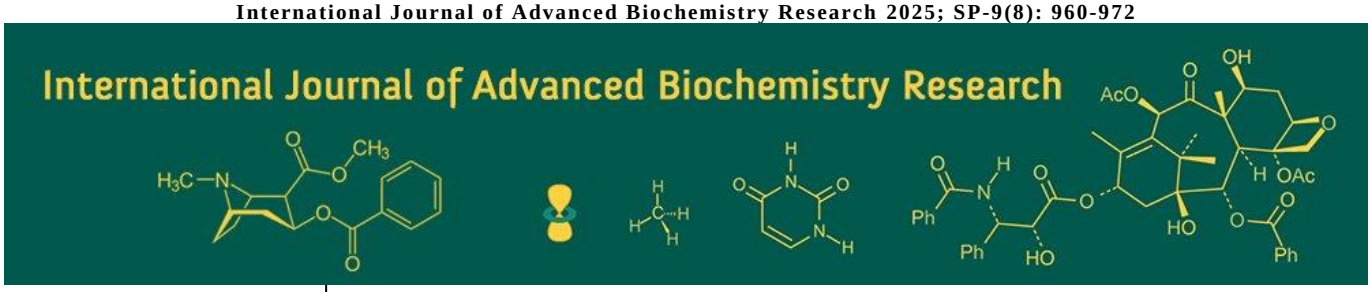

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Applications of CRISPR/Cas gene editing in vegetable crop disease and stress resistance

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Abstract
CRISPR/Cas genome editing has revolutionized plant biotechnology by enabling precise manipulation of plant genomes. In vegetable crops, CRISPR-based methods have been deployed to enhance resistance against biotic stresses (pathogenic fungi, bacteria, viruses, nematodes, and insects) and abiotic stresses (drought, salinity, heat, cold, flooding, heavy metals). This review provides a comprehensive overview of CRISPR/Cas mechanisms in plants and surveys major targets and outcomes in a wide range of vegetables, including tomato, potato, pepper, eggplant, cucumber, cabbage, lettuce, spinach, carrot, onion, garlic, beans, and peas. We discuss key susceptibility genes that have been knocked out or modified to confer resistance in each crop group, and we highlight examples of base editing, prime editing, multiplex editing, and promoter editing as advanced CRISPR applications. This detailed technical review aims to inform researchers of current progress and future opportunities in applying CRISPR/Cas to improve disease and stress tolerance in vegetables.

Keywords: CRISPR/Cas9, base editing, prime editing, powdery mildew, late blight, viruses, drought tolerance, salinity tolerance, transformation, vegetable crops

Introduction
CRISPR/Cas (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated) genome editing has emerged as a powerful tool to introduce targeted genetic changes in plants [6, 34]. Derived from a prokaryotic adaptive immune system, the CRISPR/Cas9 system typically uses a guide RNA (gRNA) to direct the Cas9 nuclease to a specific genomic locus, where Cas9 induces a double-strand break (DSB) adjacent to a protospacer adjacent motif (PAM) [19]. In plant cells, such DSBs are repaired predominantly by the error-prone non-homologous end joining (NHEJ) pathway, leading to insertions or deletions (indels) that can knock out gene function [103]. Alternatively, in the presence of a repair template, homology-directed repair (HDR) can introduce precise nucleotide changes or insertions [35]. Beyond classic Cas9, other CRISPR nucleases (such as Cas12a/Cpf1) and engineered Cas9 variants with altered PAM specificities expand the targetable genome space [61]. Recent advances include base editors (cytosine or adenine base editors) that fuse a catalytically impaired Cas nuclease to a deaminase enzyme, enabling single-base conversions (C•G→T•A or A•T→G•C) without DSBs [82, 102]. Prime editors combine a Cas9 nickase with a reverse transcriptase and a specialized pegRNA to write new sequences at the cut site, allowing all 12 types of base substitutions, small indels, or combinations thereof. These precision-editing tools can create point mutations or small sequence changes to alter protein function or regulatory elements without introducing transgenes [81].
In crop improvement, CRISPR/Cas has been used to knock out susceptibility (S) genes, introduce or recreate resistance (R) alleles, and modify quantitative trait loci (QTL) for yield and quality. In vegetables, where many desirable traits such as disease resistance and stress tolerance are polygenic or derived from wild relatives, CRISPR offers the possibility of stacking multiple resistance traits without linkage drag [20]. Unlike traditional breeding, CRISPR can target specific genes with high precision and speed. Moreover, multiplexed CRISPR strategies allow simultaneous editing of several genes or gene family members,

which is useful in cases where redundant genes control susceptibility. This article surveys the state of CRISPR/Cas applications in major vegetable species for improving resistance to pathogens (biotic stresses) and environmental stresses (abiotic stresses) [83]. We summarize known gene targets and editing strategies for each vegetable crop or crop group, highlighting successes and challenges [104]. We also discuss recent technical advances base editing, prime editing, multiplex editing, and promoter editing that are transforming plant genome engineering. Four tables detail CRISPR targets for stress resistance, key studies by institutions, and various transformation methods used in vegetables [36].

CRISPR Mechanisms in Plants

In plants, CRISPR/Cas9 is most commonly delivered as DNA constructs (via Agrobacterium or particle bombardment) or as preassembled ribonucleoprotein (RNP) complexes (via protoplast transfection or biolistics) [62]. After the Cas9/gRNA complex introduces a double-strand break at the target site, NHEJ repair often leads to small insertions or deletions (indels) that can disrupt gene coding frames, resulting in knockout alleles. For gene knock-ins or precise edits, homologous recombination or homology-directed repair (HDR) using a donor template is possible but generally less efficient in plants [36].

CRISPR/Cas9 has been adapted to many plant species, including vegetables. Cas12a (Cpf1) variants, which recognize different PAMs (TTTV) and create staggered cuts, have also been used in some crops. A new generation of engineered Cas9 enzymes (e.g. SpCas9-NG) can target relaxed PAM motifs, increasing the number of editable loci in plant genomes [84].

Base editing uses Cas9 (often a nickase variant or dead Cas9) fused to a cytidine deaminase or adenine deaminase. The deaminase converts C→T (or G→A on the opposite strand) or A→G (T→C) within a narrow window of the gRNA-binding site. Base editors have been demonstrated in some vegetables (e.g. ALS gene editing in tomato and potato) to introduce herbicide-resistant alleles and could potentially edit disease-resistance alleles [105].

