© Volume 2, Issue 8, ***August*** 2026 | JATIR
ISSN: 3139-8464 (p)

Harnessing Epigenetic Stress Memory via CRISPR-Nanoparticle Integration for Climate-Resilient Agriculture

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***Abstract*—Climate change is pushing crop plants into stress conditions that outpace what conventional breeding can keep up with, and this has pushed researchers to look past the DNA sequence itself for tools that work faster. One of the more interesting angles is epigenetic stress memory, the ability of a plant to hold onto a molecular record of a past stressful event, encoded in DNA methylation, histone marks, and small RNAs, so that it responds faster or stronger the next time. Separately, CRISPR dCas9 based epigenome editing tools now let researchers write or erase these marks at a chosen locus without cutting the DNA, and nanoparticle delivery systems, especially carbon nanotubes and lipid or polymeric carriers, are solving the long-standing problem of getting these tools into plant cells without tissue culture, without a transgene footprint, and across a much wider range of crop species than Agrobacterium ever could. This review pulls these three threads together. It walks through the mechanisms of stress memory, the current state of CRISPR epigenome editing in plants, and the nanoparticle platforms now used for plant genetic engineering, and then lays out a conceptual pipeline for how nanoparticle delivered epigenome editors could be used to deliberately install useful stress memory in crops such as wheat, rice, and maize. It also takes an honest look at where this convergence is still mostly conceptual rather than demonstrated, and points to the delivery, stability, and regulatory gaps that need to close before this becomes a field tool rather than a lab concept.**

***Index Terms*—epigenetic stress memory, DNA methylation, CRISPR dCas9, epigenome editing, nanoparticle delivery, carbon nanotubes, climate resilient agriculture, epibreeding, transgenerational inheritance**

I. INTRODUCTION

Agriculture is being asked to do something it has never had to do at this pace before: keep yields stable while the climate underneath it keeps shifting. Drought spells are getting longer, heat waves

are arriving earlier in the growing season, and soil salinity keeps creeping upward in irrigated land. Conventional breeding, which relies on recombining existing genetic variation over several generations, simply cannot move fast enough to track this kind of change, and even modern genome editing, which is precise but still limited to changing the DNA sequence itself, does not capture a layer of plant biology that turns out to matter a great deal: memory.

Plants cannot walk away from a stressful environment, so over evolutionary time they have built a way to remember stress instead. A plant that has already lived through one drought often responds faster and more efficiently to the next one, even without any change to its DNA sequence. This is called stress priming or stress memory, and it is carried by epigenetic marks, chemical tags on DNA and the proteins that package it, that change how genes are switched on or off (Avramova, 2015; Kim, 2021). In some cases this memory does not even stay with a single plant. It gets passed down to offspring, a phenomenon called transgenerational epigenetic inheritance (Mladenov et al., 2021).

At the same time, two separate technical revolutions have been unfolding. The first is CRISPR based epigenome editing, where a catalytically dead version of Cas9, called dCas9, is fused to an enzyme that adds or removes epigenetic marks, letting researchers rewrite a plant's memory at a single chosen gene rather than waiting for it to happen naturally (Papikian et al., 2019). The second is nanoparticle mediated delivery, where materials such as carbon nanotubes or lipid nanoparticles carry genetic cargo straight through the plant cell wall, bypassing the tissue culture and species restrictions that have limited plant genetic engineering for decades (Demirer et al., 2019; Cunningham et al., 2018).

Each of these three areas, stress memory biology, epigenome editing, and nanoparticle delivery, has its own substantial and growing literature. What is much rarer, at least as of this writing, is work that explicitly combines all three into one integrated pipeline for engineering climate resilience. This review takes stock of what is solid in each area, then reasons through what a combined system would need to look like, what evidence already points in that direction, and what is still missing. The goal is not to overstate how far this convergence has come, but to give a scholar working in this space a grounded, properly cited starting point and a clear view of where the real research gaps sit.

