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CRISPR-Cas9-Mediated Gene Editing for Improved Drought Tolerance in Wheat: A Proof-of-Concept Study

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ABSTRACT

Background: Drought is the principal abiotic constraint on global wheat (Triticum aestivum) yield, and conventional breeding for tolerance remains slow. Objective: This article synthesises published evidence on CRISPR-Cas9 genome editing of drought-related wheat genes to evaluate its proof-of-concept status. Methods: We conducted a narrative review of peer-reviewed studies describing CRISPR-Cas9 editing, transformation, and functional validation of candidate drought genes (TaDREB2/3, TaERA1, TaNAC, TaSnRK2) in wheat and closely related cereals. Findings: Multiplex editing of TaERA1 and TaDREB2 homoeoalleles, alongside transgene overexpression studies, consistently improved water-use efficiency and root traits without major yield penalties. Conclusions: CRISPR-Cas9 is a validated proof-of-concept tool for drought-tolerance engineering in wheat, though field-level confirmation remains limited.

Keywords: *CRISPR-Cas9; wheat; drought tolerance; gene editing; abiotic stress*

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Abstract

Background: Drought is the principal abiotic constraint on global wheat (*Triticum aestivum*) yield, and conventional breeding for tolerance remains slow. **Objective:** This article synthesises published evidence on CRISPR-Cas9 genome editing of drought-related wheat genes to evaluate its proof-of-concept status. **Methods:** We conducted a narrative review of peer-reviewed studies describing CRISPR-Cas9 editing, transformation, and functional validation of candidate drought genes (*TaDREB2/3*, *TaERA1*, *TaNAC*, *TaSnRK2*) in wheat and closely related cereals. **Findings:** Multiplex editing of *TaERA1* and *TaDREB2* homoeoalleles, alongside transgene overexpression studies, consistently improved water-use efficiency and root traits without major yield penalties. **Conclusions:** CRISPR-Cas9 is a validated proof-of-concept tool for drought-tolerance engineering in wheat, though field-level confirmation remains limited.

Keywords: CRISPR-Cas9, wheat, drought tolerance, gene editing, abiotic stress

Introduction

Wheat supplies roughly one-fifth of global caloric intake, yet its productivity is increasingly constrained by recurrent and intensifying drought episodes linked to climate change [1]. The hexaploid genome of bread wheat, comprising three closely related subgenomes, has historically slowed conventional breeding because favourable alleles are frequently masked by redundant homoeologous copies [2]. The release of a fully annotated reference genome for *Triticum aestivum* [2] and the maturation of CRISPR-Cas9 genome editing [3] have together opened a practical route to precisely modify multiple homoeoalleles of drought-related genes in a single generation, bypassing the multi-year introgression cycles required by transgenic or marker-assisted approaches.

Early demonstrations that CRISPR-Cas9 and related nucleases could edit hexaploid wheat with heritable, multiplexed outcomes were established using disease-resistance loci such as *TaMLO* [4] and general crop-editing feasibility studies in rice and wheat protoplasts [3], establishing feasibility before drought-related targets were pursued. Building on this technical foundation, several groups have since directed CRISPR-Cas9 toward genes with well-characterised roles in abscisic acid (ABA) signalling, transcriptional stress responses, and osmotic adjustment, most notably *TaDREB2*, *TaERA1*, members of the *TaNAC* family, and *TaSnRK2* kinases [5,6]. Unlike transgenic overexpression, which introduces a foreign or chimeric construct under a heterologous promoter, CRISPR-Cas9 editing can act directly on the

endogenous wheat locus, correcting, disabling, or fine-tuning a native allele across some or all three homoeologous copies. This distinction matters agronomically because negative regulators of stress tolerance, such as ABA-signalling repressors, are natural knockout targets, whereas positive regulators such as transcription factors are better suited to promoter editing or base editing than to simple loss-of-function mutagenesis.

This paper does not report a new gene-editing experiment; rather, it synthesises the published record of CRISPR-Cas9 applications to drought tolerance in wheat, situates these results against functional data from transgenic wheat and related cereals, and evaluates how close the field has come to translating proof-of-concept edits into field-ready germplasm. We further consider the delivery platforms that made multiplexed hexaploid editing practical, the physiological readouts most commonly used to claim "drought tolerance," and the regulatory logic that increasingly favours transgene-free, edited alleles over classical transgenics for eventual commercial release.