Prime editing uses a Cas9 nickase fused to reverse transcriptase, guided by a prime editing guide RNA (pegRNA) that contains a primer binding site and the desired edit. In principle, prime editors can install any small change (substitution, insertion, deletion) without a separate donor template. Prime editing is still being optimized in plants and has lower efficiency than base or NHEJ editing, but it promises to create precise alleles (e.g. known resistance alleles or regulatory variants) in the future [38].

Multiplex editing employs multiple gRNAs expressed simultaneously (often from a polycistronic tRNA-gRNA array or multiple promoters) to target several genes at once. This is useful for knocking out all members of a gene family or for editing multiple S-genes to broaden resistance. For example, multiplex CRISPR targeting of three tomato PPO genes was used to reduce tissue browning, and five gRNAs have been used in some trials to target multiple disease susceptibility genes in potato [85].

Promoter editing or cis-regulatory editing involves targeting promoters or enhancers rather than coding sequences. For instance, CRISPR can be used to mutate promoter elements that control gene expression, either to reduce unwanted gene expression (e.g. of a susceptibility gene) or to enhance expression of a defence gene [86]. Engineered transcriptional activator or repressor fusions (CRISPRa or CRISPRi) can also modulate gene expression without permanent changes, but here we focus on genome-edited promoter variants. Successful examples in plants include editing stress-responsive promoters in rice and tomato to alter drought or cold response, and similar strategies could be applied to vegetables once key regulatory motifs are identified [39].

In summary, the CRISPR/Cas toolkit in plants includes not only simple knockouts via Cas9-induced NHEJ, but also base editors, prime editors, multiplex constructs, and promoter editing. These advances greatly expand the scope of possible edits in vegetables, enabling fine-tuning of gene function and expression for disease and stress resistance [87].

CRISPR Targets for Biotic Stress Resistance

Vegetable crops are challenged by a wide array of pathogens: fungi and oomycetes cause blights and mildews; bacteria cause soft rots or blights; viruses (especially RNA viruses like potyviruses) cause mosaics and stunting; nematodes infect roots; and insects (thrips, aphids, etc.) damage foliage [63]. Classical breeding has identified numerous R-genes and QTL for disease resistance, but many of these can be overcome by evolving pathogen populations. CRISPR offers the ability to engineer durable, broad-spectrum resistance by modifying plant susceptibility genes (S-genes) or activating endogenous defense pathways [21].

Fungal and Oomycete Pathogens

Fungal diseases are a major target for CRISPR in vegetables. A common strategy is to knock out plant susceptibility genes such as MLO (Mildew Resistance Locus O), DMR (Downy Mildew Resistance-related), or other S-genes. For example, many plants carry MLO homologs that act as susceptibility factors for powdery mildew fungi [88]. In tomato (*Solanum lycopersicum*), knock-out of SlMLO1 confers nearly complete resistance to tomato powdery mildew (*Oidium neolycopersici*) [108]. The ol-2 resistance allele in tomato, which arises from a natural SlMLO1 mutation, was reproduced using CRISPR/Cas9, resulting in durable, recessive powdery mildew resistance without transgenes [40]. Similarly, pepper (*Capsicum annuum*) possesses CaMLO2, an orthologous susceptibility gene; CRISPR/Cas9 editing of CaMLO2 has been used in hot pepper cultivars to enhance resistance to powdery mildew [107]. In cucumber (*Cucumis sativus*), disruption of the CsMLO8 gene by CRISPR created transgene-free mutant lines exhibiting high resistance to powdery mildew (*Podosphaera xanthii*). These examples show that targeting MLO-family genes with CRISPR/Cas can be an effective strategy to control powdery mildew across diverse vegetables [106].

Another S-gene family is the DMR (Downy Mildew Resistance) family, specifically DMR6 (Downy Mildew Resistance 6), which encodes an enzyme that down-regulates salicylic acid (SA) accumulation [89]. The loss-of-function allele dmr6 was first identified in Arabidopsis and subsequently found in other species. In tomato, CRISPR-induced mutations in the SlDMR6-1 ortholog caused elevated SA levels and broad-spectrum resistance to multiple pathogens (including fungi, oomycetes and bacteria) without major yield penalties [109]. Potato (*Solanum tuberosum*) has two DMR6 homologs; CRISPR knockout of StDMR6-1 (but not StDMR6-2) also increased resistance to late blight (*Phytophthora infestans*) while retaining normal

growth. These studies illustrate that DMR6 genes are conserved S-genes in Solanaceae, and their CRISPR-mediated disruption can provide durable disease resistance [7].

Other susceptibility genes include DND1 (DEFENCE NO DEATH 1), a gene involved in hypersensitive response regulation [110]. Tomato and potato plants with CRISPR knockouts of DND1 showed enhanced resistance to certain fungal pathogens (powdery mildew in tomato, late blight in potato), although complete knockouts sometimes have pleiotropic dwarfing or necrotic phenotypes [90]. Recent work in tomato identified a novel partial dnd1 allele (a small in-frame deletion) with CRISPR that retained normal growth while providing powdery mildew resistance. This highlights how precise CRISPR editing (rather than full gene knockout) can mitigate fitness costs [22, 41].

In summary, major fungal and oomycete targets in vegetables have been S-genes like MLO1 and DMR6 [111]. CRISPR/Cas-mediated knockout of these genes in tomato, pepper, cucumber, and potato has conferred durable resistance to powdery mildew and late blight, often broad-spectrum and recessive in inheritance. These edits mimic natural resistant alleles found in wild relatives and can be introduced into elite cultivars without linkage drag [64].

Viral Pathogens

RNA viruses, particularly potyviruses, are significant pathogens in vegetables (e.g. Potato Virus Y in potato and pepper, Cucurbit viruses in cucumber, Lettuce Mosaic Virus in lettuce). A common and effective CRISPR target is the plant translation initiation factor eIF4E and its isoforms [112]. Many plant recessive resistance genes against potyviruses map to eIF4E or eIF(iso)4E. By disrupting specific eIF4E genes, plants can be made refractory to virus infection because the viral VPg protein can no longer hijack the host translation machinery [91].