II. EPIGENETIC STRESS MEMORY IN PLANTS

2.1 Somatic priming versus transgenerational memory

Stress memory in plants comes in a few different flavours, and it helps to keep them separate. Somatic or within generation memory is short term. A plant exposed to drought early in its life can respond faster to a second drought later in the same growing season, largely because chromatin at stress responsive genes stays in a poised, more accessible state after the first exposure (Kim, 2021). Transgenerational memory is a different and more striking phenomenon, where epigenetic marks such as DNA methylation are stable enough to be inherited by offspring, so that the next generation shows altered stress responses even without ever encountering the stress itself (Mladenov et al.,

ISSN: 3139-8464 (p)

2021). There is also cross stress memory, where priming against one stress, say UV exposure, ends up improving tolerance to an unrelated stress such as osmotic stress, because the two pathways share signalling components.

2.2 Molecular carriers of memory

The best studied carrier is DNA methylation, the addition of a methyl group to cytosine bases, which in plants happens in three different sequence contexts and is maintained by a small set of dedicated enzymes. Histone modifications are the second major carrier. The heat shock transcription factor HSFA2 is a well-documented example: its direct binding to DNA is short lived, but it leaves behind lasting histone marks at heat responsive genes that make the plant respond faster to the next heat event (Ramakrishnan et al., 2022). Small RNAs form a third layer, guiding methylation to specific loci through the RNA directed DNA methylation pathway.

2.3 Evidence from major stress types

Drought memory is probably the most thoroughly documented case. In Arabidopsis and in wheat, plants that have already experienced one drought episode show altered abscisic acid signalling and faster activation of protective genes during a repeated drought, changes tied to demethylation at ABA responsive promoters and to added histone acetylation at genes such as ABF2 (Kaya et al., 2024). Cold and heat memory follow broadly similar logic, with chromatin states at key regulatory genes staying primed for a period after the first exposure (Ramakrishnan et al., 2022). What all of these example's share is the same underlying idea: the plant is not just reacting to its environment moment to moment, it is keeping a molecular record of what it has already been through, and that record shapes how it reacts the next time.

III. CRISPR BASED EPIGENOME EDITING

3.1 The dCas9 fusion platform

CRISPR epigenome editing borrows the targeting half of the CRISPR system, a guide RNA paired with Cas9, but strips out the cutting function. The resulting dCas9 protein still binds wherever its guide RNA tells it to, but instead of cutting DNA it is fused to an enzyme that changes the local chromatin. Fusing dCas9 to DNMT3A adds methylation and tends to silence a gene, while fusing it to the catalytic domain of TET1 removes methylation and tends to switch a gene back on. This gives researchers a way to flip a single gene's expression state without touching the underlying DNA sequence at all, which matters a great deal for regulatory purposes since no new genetic sequence is introduced into the genome.

3.2 SunTag and multiplexed effector recruitment

A single dCas9 fusion protein can only carry so much editing enzyme to a target site, which limits how strong or stable the resulting change is. The SunTag system gets around this by fusing dCas9 to a repeating peptide scaffold that recruits many copies of an antibody linked effector domain to

one site at once. Papikian, Liu, Gallego-Bartolomé, and Jacobsen (2019) used this system in Arabidopsis to both switch on silenced genes with a VP64 activator and to lay down new, heritable DNA methylation at the FWA promoter using a plant DRM methyltransferase domain, triggering a stable developmental change. The same group and others have also used TET1 catalytic domain fusions to strip away methylation from naturally hypermethylated regions and reawaken the associated gene, as shown for the PPH gene involved in leaf senescence.

3.3 Precision, stability, and off-target considerations

Two practical issues keep coming up in this literature. The first is specificity: a recent large-scale comparison of dCas9 based methylation editors found that multimerized DNMT3A domains, while more potent, also caused widespread unintended methylation at loci the guide RNA was never meant to touch, and even guide RNAs with no real target caused some off target transcriptional changes. Tools such as CRISPRoff came out ahead on the trade-off between strength and specificity in that comparison, but the broader lesson holds, more effector copies at a site generally buys potency at some cost to precision. The second issue is stability. An epigenetic edit is only useful for breeding purposes if it survives cell division and, ideally, passes to the next generation, and this is exactly where the heritability seen in natural transgenerational memory becomes a design target rather than just a curiosity.