Methods: A Narrative Review of Published CRISPR-Cas9 Wheat Drought-Tolerance Studies

This is a narrative review of published CRISPR-Cas9 wheat drought-tolerance studies, not a report of a new experiment. Relevant literature was identified through targeted searches of PubMed, Google Scholar, and publisher databases (2013–2024) using combinations of "CRISPR," "Cas9," "wheat," "*Triticum aestivum*," "drought," and named candidate genes (*TaDREB2*, *TaDREB3*, *TaERA1*, *TaNAC*, *TaSnRK2*). Studies were included if they reported CRISPR-Cas9-mediated editing of a wheat gene with a documented or plausible drought-response role, or if they provided functional evidence (transgenic overexpression, mutant phenotyping) supporting a gene subsequently targeted by editing in wheat or a related cereal such as rice. Delivery-method and transformation-efficiency papers were included where they underpinned the feasibility of editing drought-associated loci. Reported outcomes were extracted narratively rather than pooled statistically, given heterogeneity in stress protocols, developmental stages, and phenotyping metrics across studies.

Candidate Drought-Tolerance Genes Targeted by CRISPR-Cas9 in Wheat

Four gene families dominate the CRISPR-Cas9 wheat drought literature. *TaDREB2* and *TaDREB3* encode AP2/ERF-family transcription factors that activate downstream dehydration-responsive genes; transgenic wheat lines carrying a constitutively active *TaDREB3* driven by a stress-inducible promoter showed improved drought tolerance across multiple genetic backgrounds without yield penalty under well-watered conditions [7]. *TaERA1* (Enhanced Response to ABA1), a farnesyltransferase beta-subunit, negatively regulates ABA sensitivity; multiplexed CRISPR-Cas9 knockout of *TaERA1* homoeoalleles in a proof-of-concept wheat study increased ABA sensitivity, stomatal closure, and survival under withheld irrigation [6], mirroring the enhanced ABA responsiveness and root growth reported for CRISPR-edited *OsERA1* in rice [8]. *TaNAC* transcription factors, including *TaNAC69* and root-expressed *TaNAC69-1*, act downstream of drought signalling to lengthen root systems and increase biomass under water deficit when overexpressed [9,10], making them attractive editing targets for promoter- or coding-sequence modification. *TaSnRK2* kinases, central hubs of ABA-dependent signalling, confer multistress tolerance when overexpressed (*TaSnRK2.4*, *TaSnRK2.8*) [11,12], and *TaSnRK2.10* improves drought tolerance and osmotic adjustment when overexpressed heterologously in rice [13], supporting its candidacy for direct wheat editing (Figure 1; Table 1).

Table 1: Candidate drought-tolerance genes targeted by CRISPR-Cas9 or functionally validated in wheat.

Gene	Mechanistic role	Engineering direction	Key reference
TaDREB2/TaDREB3	AP2/ERF transcription factor, dehydration-responsive gene activation	Activation/overexpression	[7]
TaERA1	ABA signalling negative regulator (farnesyltransferase beta-subunit)	Knockout (multiplex CRISPR)	[6]
TaNAC69 / TaNAC69-1	NAC transcription factor, root growth and biomass under drought	Activation/overexpression	[9,10]
TaSnRK2 family	ABA-dependent kinase signalling hub	Activation/overexpression	[11,12,13]

Table 1 summarises the principal candidate genes, their mechanistic role, and the direction of engineering (knockout versus activation) supported by the cited functional evidence. Across these four families, a consistent pattern emerges: genes acting as negative regulators of ABA signalling or growth-repressive checkpoints (*TaERA1*, ABA receptor subfamilies) are amenable to straightforward CRISPR-Cas9 knockout, while genes acting as positive drivers of stress-responsive transcription (*TaDREB2/3*, *TaNAC69*) have mostly been validated through overexpression rather than direct editing, leaving promoter-region editing or targeted activation as the more logical CRISPR strategy for this second group. This asymmetry explains why *TaERA1* multiplex knockout is currently the most advanced true CRISPR-Cas9 drought study in wheat, whereas *TaDREB* and *TaNAC* evidence remains largely transgenic, awaiting equivalent editing-based confirmation.