For instance, tomato has two paralogs (eIF4E1 and eIF4E2). CRISPR knockout of eIF4E1 in tomato conferred resistance to certain potyviruses such as Pepper mottle virus (PepMoV) and reduced susceptibility to Potato virus Y (PVY) [113]. Similarly, in potato (tetraploid), CRISPR-mediated knockout of the eIF4E1 gene broadened the resistance of a commercial cultivar (Desirée) to PVY strains that normally overcome existing R genes [42]. In cucumber, CRISPR editing of the single eIF4E gene produced non-transgenic mutants that were immune to Cucumber vein yellowing virus (CVYV) and showed resistance to Zucchini yellow mosaic virus (ZYMV) and Papaya ringspot virus (PRSV-W) [92]. In Chinese cabbage (Brassica rapa), editing of one of the eIF(iso)4E genes by CRISPR/Cas9 provided resistance to Turnip mosaic virus (TuMV). These examples demonstrate that translation initiation factors are conserved viral susceptibility genes across vegetable families, and their targeted mutagenesis is a versatile strategy for virus resistance [23, 65].

Other viral resistance strategies include editing *S-genes* specific to a virus family or editing recognition sites. For cucurbits, CRISPR/Cas9 targeting of key replication genes in an Arabidopsis model showed potential for constructing mosaic-resistant plants, though such approaches are still nascent [114]. In general, CRISPR has not been used widely to directly edit viral genomes in vegetable hosts (unlike the CRISPR antiviral approaches in animals), but knocking out the plant co-factors remains a practical path [115].

Bacterial Pathogens

Bacterial diseases such as soft rots and wilts are also targeted by CRISPR strategies. A similar approach of knocking out susceptibility genes has been applied for bacterial pathogens [116]. For example, tomato SlDMR6-1 knockout not only reduced fungal disease, but also enhanced resistance to bacterial pathogens like Pseudomonas syringae and Xanthomonas spp [93]. In potato, mutation of DND1 via CRISPR reduced susceptibility to the bacterial pathogen *Ralstonia solanacearum*. These cases reflect that S-genes often play roles in multiple pathogen interactions. To date, relatively few vegetable genes are identified exclusively for bacterial resistance, but CRISPR can mimic bacterial R-genes (if known) or disrupt bacterial toxin targets [1, 43].

Nematode and Insect Pests

Parasitic nematodes (e.g. root-knot nematodes) and insects are major agricultural pests, but CRISPR applications in vegetables against these are still emerging. Nematode resistance might be achieved by editing plant genes involved in feeding site formation or hormonal responses [94]. For example, in other crops CRISPR has been used to knock out nematode-susceptibility genes (such as Mi-1 homologs), but in vegetables this remains largely unexplored [117]. Similarly, insect resistance might involve editing genes that deter feeding or produce anti-insect compounds. No prominent vegetable CRISPR studies on insect resistance have been reported yet; this is a frontier for future work [66].

In summary, vegetable biotic stress targets for CRISPR include susceptibility genes (MLO, DMR6, eIF4E, DND1, etc.) conserved across species, defence regulators (e.g. transcription factors modulating SA or JA pathways), and specific host factors required by pathogens [44]. By knocking out or modifying these genes, researchers have obtained tomato, pepper, cucumber, potato and Brassica lines with enhanced resistance to fungi, bacteria and viruses. Table 1 below summarizes notable crop-target-gene combinations for biotic stress resistance [24].

CRISPR Targets for Abiotic Stress Resistance

Abiotic stresses such as drought, salinity, high temperature, cold, flooding and heavy metals greatly impact vegetable yields. CRISPR/Cas can improve tolerance by editing genes related to stress signalling, ion transport, water use, or protective metabolites [25, 67]. Below we discuss known and potential CRISPR targets for major vegetables under different abiotic stresses.

Drought Tolerance

Water deficit affects tomato, lettuce, beans and many other vegetables. Several transcription factors and signalling genes have been identified by CRISPR editing as drought-related targets. In tomato, loss-of-function mutations in the trihelix transcription factor SlGT-2 or SlGT-30 via CRISPR reduced stomatal density, resulting in lower water loss and improved drought tolerance (as well as increased fruit size via end reduplication) [45]. Another example is editing of the lateral organ boundaries domain gene SlLBD40, which enhanced drought resistance by modulating hormone signalling. Knocking out SlNAC family members or ethylene response factors by CRISPR has also produced plants with better performance under water stress [118]. In pepper, CRISPR disruption of SlGT homologs or other stress-responsive TFs may yield similar benefits (studies are ongoing) [68].