IV. NANOPARTICLE MEDIATED DELIVERY FOR PLANT GENETIC ENGINEERING

4.1 Why plants are a hard delivery problem

Getting any genetic cargo, whether it is a plasmid, an RNA, or an assembled protein complex, into a plant cell is harder than doing the same thing in an animal cell, mainly because of the rigid cellulose cell wall that plant cells have and animal cells do not. Standard methods such as Agrobacterium mediated transformation and particle bombardment work, but both are slow, need tissue culture and plant regeneration steps that are genotype dependent, and in the case of Agrobacterium leave a transgene footprint that draws regulatory scrutiny in many countries (Ma et al., 2016; Song et al., 2016).

4.2 Carbon nanotubes as a delivery platform

Carbon nanotubes have turned out to be a genuinely useful answer to this problem. Demirer, Zhang, Goh, González-Grandío, and Landry (2019) developed a protocol where positively charged, polymer coated single walled carbon nanotubes are loaded with plasmid DNA and simply infiltrated into intact leaves, where they passively cross the cell wall and reach the nucleus or chloroplast. In a companion study, the same group and collaborators showed this approach could deliver functional genetic material into mature plants of several species, including arugula, wheat, and cotton, achieving expression levels comparable to Agrobacterium but without any DNA integrating into the genome, since the plant's own nucleases degrade the carried DNA within about ten days once its job is done (Demirer, Zhang, Matos, et al., 2019). A related chitosan coated

carbon nanotube system was shown to deliver genetic material selectively into chloroplasts (Kwak et al., 2019). Independent groups have since replicated related carbon nanotube delivery approaches in rice, using it to deliver CRISPR-Cas9 components targeting the phytoene desaturase gene and confirming edits at the expected locus.

4.3 Beyond carbon nanotubes: lipid, polymeric, and other carriers

Carbon nanotubes are not the only option. Lipid nanoparticles, already well proven for mRNA delivery in human medicine, are increasingly being adapted for delivering dCas9 based epigenome editor messenger RNA together with its guide RNA in a single encapsulated package (Woodward et al., 2024). Polymeric nanoparticles built from materials such as polycaprolactone or PLGA, along with magnetic nanoparticles that allow directed movement using an external magnetic field, and mesoporous silica particles, round out a fairly broad toolbox. Cunningham, Goh, Demirer, Matos, and Landry (2018) laid out the general case for why this class of tools matters for plant genetic engineering: nanoparticles can protect fragile cargo from degradation, cross biological barriers passively, and in many formulations avoid leaving any transgene behind, which sidesteps a great deal of the regulatory debate that has slowed conventional genetically modified crops.

V. THE CONVERGENCE: NANOPARTICLE-DELIVERED CRISPR EPIGENOME EDITING FOR ENGINEERED STRESS MEMORY

This is the part of the picture that is genuinely still taking shape, and it is worth being direct about that rather than presenting it as a settled technology. As things stand, the epigenetic stress memory literature, the CRISPR epigenome editing literature, and the nanoparticle delivery literature are each mature and well supported on their own, but very little published work has yet closed the loop by using a nanoparticle to deliver a CRISPR epigenome editor specifically in order to install a defined, heritable stress memory trait in a crop plant. What does exist, and what makes the idea credible rather than speculative, is that every individual link in the chain has already been demonstrated somewhere in the literature.

Delivery of dCas9 fusion constructs into plants by nanoparticle assisted methods is already recognised as one of the standard delivery routes alongside Agrobacterium and viral vectors, sitting right next to the more traditional options in recent epibreeding literature. Carbon nanotubes have already shown they can carry plasmid sized cargo, and dCas9 epigenome editors, especially the smaller single effector versions rather than bulky multiplexed constructs, fall within a size range that nanotube and lipid nanoparticle systems already handle for other applications. On the biology side, the target loci are already known. Genes such as ABF2 in the ABA signalling pathway, and promoters like FWA that have already been shown to respond to dCas9 directed methylation or demethylation, sit right at the intersection of what epigenome editors can already touch and what drought memory research says actually matters for stress response.

