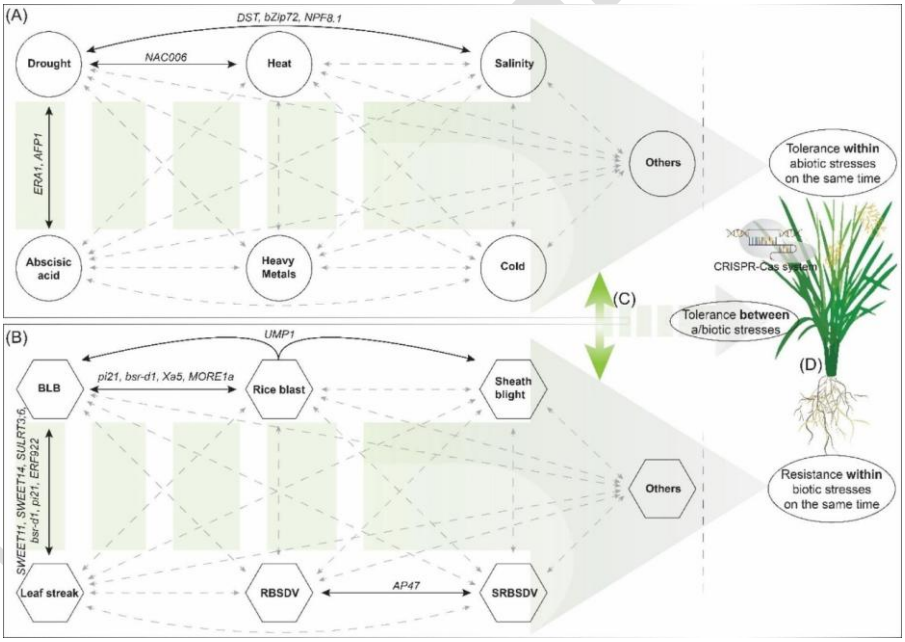

Fig.2. An example of the proof-of-concept for creating rice lines that are resistant to climate change through simultaneous manipulation of one, two, or more genes, as well as potential future developments.

Comment [NSN19]: Corrected

Abiotic stress networks (A), biotic stress networks (B), combined abiotic and biotic stress tolerance (C), and CRISPR-edited rice lines with simultaneous stress tolerance (D). Solid lines indicate proof-of-concepts for the simultaneous creation of resistance or tolerance to several stresses; dotted lines show potential directions and sites for more rice gene editing research. BLB, or bacterial leaf blight; SRBSDV, or southern additionally known as the rice black streak, dwarf virus (RBSDV).

Either they are still discussing the new genomics and precision breeding technologies, or they are drafting regulations and proposals, as those for Pakistan, the Kingdom of Saudi Arabia, etc.

Though producing transgene-free plants through outcrossing is a time-consuming and tedious operation, current developments in CRISPR systems may eventually replace it.

6. CONCLUSION

Comment [NSN20]: Number Section a dded

Versatile findings from 2022 and 2023 about the ability of the CRISPR-CoV simultaneously kill off and fine-tune gene expression are probably going to pave the way for their easy application in precision breeding and agricultural speed initiatives, including rice.In conclusion, the fact that Despite their limits and slower rate of action, traditional plant breeding techniques, are having trouble meeting the world's food needs in addition to genetic engineering and transgenics. Geeks with a CRISPR-Cas base are among the latest, quick, and precise breeding methods needed for rice breeding operations. The CRISPR-Cas based GE, however system has some drawbacks, issues, and difficulties, the applications and successes documented in this review demonstrate.This strategy may allow the development of tolerance for dealing with several biotic and abiotic obstacles at once (both inside and between various environmental stressors)., thus saving researchers time and money in the future. A few proof-of-concepts have been shown to be tolerant or resistant to two or more pressures in this regard. As we conclude up our discussion, we hypothesise that developing and characterizing CRISPR-edited plants simultaneously under varying stress circumstances holds enormous potential for revolutionizing and expediting upcoming breeding projects.

Comment [NSN21]: Corrected

COMPETING INTERESTS

Comment [NSN22]: Section added

The authors have declared that no competing interests exist.

Comment [NSN23]: Added

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Comment [NSN24]: Corrected

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