Table 1: Vegetable crops with CRISPR targets for biotic stress resistance

Crop	Biotic Stress (Pathogen)	Targeted Gene(s)	Notes and Outcomes
Tomato	Powdery mildew (Oidium sp.)	*SlMLO1*, *SlDND1*	Loss-of-function mutants confer near-complete resistance to powdery mildew; low-pleiotropy alleles identified.
	Late blight (Phytophthora infestans)	*SlDMR6-1*	Knockout increases resistance (due to higher SA levels) with minimal growth penalty.
	Bacterial speck/blight (*Pseudomonas*/*Xanthomonas*)	*SlDMR6-1*, *SlEDS1* (regulator)	*SlDMR6-1* KO reduces susceptibility; *SlEDS1* editing can modify defense pathways.
	Potyviruses (TEV, PVY, PepMoV)	*SlEIF4E1*, *SlEIF4E2*	*eIF4E1* knockout confers resistance to Pepper mottle virus; may require stacking with *eIF*(*iso*)*4E* for full spectrum.
Potato	Late blight (P. infestans)	*StDMR6-1*, *StDND1*, *StCHL1*	CRISPR KO of *StDMR6-1* and *StDND1* yields enhanced late blight tolerance; *StCHL1* KO also effective.
	Potato virus Y (PVY)	*StEIF4E1*	CRISPR knockout broadens PVY resistance in cultivars already carrying N genes, reducing viral accumulation.
	Bacterial wilt (Ralstonia solanacearum)	*StDND1* (homolog)	Mutant lines show reduced susceptibility, though may have mild phenotype.
Pepper	Powdery mildew (Leveillula taurica)	*CaMLO2*	Editing of *CaMLO2* in multiple cultivars produces mutants with enhanced mildew resistance.
	Potyviruses (PepMV, ChiVMV, TMV)	*CaEIF4E* (pvr1 locus)	Targeting *eIF4E* homologs (pvr loci) is expected to confer broad potyvirus resistance; CRISPR studies pending.
	Bacterial spot (Xanthomonas spp.)	*CaDMR6-1*	Putative editing target based on analogies to tomato, not yet reported.
Eggplant	Phytophthora blight (P. capsici/infestans)	*SmDMR6-1*	CRISPR knockout of *SmDMR6-1* aims to enhance oomycete tolerance; first-generation mutants are being evaluated.
	Fruit/leaf spots (Colletotrichum spp.)	*SmMLO* (putative)	Editing homologs of MLO may reduce susceptibility; untested in eggplant so far.
Cucumber	Powdery mildew (Podosphaera xanthii)	*CsaMLO8*	CRISPR mutants (targeting *CsaMLO8*) showed high powdery mildew resistance in field trials.
	Potyviruses (ZYMV, PRSV, CMV, CVYV)	*CsEIF4E*	Knockout mutants are immune or tolerant to multiple cucurbit viruses (CVYV, ZYMV, PRSV, etc.).
Cabbage	Turnip mosaic virus (TuMV, potyvirus)	*BrEIF4E*(*iso*) (Bra035393)	Editing *eIF*(*iso*)*4E* gene confers resistance to TuMV in Chinese cabbage (B. rapa) cultivars.
	Black rot (Xanthomonas campestris)	*BrDMR6* (putative)	Potential target analogous to tomato, not yet implemented.
Lettuce	Lettuce mosaic virus (LMV)	*LsEIF4E*	Natural LMV resistance maps to *eIF4E*; CRISPR editing of *LsEIF4E* could recreate this.
	Downy mildew (Bremia lactucae)	*LsMLO* (candidate)	Susceptibility factors likely exist; in Arabidopsis *MLO2* is S-gene for downy mildew.
Spinach	Downy mildew (Peronospora effusa)	*SoDMR6* or *SoMLO* (putative)	Editing homologs of known S-genes (like DMR6 or MLO) is a prospective strategy; not yet demonstrated.
Carrot	Carrot fly damage (Psila rosae)	*DcSPL* (DEFORMED ROOTS-like gene) [citation pending]	Natural *DTR1* mutation confers carrot fly resistance; CRISPR could recreate *dtr1* allele.
	Alternaria leaf blight (Alternaria dauci)	*DcAL13-like* (putative)	Candidate genes (e.g. detoxification enzymes) could be targeted; no reports yet.
Onion/Garlic	Downy mildew (Peronospora destructor)	*AcMLO* (predicted)	Editing onion MLO homologs could confer resistance; transformation system now established.
	Onion yellow dwarf virus (BYDV)	*AcEIF4E* (predicted)	Co-factor for BYDV is unknown; eIF4E editing is theoretical.
Beans (Phaseolus)	Bean common mosaic virus (BCMV)	*PvEIF4E* (bc-3 locus)	Recessive resistance bc-3 is a mutant *eIF4E*; CRISPR of *PvEIF4E* could simulate bc-3 resistance.
	Anthracnose (Colletotrichum lindemuthianum)	*PvDMR6* (putative)	No specific CRISPR targets reported; possible S-genes like *MLO* family.
Peas (Pisum)	Pea seed-borne mosaic virus (PSbMV)	*PsEIF4E* (sbm1 locus)	The *sbm1* allele encodes a modified eIF4E; CRISPR could be used to create this resistant allele.
	Downy mildew (Peronospora pisi)	*PsMLO2* (candidate)	Homologs of Arabidopsis MLO contribute to downy mildew susceptibility; potential CRISPR targets.

In leafy vegetables like lettuce, drought tolerance has been explored through natural variation rather than CRISPR; however, candidate genes include abscisic acid (ABA) receptors (PYR/PYL/RCAR family) and stress-related kinases (SnRK2s) [9]. Editing an ABA receptor in tomato has been shown to improve drought response, suggesting a similar strategy could apply to lettuce or spinach [8].

Carrot is relatively tolerant of moisture, but under drought stress some studies suggest upregulation of DcNCED (ABA biosynthesis) genes [70]. CRISPR knockdown of negative regulators of ABA or stomatal density (e.g. SDD1 or EPF peptides) could enhance water conservation in carrot. Peas

and beans also respond to drought via ABA pathways; CRISPR editing of key regulators (e.g. *AREB/ABF* transcription factors) may be a future direction [46].

Overall, tomato is a prime example where CRISPR has directly produced drought-tolerant lines by editing specific TFs (SlGT30, SlLBD40, SlHYPRP1, etc.) [69]. For other crops, analogous genes (orthologs of these tomato genes or universal drought regulators) are logical targets. Moreover, multiplex editing of gene families (e.g. several NAC or bZIP factors) may be needed to achieve substantial drought tolerance in multigenic traits [2].

Salinity Tolerance

High soil salinity affects tomato, cucumber, lettuce and brassicas. A key strategy is to regulate ion homeostasis by editing transporter genes. In tomato, the SlHYPRP1 gene (a hybrid proline-rich protein) was precisely edited by CRISPR to remove functional domains; mutants lacking the proline-rich domain showed improved salt tolerance and also enhanced heat tolerance. This approach indicates that editing single genes can affect multiple abiotic traits [71].

In cucumber, CRISPR studies have highlighted the potassium transporter CsAKT1. Mutants lacking CsAKT1 showed altered K^+ uptake under salt stress, confirming its role in maintaining a favourable K^+/Na^+ balance in plants treated with salt [72]. Disrupting Na^+ transporters is another approach: for example, *HKT1;1* homologs (Na^+ retrieval from xylem) could be edited to reduce Na^+ accumulation in shoots [47, 48]. While not yet reported in vegetables, CRISPR knockout of SlSOS1 (a Na^+/H^+ antiporter) might increase salinity sensitivity, whereas activating such transporters could confer tolerance (e.g. by promoter editing) [26].

In lettuce and spinach, less CRISPR work has been done. However, genes like SOS3, NHX1 and ALMT (anion channels) are known from Arabidopsis to regulate salt stress [119]. Similar targets in lettuce may be engineered. For example, downregulating an Na^+ transporter or overexpressing an Na^+/H^+exchanger via promoter editing could improve salt tolerance in leafy greens [95].

Overall, CRISPR in tomato and cucumber has validated ion transporters and stress regulators as targets for salinity tolerance. Future work may edit homologous genes in other vegetables or use CRISPR activation (CRISPRa) to boost expression of salt-protective genes [49].