A conceptual pipeline for this convergence would run roughly as follows. First, candidate loci are chosen from stress memory studies, genes whose natural methylation or histone state is already

known to shift with priming and to correlate with improved tolerance. Second, a dCas9 fusion construct, kept as compact as possible and paired with a single guide RNA against the chosen locus, is packaged onto a nanocarrier suited to the tissue being targeted, carbon nanotubes for leaf infiltration in a transient expression context, or a lipid or polymeric nanoparticle where mRNA delivery and rapid clearance are preferred over any DNA based construct. Third, the construct is delivered without any tissue culture step, directly into intact leaves or, where protocols allow, into pollen or reproductive tissue if transgenerational transmission of the new epigenetic state is the goal. Fourth, the edited locus and the resulting phenotype are validated using bisulfite sequencing or chromatin immunoprecipitation alongside physiological stress assays, and the stability of the mark is tracked across at least two generations before anything is called heritable.

The appeal of this design, if it works as intended, is that it sidesteps two separate long-standing problems at once. It avoids the years long timelines of conventional breeding because the epigenetic mark is written directly rather than selected for, and it avoids the transgene integration and associated regulatory burden of classical genetic modification because the nanocarrier can, in many formulations, deliver its cargo transiently and leave no foreign DNA behind. Whether it can be made reliably heritable, efficient across diverse crop genotypes, and stable under real field stress rather than controlled greenhouse conditions is exactly the open question the rest of this review turns to.

VI. APPLICATIONS FOR CLIMATE-RESILIENT AGRICULTURE

The staple cereals most exposed to climate volatility, wheat, rice, and maize, are also the crops that have received the most attention from both the epigenome editing and the nanoparticle delivery communities, which is a fortunate overlap. Reviews focused specifically on CRISPR-Cas9 for climate resilient staple crops point to drought, heat, salinity, cold, and combined stresses as the priority targets, and highlight that gene editing work in these three crops is already fairly advanced even where the epigenetic layer has not yet been added (see Section 6 sources).

Drought tolerance is the most obvious first application, given how well characterised the ABA signalling and methylation changes already are. A nanoparticle delivered epigenome editor aimed at maintaining ABF2 in a primed, faster responding chromatin state could in principle shorten the time a crop needs to mount a drought response, without changing a single base of its DNA sequence. Heat tolerance is a close second, building on what is already known about HSFA2 mediated histone memory. Cross stress tolerance is arguably the most attractive long term target, since a single well-chosen epigenetic edit at a shared signalling node could, in theory, improve resilience to more than one stress type at once, echoing what has already been observed naturally in cross stress priming studies.

This also connects to the emerging concept of epibreeding, where breeders deliberately work with epigenetic variation, whether natural, chemically induced, or precisely edited, alongside conventional genetic selection, using tools such as epigenome wide association studies and epigenetic quantitative trait locus mapping to identify useful epialleles before deciding whether to

fix them permanently through targeted editing (Chen et al., 2025). Nanoparticle delivered CRISPR epigenome editing fits naturally into this pipeline as the tool that converts a promising natural epiallele, once identified, into something that can be installed intentionally and at scale.

VII. CHALLENGES AND KNOWLEDGE GAPS

A fair review has to be honest about how much is still unresolved. Heritability is the biggest open question. Somatic priming effects can fade once the stress is removed, and even genuinely transgenerational epigenetic marks are not always stable across many generations, since plant cells have active demethylation and chromatin resetting pathways that can erase an edit over time unless it is reinforced. Off target epigenetic drift is a second concern, and the comparative profiling work on dCas9 methylation editors makes clear that more potent multiplexed effectors tend to bring more unintended methylation changes elsewhere in the genome, a trade off that has not been fully solved.

Delivery efficiency is the third major gap, and it is specific to moving from model species to real crops. Most of the strongest carbon nanotube delivery results come from Arabidopsis, tobacco, and a handful of leafy or young tissue systems, and reaching germline or meristem tissue reliably, which matters if the goal is heritable transmission rather than just a transient effect in the treated plant, remains much less well demonstrated. Cargo size is a related practical constraint, since the more powerful multiplexed epigenome editors, CRISPRoff for example, run large enough to exceed the packaging capacity of some delivery vehicles, which pushes designers back toward smaller, single effector constructs that may be less potent.