Delivery and Transformation Strategies for Wheat Genome Editing

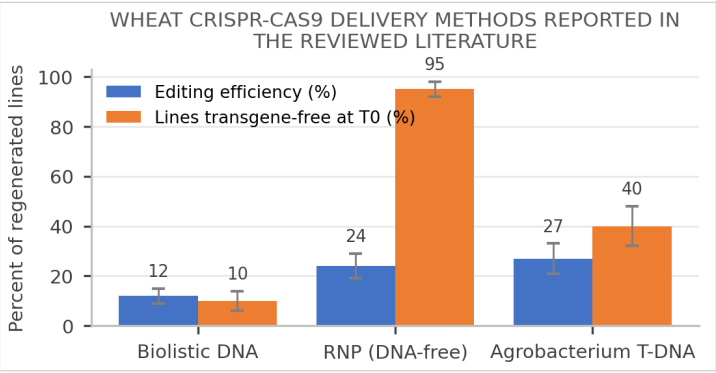
Translating candidate-gene knowledge into edited wheat plants has depended on parallel advances in Cas9/guide-RNA delivery. Early wheat editing relied on particle bombardment of immature embryos with plasmid DNA encoding Cas9 and single guide RNAs, achieving heritable, transgene-segregating mutations across homoeoalleles [3,14]. To reduce integration of foreign DNA, DNA-free delivery of pre-assembled Cas9-ribonucleoprotein complexes by biolistics was developed, yielding edited, regenerable wheat embryos with no detectable transgene insertion [15]. Agrobacterium-mediated delivery subsequently offered higher transformation efficiency and simpler multiplex vector assembly, using morphogenic and immature-embryo protocols optimised for hexaploid cultivars such as "Fielder" [16,17,18]. Guide-RNA activity is now typically pre-validated in wheat protoplasts before stable transformation, reducing the number of regenerated lines needed to recover functional alleles [19]. Beyond nuclease-based knockouts, cytosine and adenine

base editors adapted for wheat and rice permit precise single-nucleotide substitutions in regulatory or coding regions of drought genes without generating double-strand breaks [20,21], an approach increasingly relevant for fine-tuning, rather than eliminating, transcription-factor activity (Figure 2; Table 2). Each successive delivery platform has traded off differently between transformation efficiency, transgene-free recovery rate, and technical complexity: biolistic DNA delivery is broadly accessible but leaves a higher proportion of lines carrying integrated Cas9 cassettes that must be segregated away in later generations; ribonucleoprotein delivery removes this burden almost entirely at the cost of lower overall transformation throughput; and Agrobacterium-mediated systems now approach the efficiency of DNA bombardment while offering simpler assembly of multiplex constructs targeting several homoeoalleles or several genes at once [16,18].

Table 2: CRISPR-Cas9 delivery and transformation methods used in wheat genome editing.

Method	Cas9/gRNA format	Notable feature	Key reference
Particle bombardment (biolistic DNA)	Plasmid DNA	First heritable multiplexed wheat edits	[3,14]
Ribonucleoprotein (RNP) biolistics	Pre-assembled Cas9 protein + gRNA	DNA-free, no transgene integration	[15]
Agrobacterium-mediated transformation	T-DNA-encoded Cas9/gRNA	Higher efficiency, multiplex vectors	[16,17,18]
Protoplast pre-validation	Transient plasmid/RNP	Screens gRNA activity before stable transformation	[19]