Heat Stress Tolerance

High temperature can damage vegetables like tomato, pepper, cucumber, and lettuce. CRISPR targets for heat tolerance include heat-shock factors and protective proteins. In tomato, the edited *SlHYPRP1* variants (described above) also showed enhanced heat tolerance: removal of certain domains allowed plants to withstand several days at 42-45°C with recovery [10]. This suggests HYPRP1 normally has a repressive role under heat, and its editing yielded heat-resistant lines [96].

Editing heat shock transcription factors (HSFs) is another strategy. In tomato, CRISPR knockout of *SlHsfA1a* (a master heat-shock regulator) caused temperature sensitivity, confirming its importance. Conversely, cis-engineering of HSP promoters (e.g. HSP70) to increase expression might improve thermos tolerance [50].

Pepper and eggplant are also susceptible to heat. Although specific CRISPR studies are lacking, orthologous HSF genes (like Capsicum *CaHsfA2*) or DREB family TFs are plausible targets [120]. Cabbage and other brassicas often have cold tolerance but can suffer heat; editing EIN3 or ethylene-related genes via CRISPR could modify heat responses. Lettuce wilts at moderate heat, so downregulating negative regulators of heat tolerance (e.g. *HSFA2* or *CBF* which cross-talks with heat) could help [26].

In summary, CRISPR editing of heat stress genes (such as HYPRP1, HSFs, or cell cycle factors like SlGT30) has begun to yield multi-stress tolerant tomato lines. Extending these findings, any crop orthologs of these targets (e.g. pepper *CaHYPRP*, lettuce *LsHsfA*) are candidates [97]. Because heat tolerance is complex, multiplex editing of several genes (combining improved transpiration control, chaperone expression, and hormone signalling) may be required for robust effects [73].

Cold (Chilling/Frost) Tolerance

Cold stress is less of an issue for warm-season crops but important for overwintering or spring planting vegetables. Key cold tolerance genes in plants include the C-repeat binding factors (CBFs) and ICE regulators [121]. In tomato, CRISPR knockout of *SlCBF1* reduced chilling tolerance, demonstrating its necessity [98]. To improve cold tolerance, one could use CRISPR to create gain-of-function alleles (e.g. in promoters of *CBF*) or edit regulatory elements to prolong cold-induced gene expression. Alternatively, knocking out repressors of cold responses (like genes that normally degrade ICE proteins) might be beneficial [11].

For temperate vegetables like lettuce and spinach, enhancing *CBF* pathway genes by CRISPR is a theoretical approach [122]. For root vegetables like carrot, engineering cold tolerance could involve genes that control root cellular osmolytes (e.g. fructans) via CRISPR, though this is speculative. Since examples are limited, editing known stress regulators (NAC, MYB, zinc finger TFs that control cold response) may be an avenue for the future [27, 51].

Flooding (Waterlogging) Tolerance

Flooding stress (oxygen deprivation) is relevant to low-lying production or heavy rains. Few reports exist for vegetables, but CRISPR could target the ERF-VII family (submergence tolerance genes) or genes like *PDC1* (pyruvate decarboxylase) [99]. In rice, *SUB1* confers submergence tolerance; vegetables have no direct homolog but editing ethylene receptors (like SlETR in tomato) might influence flood responses. This area remains largely unexplored in vegetables [12].

Heavy Metal Tolerance

Heavy metal toxicity (cadmium, lead, arsenic) can limit safe cultivation of vegetables. Genes controlling metal uptake (e.g. transporters of the NRAMP or HMA families) are logical targets. For instance, knockout of a cadmium transporter could reduce Cd accumulation in edible tissues [52]. In Brassica species (cabbage, Chinese cabbage), which sometimes accumulate arsenic from soil, CRISPR of aquaporin channels (like Lsi1 analogs) or *HMA2/4* could be tested [74]. In tomato, modifying Zn/Cd transport genes (SlHMA3) might alter uptake. So far, specific CRISPR edits for metal tolerance in vegetables have not been reported, but the principle is analogous to findings in model plants and cereals [3, 28].