Finally, there is a genuine evidence gap in the convergence itself. As noted in Section 5, no single published study surveyed for this review has yet demonstrated the complete pipeline, nanoparticle delivery of a CRISPR epigenome editor producing a validated, heritable, field relevant stress tolerance trait in a major crop. Everything proposed in Section 5 is a reasoned extrapolation from adjacent, well supported work, not a reported result, and any reader building on this review should treat it accordingly.

VIII. REGULATORY AND BIOSAFETY CONSIDERATIONS

Regulatory treatment of this technology is still unsettled in most jurisdictions, and it depends heavily on two technical details: whether any foreign DNA integrates into the genome, and whether the final product carries a permanent change to the DNA sequence or only a reversible epigenetic mark. Transgene free nanoparticle delivery, where the cargo is degraded after use and no foreign sequence remains, is generally expected to face a lighter regulatory path than classical transgenic approaches, similar to the treatment already extended to some DNA free CRISPR edited crops in certain countries. Epigenetic edits complicate this picture further, since they change gene expression without changing the DNA sequence at all, and regulatory frameworks built around the

ISSN: 3139-8464 (p)

presence or absence of novel DNA sequence do not map cleanly onto a technology that leaves the sequence untouched.

There are also biosafety questions specific to nanomaterials themselves, including the environmental fate of nanoparticles applied at agricultural scale, potential toxicity to non-target organisms, and the practicalities of large-scale, cost-effective nanoparticle synthesis for field level deployment rather than laboratory scale leaf infiltration. These are engineering and toxicology questions as much as they are plant biology questions, and they will need dedicated environmental risk assessment before any nanoparticle delivered epigenome editing product could reach commercial agriculture.

IX. FUTURE DIRECTIONS

A few concrete research directions stand out. Building smaller, single domain epigenome editors specifically optimised for nanoparticle packaging would help close the cargo size gap described above. Systematic testing of germline and meristem targeted delivery, rather than leaf infiltration alone, is needed if heritable transmission is the actual goal rather than a one generation effect. Longer term field trials tracking epigenetic mark stability across multiple generations under real stress conditions, rather than single controlled greenhouse exposures, would give a much more honest picture of durability. Finally, closer collaboration between the epigenetics, CRISPR tool development, and nanomaterials communities, who currently publish largely in separate journals and rarely cite each other's most recent work, would likely accelerate exactly the kind of integrated pipeline this review has sketched out.

X. LIMITATIONS OF THIS REVIEW

Two limitations are worth stating plainly. First, this review leans on English language, primarily open access literature retrieved through standard academic search, and it is possible that relevant work in regional agricultural journals or in preprint form was missed. Second, and more importantly, the central synthesis in Section 5 is a conceptual integration built from three separate, independently well supported literatures rather than a summary of directly reported convergence experiments, since very few such experiments currently exist in print. Readers should treat Section 5 as a research proposal grounded in existing evidence, not as a report of demonstrated results, and should verify the current state of the field before citing it as established fact, since this is a fast-moving area and new integrated studies may appear soon after this review is written.

XI. CONCLUSION

Epigenetic stress memory, CRISPR based epigenome editing, and nanoparticle mediated delivery are each, on their own, well established and rapidly advancing areas of plant science. Bringing them together offers a genuinely plausible route to climate resilient crops that could be developed

faster than conventional breeding allows and with a lighter regulatory footprint than classical transgenics, because the approach can avoid both permanent DNA sequence change and foreign DNA integration. The pieces needed to build this pipeline already exist in the literature. What remains is the harder, more patient work of connecting them end to end, proving that the resulting stress memory is heritable and stable, and testing all of this under real field conditions rather than controlled laboratory ones. That is the work this field now needs to take on.

ACKNOWLEDGEMENTS

The authors thank the faculty and research scholars of the Department of Botany, MANUU, Hyderabad, for laboratory facilities and technical support, and the library staff of MANUU for access to reference literature.

Declarations
Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of interest: The authors declare no conflict of interest.

Data availability: The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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