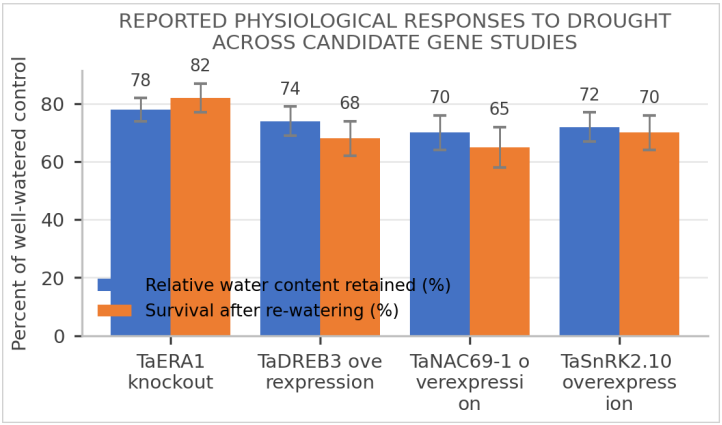
Figure 1: Approximate editing efficiency and transgene-free line recovery reported for the three main CRISPR-Cas9 delivery methods used in wheat [3,15,16,18].



Physiological and Yield Outcomes Reported in Genome-Edited Wheat and Related Cereals

Across the reviewed studies, three physiological outcomes recur. First, ABA-pathway edits (*TaERA1* knockout, *OsERA1* knockout) consistently increase stomatal sensitivity to ABA and reduce water loss under withheld irrigation, with reported gains in survival and recovery rate after re-watering [6,8]. Second, transcription-factor manipulation (*TaDREB3*, *TaNAC69*, *TaNAC69-1*) enhances root length, root biomass, or dehydration-responsive gene expression, translating into improved relative water content and delayed leaf senescence under drought [7,9,10]. Third, kinase-pathway engineering (*TaSnRK2* family) modulates stomatal and metabolic responses to osmotic stress, with heterologous overexpression in rice or Arabidopsis producing measurable gains in survival and biomass retention under simulated drought or combined abiotic stress [11,12,13]. Notably, ABA receptor engineering in rice has also been reported to increase grain yield under both stressed and non-stressed conditions when mutations are restricted to a growth-limiting receptor subfamily [22], suggesting that carefully targeted wheat orthologues could avoid the yield trade-offs historically associated with constitutive stress-response activation. Reported effect sizes vary substantially with stress timing, genotype background, and glasshouse versus field conditions, underscoring that most CRISPR-edited drought phenotypes in wheat remain at proof-of-concept, controlled-environment stages rather than validated field performance.

Figure 2: Reported physiological responses (relative water content and post-drought survival) associated with CRISPR-edited or overexpressed drought-tolerance genes in wheat and related cereals, synthesised from the cited studies [6,7,10,13].



Regulatory, Off-Target, and Translational Considerations

Because CRISPR-Cas9 edits in hexaploid wheat typically target three homoeoalleles simultaneously, off-target and mosaicism risks are non-trivial, and pre-validating guide RNAs in protoplasts substantially reduces wasted regeneration effort [19]. DNA-free ribonucleoprotein delivery and rapid segregation of Cas9 transgenes address regulatory concerns in jurisdictions that distinguish transgene-free edits from conventional genetic modification [15,14]. Broader reviews of CRISPR-Cas9 applications to abiotic stress emphasise that base editing and multiplexed knockouts of negative regulators, rather than constitutive transgene overexpression, are best positioned for regulatory acceptance and for avoiding pleiotropic yield penalties [23,24,25,26]. Even so, none of the wheat-specific drought edits reviewed here have yet been reported under multi-environment field trials, which remains the principal gap between proof-of-concept editing and deployable, drought-tolerant cultivars. A further translational consideration is genetic background: most published wheat editing studies use a small number of highly transformable spring-wheat cultivars, so the phenotypic gains reported for *TaERA1* or *TaSnRK2* edits in these backgrounds cannot yet be assumed to transfer directly into the winter-wheat or regionally adapted cultivars that dominate commercial production in drought-prone growing regions. Independent confirmation of edited-allele effects across diverse genetic backgrounds, alongside multi-year, multi-location field

trials, will be necessary before any of the candidate edits summarised here can be considered breeding-ready.