Table 2: Vegetable crops with CRISPR targets for abiotic stress resistance

Crop	Abiotic Stress	Targeted Gene(s)	Notes and Outcomes
Tomato	Drought	*SlGT30*, *SlLBD40*, *SlERF* (various)	CRISPR knockout of *SlGT30* or *SlLBD40* reduces stomatal density and improves drought tolerance; *SlERF* family edits modulate water-use.
	Salinity	*SlHYPRP1* (domain edited), *SlHKT1;2*, *SlSOS1*	Precise editing of *SlHYPRP1* domains yielded salt-tolerant plants; engineering of ion transporter genes is a future strategy.
	Heat	*SlHYPRP1* (8CM domain), *SlHSFs*	Edited *SlHYPRP1* variants conferred heat resilience; targeting heat-shock factor genes is another approach.
	Cold/Chilling	*SlCBF1*, *SlICE* (putative)	Knockout of *SlCBF1* reduces cold tolerance; prime editing of promoter *CBF* motifs might enhance resilience.
Potato	Drought	*StNOA1* (NOS homolog), *StCDPK* (calcium kinase)	Candidates from stress studies; untested by CRISPR in potato.
	Salinity	*StPDH* (pyruvate dehydrogenase)	Potential target based on metabolic role; no reports yet.
Pepper	Drought	*CaERF5*, *CaNAC* (various TFs)	CRISPR to knock out repressors or activate stress TFs is theoretical; pleiotropic effects must be assessed.
	Salinity	*CaABI1* (ABA signalling), *CaHKT1* (Na transporter)	Editing ABA pathway genes or Na^+ transporters could improve salt tolerance; research pending.
Eggplant	Heat	*SmHSF* genes, *SmLea* (late embryogenesis proteins)	Targets inferred from other species; CRISPR screening of candidate HSFs is possible.
	Drought	*SmDREB*, *SmNAC2*	Transcription factors known for drought response; editing could enhance water stress tolerance.
Cucumber	Salt	*CsAKT1*	CRISPR validated *CsAKT1* role: mutants confirmed its importance for K^+ uptake under salt stress.
	Drought	*CsAREB1* (ABA-responsive element), *CsSLAH* (anion channel)	Candidates from stress transcriptomics; not yet edited.
	Temperature (HVOC production)	*CsTPS5* (terpene synthase)	Knockout alters volatile profile under heat stress; studied via RNAi rather than CRISPR.
Cabbage	Salinity	*BrNAC1*, *BrMYB31*	Putative regulators of osmolyte production; CRISPR not reported.
	Cold hardiness	*BrCBF3*, *BrGolS* (galactinol synthase)	Potential targets to enhance frost tolerance; experimental validation needed.
Lettuce	Drought	*LsPYL* (ABA receptor), *LsSPL*	ABA receptor knockout might increase sensitivity; one study found *LSPYR1* mutation boost tolerance via ABA.
	Heat	*LsPIF4* (phytochrome-interacting factor), *LsHsfA2*	Analogous to findings in Arabidopsis/tomato; editing could alter thermosensing.
	Salinity	*LsSOS3*, *LsNHX1*	Ion homeostasis genes; CRISPR of orthologs might regulate Na^+ export/storage.
Spinach	Drought	*SobZIP* TFs, *SoDHN* (dehydrins)	Unknown; dehydrin gene editing for osmoprotection is theoretical.
	Heavy metals (arsenic)	*SoPIP* (aquaporin), *SoNRAMP*	Candidate transporters for metal uptake; untested.
Carrot	Drought/Salinity	*DcDREB2*, *DcCAT1* (catalase)	Potential targets based on stress signalling studies.
	Cold (freeze tolerance)	*DcAFP1* (antifreeze protein)	A carrot antifreeze gene could be enhanced via promoter editing.
Onion/Garlic	Drought	*AcNAC*, *AsNCED*	Candidate NAC TFs and ABA biosynthesis genes; transformation now possible to test these.
	Flooding	*AcPDC*, *AcADH*	Anaerobic metabolism enzymes; CRISPR to enhance flood tolerance has not been reported.
Beans (Phaseolus)	Drought	*PvXTH1* (xyloglucan endotransglucosylase), *PvPep1*	Cell wall modification and peptide signalling genes; theoretical targets.
	Salinity	*PvSOS1*, *PvNHX1*	Orthologs of Arabidopsis salt tolerance genes; potential for CRISPR editing.
Peas (Pisum)	Drought/Heat	*PsSPCH* (stomatal development), *PsDREB2*	Genes controlling stomata and dehydration response; editing may alter transpiration rates.
	Heavy metals (Al)	*PsALMT1* (aluminum transporter)	Pea analog of wheat TaALMT1; modifying activity could affect Al tolerance.

Advances in CRISPR Technologies for Vegetables

In addition to standard knockout editing, several advanced CRISPR approaches are being applied or explored in vegetables:

- **Base Editing (BE):** Base editors have been used in tomato and potato to introduce precise point mutations. For example, cytidine base editors (CBEs) were applied to the acetolactate synthase (*ALS*) gene in tomato and potato, creating herbicide resistance alleles without DSBs [29]. Similarly, BE could be used to create disease-resistant alleles (e.g. mimicking natural SNPs in susceptibility genes or R-genes) [53, 100]. Adenine base editors (ABEs) can make A•T→G•C changes, useful for editing regulatory motifs or creating start codon changes. Base editing avoids tissue-culture-based regeneration by sometimes allowing transient RNP or vector delivery with point editing, which in principle could yield transgene-free events at high precision [75, 123].
- **Prime Editing (PE):** Prime editing holds promise for precise editing in vegetables, though it has been mostly demonstrated in rice and Arabidopsis so far. In tomato, prime editing could theoretically install resistance-conferring single-nucleotide polymorphisms (e.g. in

promoter of *CBF* or coding region of *MLO*), or insert small tags [30, 54]. The challenge is delivery of the larger PE machinery and sufficient efficiency, but progress is being made (for example, prime editing of *SlALS* in tomato has been reported with modest efficiency). As protocols improve, prime editing may allow one-step introduction of any allele variant, which could be transformative for complex traits [13, 76].

- **Multiplex Gene Editing:** Vegetables often have gene families or multiple pathogen effectors, so editing multiple targets at once is valuable. Multiplex CRISPR (expressing two or more gRNAs) has been used in eggplant to knock out three polyphenol oxidase (PPO) genes simultaneously, reducing fruit browning [77]. Multiplex editing can also combine biotic and abiotic trait targets; e.g. one could simultaneously knock out a *DMR6* gene for disease and an *HKT* transporter for salt tolerance in tomato by a single construct. Delivery of multiplex gRNAs via tRNA-processing systems or Csy4 systems is feasible in plant transformation, and such approaches are increasingly utilized in breeding pipelines [55, 124].
- **Promoter and Regulatory Editing:** Editing promoters of key genes can modulate expression levels or stress induction. For example, in rice a drought-responsive NAC transcription factor's promoter has been modified by CRISPR to enhance its expression under stress. In vegetables, similar strategies are on the horizon [78]. One could edit W-box (WRKY TF binding) or ABRE (ABA-responsive) elements in promoters of defence genes or stress genes. For instance, editing the promoter of a tomato *CBF* gene to remove repressor motifs might increase cold induction. Likewise, deletion of a microRNA binding site in a UTR could stabilize a stress mRNA [56]. Although these uses are still rare in vegetables, they represent a powerful addition to the CRISPR toolkit [4].

Collectively, these advanced editing modalities greatly expand the types of genetic changes possible. In vegetables, where complex traits often depend on subtle allelic variants or stacked genes, base editing and prime editing enable precise allele creation, while multiplex editing enables trait stacking [100, 125]. Promoter editing allows fine-tuning gene expression. Together, they accelerate the development of new vegetable varieties with tailored resistance and stress tolerance [31].

Notable CRISPR Studies and Success Cases

Numerous research groups worldwide have demonstrated successful CRISPR editing in vegetables. Table 3 lists notable examples, including the crop, gene target, institution and outcome [14, 18]. These cases highlight the diversity of projects from model studies to field-relevant trials and the global effort in crop genome editing.