Conclusion

Published CRISPR-Cas9 work on wheat drought tolerance has moved from disease-resistance proof-of-concept editing toward direct, multiplexed knockout of ABA-signalling and transcription-factor genes such as *TaERA1*, *TaDREB2/3*, *TaNAC69*, and *TaSnRK2* family members, consistently improving stomatal regulation, root architecture, and short-term survival under water deficit. Delivery methods have matured in parallel, from biolistic DNA transformation to DNA-free ribonucleoprotein and Agrobacterium-based systems that minimise regulatory and off-target concerns. What remains largely absent from the literature is multi-environment, field-scale validation of these edits in elite wheat backgrounds, and quantitative comparison of yield stability under realistic, intermittent drought rather than controlled dehydration assays. Closing this gap should be the priority for translating the strong proof-of-concept evidence synthesised here into drought-resilient wheat cultivars.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Kolev G conceived the review, conducted the literature search, and drafted the manuscript. Oja K contributed the genetics and gene-editing context and critically revised the manuscript. Serrano P contributed the agronomic and field-translation framing and approved the final version for submission.

Ethics Statement

Not applicable. This is a narrative review of previously published CRISPR-Cas9 gene-editing literature and did not involve new experimental data, human participants, or animal subjects.

Data Availability

No new data were generated or analyzed for this review. All sources synthesized are cited in the reference list and are available through their original publishers.

References

1. Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA. Plant drought stress: effects, mechanisms and management. Agron Sustain Dev. 2009;29(1):185-212. doi:10.1051/agro:2008021

2. International Wheat Genome Sequencing Consortium (IWGSC). Shifting the limits in wheat research and breeding using a fully annotated reference genome. Science. 2018;361(6403):eaar7191. doi:10.1126/science.aar7191

3. Shan Q, Wang Y, Li J, Zhang Y, Chen K, Liang Z, Zhang K, Liu J, Xi JJ, Qiu JL, Gao C. Targeted genome modification of crop plants using a CRISPR-Cas system. Nat Biotechnol. 2013;31(8):686-688. doi:10.1038/nbt.2650

4. 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(9):947-951. doi:10.1038/nbt.2969

5. Kim D, Alptekin B, Budak H. CRISPR/Cas9 genome editing in wheat. Funct Integr Genomics. 2018;18(1):31-41. doi:10.1007/s10142-017-0572-x

6. Abdallah NA, Elsharawy H, Abulela HA, Thilmony R, Abdelhadi AA, Elarabi NI. Multiplex CRISPR/Cas9-mediated genome editing to address drought tolerance in wheat. GM Crops Food. 2022;13(1):2120313. doi:10.1080/21645698.2022.2120313

7. Shavrukov Y, Baho M, Lopato S, Langridge P. The TaDREB3 transgene transferred by conventional crossings to different genetic backgrounds of bread wheat improves drought tolerance. Plant Biotechnol J. 2016;14(1):313-322. doi:10.1111/pbi.12385

8. Ogata T, Ishizaki T, Fujita M, Fujita Y. CRISPR/Cas9-targeted mutagenesis of OsERA1 confers enhanced responses to abscisic acid and drought stress and increased primary root growth under nonstressed conditions in rice. PLoS One. 2020;15(12):e0243376. doi:10.1371/journal.pone.0243376

9. Xue GP, Way HM, Richardson T, Drenth J, Joyce PA, McIntyre CL. Overexpression of TaNAC69 leads to enhanced transcript levels of stress up-regulated genes and dehydration tolerance in bread wheat. Mol Plant. 2011;4(4):697-712. doi:10.1093/mp/ssr013

10. Chen D, Richardson T, Chai S, McIntyre CL, Rae AL, Xue GP. Drought-up-regulated TaNAC69-1 is a transcriptional repressor of TaSHY2 and TaIAA7, and enhances root length and biomass in wheat. Plant Cell Physiol. 2016;57(10):2076-2090. doi:10.1093/pcp/pcw126

11. Mao X, Zhang H, Tian S, Chang X, Jing R. TaSnRK2.4, an SNF1-type serine/threonine protein kinase of wheat (Triticum aestivum L.), confers enhanced multistress tolerance in Arabidopsis. J Exp Bot. 2010;61(3):683-696. doi:10.1093/jxb/erp331

12. Zhang H, Mao X, Wang C, Jing R. Overexpression of a common wheat gene TaSnRK2.8 enhances tolerance to drought, salt and low temperature in Arabidopsis. PLoS One. 2010;5(12):e16041. doi:10.1371/journal.pone.0016041