Table 3: Notable CRISPR/Cas experimental studies or success cases in vegetable crops

Institution / Country	Crop	Target Gene(s) / Trait	Outcome / Remarks
Swedish Univ. of Agricultural Sci., Sweden (2021)	Potato	*StDMR6-1*, *StDND1*, *StCHL1* (S-genes)	Generated knockout potatoes with increased late blight resistance, validating S-gene editing
Wageningen Univ. & Univ. Torino, NL/Italy (2024)	Tomato	*SlDND1* (powdery mildew susceptibility)	Developed tomato dnd1 mutants via Agrobacterium; identified an allele with minimal growth penalty and strong mildew resistance
Agricultural Research Org., Israel (Volcani Ctr.) (2016, 2023)	Cucumber	*CsEIF4E*, *CsaMLO8* (virus, powdery mildew)	Non-transgenic cucumber mutants resistant to multiple viruses (CVYV, ZYMV, PRSV) via *CsEIF4E* knockout; *CsaMLO8* knockout lines with powdery mildew resistance
Frontis & RDA Korea (2020)	Tomato	*SlEIF4E1* (Pepper mottle virus)	CRISPR/Cas9 knockout of *eIF4E1* in tomato conferred resistance to PepMoV
Nanjing Agri. Univ. & RDA Korea (2023)	Chinese cabbage (Brassica rapa)	*BrEIF4E*(*iso*) (Turnip mosaic virus)	Edited eIF(iso)4E gene via CRISPR; T1 lines with high editing frequency showed strong TuMV resistance
China Agr. Univ. (2024)	Tomato	*SlGT30* (trihelix TF for drought/fruit)	Knockout of *SlGT30* reduced stomatal density, increased cell ploidy, enhancing drought resistance and fruit size
Gyeongsang Natl. Univ. & collaborators, Korea/Vietnam (2023)	Tomato	*SlHYPRP1* (salt stress protein)	Domain-specific editing of *SlHYPRP1* (precise deletion of PRD and 8CM domains) created alleles with multi-stress tolerance (salt, drought, heat) and reduced bacterial growth
Kangwon Natl. Univ., Korea (2023)	Pepper	*CaMLO2* (powdery mildew)	Compared CRISPR/Cas9 RNP editing efficiency across six hot pepper cultivars; achieved up to 17% indel frequency with one sgRNA, validating *CaMLO2* editing potential
Torino Univ., Italy & WUR, Netherlands (2024)	Tomato	*SlDMR6-1* (broad-spectrum disease)	Confirmed that CRISPR knockout of SlDMR6-1 increases SA levels and broad disease resistance; one allele with partial deletion had no cas9 transgene and minimal off-targets
ENEA & CREA, Italy/Hungary (2022)	Potato	*StEIF4E1* (Potato Virus Y)	Produced tetraploid *eIF4E1* KO lines in cultivar Desirée via protoplast editing; mutants showed reduced PVY^NTN accumulation and milder symptoms, extending viral resistance spectrum.
CNRS & Univ. Toulouse, France (2019)	Arabidopsis (model)	*DND1*, *DMR6*, *PMR4* (general S-genes)	Demonstrated that CRISPR knockout of various S-genes confers recessive pathogen resistance; served as framework for targeting homologs in vegetables
Volcani Ctr., Israel (2016)	Cucumber	*CsEIF4E* (viral susceptibility)	Created transgene-free *eif4e* mutants resistant to Cucumber vein yellowing virus and zucchini yellow mosaic virus, using Agrobacterium and segregation
Nicolaus Copernicus Univ., Poland (2022)	Cucumber	*CsaMLO1/8* (powdery mildew)	Used CRISPR to generate cucumber lines with reduced powdery mildew lesions by mutating clade V MLO genes; validated that *CsaMLO1* and *CsaMLO8* redundancy determines susceptibility
EurekAlert News (Nanjing Univ.) (2023)	Chinese cabbage	*BrEIF4E*(*iso*) (virus)	Highlighted the editing of *eIF*(*iso*)*4E* in Chinese cabbage and its confirmed TuMV resistance, underscoring CRISPR's role in rapid breeding.
Univ. Florida, USA (2016)	Tomato	*SlMlo1* (powdery	Early demonstration of CRISPR knockout of *MLO* homolog (ol-2)

		mildew)	producing near-immune powdery mildew tomato

CRISPR Delivery Methods and Transformation Systems
Efficient delivery of CRISPR components into vegetable cells is crucial. Different vegetables use species-appropriate transformation and regeneration systems.

Agrobacterium tumefaciens-mediated transformation is widely used in dicotyledonous vegetables. Leaf explants or hypocotyl segments are co-cultured with Agrobacterium carrying binary CRISPR vectors [15, 57]. This method has produced stable edited lines in tomato, potato, eggplant, pepper, cucumber, lettuce, cabbage and others [101]. For example, tomato and potato use Agrobacterium on cotyledon or leaf pieces; hot pepper and eggplant often use cotyledon or epicotyl segments. Chinese cabbage and lettuce have been transformed via Agrobacterium on hypocotyl or protoplasts [32].

- **Protoplast transfection:** (PEG-mediated) allows DNA or RNP delivery to isolated plant cells, followed by plant regeneration from single cells or tissue. This has been done in tomato, potato, and lettuce as a transgene-free approach [79]. For instance, tomato RNPs have been introduced into mesophyll protoplasts and regenerated into edited plants. Cucumber and lettuce can also be protoplast-transformed and regenerated [58].
- **Ribonucleoprotein (RNP) delivery:** Purified Cas9 protein complexed with synthetic gRNAs can be delivered by PEG transfection into protoplasts or by particle bombardment into tissue. RNP delivery avoids DNA insertion, often yielding non-transgenic mutants. Pepper *CaMLO2* editing was demonstrated using RNPs in protoplasts, and cucumber *eIF4E* mutants were recovered from DNA-free systems [33].
- **Biolistic (gene gun):** Particle bombardment of embryos or callus is used for monocots and recalcitrant dicots. Onion, which is hard to transform by Agrobacterium, has been edited by biolistics (via embryogenic callus) targeting the PDS gene. Biolistic delivery is also used in garlic and some lettuce transformations [17].
- **Virus-mediated delivery:** Though less common for vegetables, some CRISPR systems use modified plant viruses (e.g. geminivirus replicons) to deliver Cas9 and gRNA to speed editing. Cucumber mosaic virus has been engineered to carry gRNAs in some proof-of-concept studies [80].
- **Agrobacterium rhizogenes (hairy root):** Rarely used for whole-plant editing, but can produce transgenic roots expressing CRISPR for study of root pathogens or gene function.
- **In planta infiltration:** Direct infiltration of Cas9-gRNA complexes into developing buds or pollen (as in Arabidopsis floral dip) is not established in major vegetables [81].
- **Gene editing in elite/variety backgrounds:** For commercial breeding, transformation of elite lines is important. Some protocols combine Agrobacterium transformation with transient geminivirus replicons to increase editing rates (demonstrated in tomato) [59].