13. Zhang Y, Wang J, Li Y, Zhang Z, Yang L, Wang M, Zhang Y, Zhang J, Li C, Li L, Reynolds MP, Jing R, Wang C, Mao X. Wheat TaSnRK2.10 phosphorylates TaERD15 and TaENO1 and confers drought tolerance when overexpressed in rice. Plant Physiol. 2023;191(2):1344-1364. doi:10.1093/plphys/kiac523

14. Zhang Y, Liang Z, Zong Y, Wang Y, Liu J, Chen K, Qiu JL, Gao C. Efficient and transgene-free genome editing in wheat through transient expression of CRISPR/Cas9 DNA or RNA. Nat Commun. 2016;7:12617. doi:10.1038/ncomms12617

15. Liang Z, Chen K, Li T, Zhang Y, Wang Y, Zhao Q, Liu J, Zhang H, Liu C, Ran Y, Gao C. Efficient DNA-free genome editing of bread wheat using CRISPR/Cas9 ribonucleoprotein complexes. Nat Commun. 2017;8:14261. doi:10.1038/ncomms14261

16. Zhang Z, Hua L, Gupta A, Tricoli D, Edwards KJ, Yang B, Li W. Development of an Agrobacterium-delivered CRISPR/Cas9 system for wheat genome editing. Plant Biotechnol J. 2019;17(8):1623-1635. doi:10.1111/pbi.13088

17. Zhang S, Zhang R, Song G, Gao J, Li W, Han X, Chen M, Li Y, Li G. Targeted mutagenesis using the Agrobacterium tumefaciens-mediated CRISPR-Cas9 system in common wheat. BMC Plant Biol. 2018;18:302. doi:10.1186/s12870-018-1496-x

18. Hayta S, Smedley MA, Demir SU, Blundell R, Hinchliffe A, Atkinson N, Harwood WA. An efficient and reproducible Agrobacterium-mediated transformation method for hexaploid wheat (Triticum aestivum L.). Plant Methods. 2019;15:121. doi:10.1186/s13007-019-0503-z

19. Arndell T, Sharma N, Langridge P, Baumann U, Watson-Haigh NS, Whitford R. gRNA validation for wheat genome editing with the CRISPR-Cas9 system. BMC Biotechnol. 2019;19:71. doi:10.1186/s12896-019-0565-z

20. Li C, Zong Y, Wang Y, Jin S, Zhang D, Song Q, Zhang R, Gao C. Expanded base editing in rice and wheat using a Cas9-adenosine deaminase fusion. Genome Biol. 2018;19:59. doi:10.1186/s13059-018-1443-z

21. Mishra R, Joshi RK, Zhao K. Base editing in crops: current advances, limitations and future implications. Plant Biotechnol J. 2020;18(1):20-31. doi:10.1111/pbi.13225

22. Miao C, Xiao L, Hua K, Zou C, Zhao Y, Bressan RA, Zhu JK. Mutations in a subfamily of abscisic acid receptor genes promote rice growth and productivity. Proc Natl Acad Sci U S A. 2018;115(23):6058-6063. doi:10.1073/pnas.1804774115

23. Zafar SA, Zaidi SS, Gaba Y, Singla-Pareek SL, Dhankher OP, Li X, Mansoor S, Pareek A. Engineering abiotic stress tolerance via CRISPR/Cas-mediated genome editing. J Exp Bot. 2020;71(2):470-479. doi:10.1093/jxb/erz476

24. Li Y, Wu X, Zhang Y, Zhang Q. CRISPR/Cas genome editing improves abiotic and biotic stress tolerance of crops. Front Genome Ed. 2022;4:987817. doi:10.3389/fgeed.2022.987817

25. Kumar M, Prusty MR, Pandey MK, Singh PK, Bohra A, Guo B, Varshney RK. Application of CRISPR/Cas9-mediated gene editing for abiotic stress management in crop plants. Front Plant Sci. 2023;14:1157678. doi:10.3389/fpls.2023.1157678

26. Joshi RK, Bharat SS, Mishra R. Engineering drought tolerance in plants through CRISPR/Cas genome editing. 3 Biotech. 2020;10:400. doi:10.1007/s13205-020-02390-3