Each method has trade-offs in efficiency, chimerism, and regeneration requirement. Agrobacterium transformation remains the most common route for stable editing in dicots (as it allows selection and regeneration), while RNP methods are promising for avoiding transgene integration [60]. Ongoing improvements (e.g. biolistic Cas9 RNPs in lettuce cotyledons, pollen magnetofection) continue to expand delivery options [5, 16, 126].

Table 4: CRISPR delivery methods and transformation systems used in vegetable crops

Delivery/Transformation System	Crops Used	Details / Notes
Agrobacterium-mediated stable transformation	Tomato, Potato, Pepper, Eggplant, Cabbage, Lettuce, Spinach, Chinese Cabbage, Radish, (most dicots)	Binary vectors encoding Cas9/gRNA introduced into leaf/hypocotyl explants; T-DNA integrates or transiently expresses CRISPR; subsequent tissue culture to regenerate edited plants. Highly efficient in tomato, tobacco family, brassicas, lettuce.
Protoplast transfection (DNA)	Tomato, Potato, Lettuce, Eggplant	Isolated mesophyll or callus protoplasts treated with PEG and plasmid DNA (Cas9 + gRNA constructs); regenerable in some species (tomato, lettuce). Can yield transgene-free mutations if no T-DNA integration.
Protoplast transfection (RNP)	Tomato, Cucumber, Lettuce, Spinach	Purified Cas9 protein and gRNA RNP complexes delivered by PEG into protoplasts. Mutations induced without DNA; protoplasts regenerated (e.g. tobacco mosaic technique, though regeneration can be genotype-dependent).
Particle bombardment (biolistics)	Onion, Garlic, Lettuce, Carrot, Cabbage	Gold/tungsten particles coated with CRISPR plasmid DNA or RNP shot into embryogenic callus or meristems. Used where Agrobacterium is inefficient (onion) or for transient editing.
Agrobacterium RNP/RNA delivery	Tomato, Rice (for reference), Cabbage	In some protocols, Agrobacterium is engineered to deliver Cas9 protein or RNA directly, but this is still experimental.
Virus vector delivery	Tobacco (model), limited vegetables	Viral replicons (e.g. TRV, geminivirus) carrying gRNA can infect plants; Cas9 provided by transgene. Example: TRV-CRISPR used in tomato for photobleaching gene. Not widely used commercially in vegetables yet.
Rhizobium rhizogenes (hairy root)	Tomato, Tobacco, Bean	Transforms roots to create hairy roots expressing Cas9/gRNA. Useful for rapid gene function analysis (e.g. nematode resistance genes) but does not produce whole-plant edits.
Direct plant tissue infiltration	Limited (N. benthamiana model)	Vacuum or syringe infiltration with CRISPR plasmid or RNP; mostly used in plant models for quick testing of gRNA activity; rarely used for stable editing in vegetables due to regeneration challenges.
Protoplast-derived RNP (transgene-free)	Lettuce, Petunia (proxy), Cucumber	Lettuce cotyledon protoplasts transfected with RNP yield transgene-free edited plants after regeneration. Demonstrated in Nicotiana and also lettuce (e.g. LsNCED1 edited by RNPs).
Gemini-vector-mediated delivery	Tomato, Grapevine	Use of geminivirus replicons to carry donor templates or gRNAs to boost HDR. Recently applied in tomato to edit fruit genes with donor DNA. Offers high copy number for editing.
Double-stranded RNA and	Spinach (hypothetical),	Emerging methods include magnetic nanoparticle or carbon nanotube delivery of

protein bombardment	lettuce	RNP into seedlings; not yet standard in vegetables.
Combination methods	Various (e.g. Agro + protoplast)	Some pipelines use Agrobacterium to introduce CRISPR cassette, then segregate out transgenes in T1 to obtain edited events, thus achieving transgene-free status.

Conclusion

CRISPR/Cas genome editing has rapidly become a cornerstone of vegetable crop improvement for disease and stress resistance. In the past few years, researchers worldwide have applied CRISPR to virtually every major vegetable species of economic importance. By targeting susceptibility genes (e.g. MLO, DMR6, eIF4E) and stress-regulatory genes, CRISPR edits have conferred resistance to fungal and oomycete pathogens (powdery mildew, late blight, downy mildew), bacterial infections, and multiple plant viruses. Advanced editing approachesincluding base editing, prime editing, and multiplex CRISPR constructsare now enabling even finer control, such as single-nucleotide changes, allele stacking, and gene expression modulation.We have seen that in Solanaceous vegetables (tomato, potato, pepper, eggplant) many S-genes are conserved, and CRISPR knockouts yield broad-spectrum resistance with often minimal fitness costs. Cucurbits (cucumber) respond well to knockouts of MLO and eIF4E homologs. Brassicas and leafy crops are next in line to benefit as transformation methods improve. Even root and bulb crops (carrot, onion, garlic) now have the first CRISPR protocols established, opening the door to targeting their unique diseases. Although some areas such as insect resistance or flooding tolerance are still emerging, the rapid evolution of CRISPR tools (gene editors, delivery methods, transgene-free systems) promises solutions. Notably, many CRISPR-induced alleles recapitulate natural resistance variations (e.g. recessive virus resistance alleles) or reveal entirely new possibilities (e.g. domain deletions in stress proteins). With careful design, undesirable pleiotropy can be minimized by precise edits, allele dosage control (e.g. editing one allele in polyploids), or promoter tweaking. The availability of high-quality vegetable genomes, combined with CRISPR's accuracy, means breeding programs can now pyramid multiple resistances at the molecular level.In conclusion, CRISPR/Cas technology has proven its versatility in vegetable crops for enhancing resistance to both biotic and abiotic stresses. Future work will likely focus on expanding editing in under-represented species (onion, spinach), combining multiple traits in single cultivars through multiplex editing, and utilizing new base/prime editors for precision breeding. As regulatory landscapes evolve and public acceptance grows, CRISPR-edited vegetable varieties are poised to play a key role in sustainable agriculture by reducing pesticide use and increasing resilience to climate challenges, ultimately improving food security and quality.

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