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RESEARCH NOTE

Gamma Irradiation Stimulates HDA14 Expression: Perspectives in Radiation Epigenetics for Plant Breeding

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Gamma irradiation is a commonly used technique to induce genetic variations in crops for plant breeding programs, but its unpredictable nature and potential for unwanted mutations hinder its widespread application. Epigenetics, however, provides an alternative approach for developing desirable plant traits without altering the DNA sequence. In this study, we investigated the impact of ionizing radiation on the epigenome by performing a meta-analysis on the expression levels of Class II histone deacetylases (HDAs). We found that 200-Gy exposure of *Arabidopsis* seedlings downregulated Class II HDAs while upregulating AtHDA14 expression in mature rosette leaves, suggesting a developmental stage-specific response to radiation. The increased expression of AtHDA14 stimulated multiple biological processes and molecular functions, potentially generating transgenerational epigenetic variants associated with enhanced stress resilience and improved crop performance. This finding supports the concept of "radiation epigenetics" as a promising paradigm for plant breeding. However, further empirical testing is necessary to validate the effectiveness and safety of this approach. Harnessing the potential of ionizing radiation to modulate Class II HDA expression and modify epigenetic marks could revolutionize plant breeding practices, hence offering new avenues for developing stress-tolerant and high-yielding crop varieties.

Keywords: *Arabidopsis thaliana*, Class II HDA, epigenetics, HDA14, histone deacetylases, ionizing radiation

Ionizing radiation has long been employed as a technique to induce genetic mutations for crop improvement, thus playing a crucial role in plant breeding programs (Kim *et al.* 2019). This approach has facilitated the development of numerous crop varieties adapted to specific environmental conditions or possessing desirable traits such as increased yield, disease resistance, and stress tolerance. Remarkably, radiation mutation breeding has led to the creation of over 3,400 varieties from 200 plant species – including rice, wheat, barley, soybean, and tomato (FAO/IAEA 2022). However, while mutations can broaden phenotypic diversity, it is now recognized that phenotypic variations can arise without alterations in the DNA sequence through the realm of epigenetics.

Epigenetics has revolutionized plant biology in recent years, hence providing valuable insights into how plants respond and adapt to diverse environmental cues and stresses. Epigenetic modifications encompassing DNA methylation, histone modifications, and small RNA-mediated gene silencing play pivotal roles in regulating gene expression and shaping phenotypic variation in plants. Notably, these modifications can be heritable, thereby serving as a form of "memory" that enables plants to recall past environmental conditions and elicit appropriate responses. Given the advances in our understanding of

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epigenetics, exploring epigenetic variation or employing epigenome editing in plant breeding presents a promising avenue for crop improvement. In this study, we sought to investigate the potential impact of ionizing radiation on the plant epigenome by manipulating the expression of a specific epigenetic regulator – Class II histone deacetylases (HDAs).

To investigate the expression profiles of Class II HDAs in response to gamma irradiation, we conducted a comprehensive analysis using microarray data from Genevestigator, a valuable resource for transcriptome meta-analysis (Hruz *et al.* 2008). By querying the GeneChip® *Arabidopsis* ATH1 Genome Array (Affymetrix), we obtained two relevant microarray datasets involving gamma treatment on mature *Arabidopsis* leaves (Bourbousse *et al.* 2018) and seedlings (Kim and Kim 2013). Our analysis unveiled that all five Class II HDAs exhibited ubiquitous expression across various vegetative parts and developmental stages of *Arabidopsis thaliana*, thus underscoring their active involvement in diverse physiological and molecular processes throughout plant growth and development.

Remarkably, exposure to gamma radiation at a dose of 200 Gy elicited distinct changes in the expression of Class II HDAs across different developmental stages (Figure 1). In irradiated seedlings, a significant reduction in HDA expression was observed, ranging from 6–8-fold compared to control seedlings. This downregulation implies that ionizing radiation adversely affects the expression of Class II HDAs in *Arabidopsis* seedlings, thereby potentially resulting in alterations in histone acetylation status and subsequent perturbations in gene expression patterns. Conversely, irradiated rosette leaves in mature plants displayed a general upregulation of Class II HDAs, with a particular emphasis on AtHDA14. Notably, AtHDA14 and its orthologs are exclusive to plants, suggesting their potential role in protecting against radiation-induced damage in plant-specific metabolic processes such as photosynthesis and redox regulation (Alinsug *et al.* 2009).

To gain insights into the functional role of AtHDA14, we employed the STRING database (Szklarczyk *et al.* 2019) to identify potential binding partners. Additionally, we conducted Gene Ontology queries to investigate the biological processes, molecular functions, and cellular components associated with AtHDA14. Furthermore, we utilized OrthoDB (Kuznetsov *et al.* 2023) to identify orthologs of AtHDA14 in other plant systems.

As illustrated in Figure 2A, our analysis revealed several interacting proteins of AtHDA14, with most having validated experimentally using various techniques that elucidate protein-protein interactions. The AtHDA14 interactome (Figure 2B) is composed of HDA17 and HDA10, NAD-dependent protein deacetylase sirtuin 2 (SRT2), mRNA splicing protein AT3G52250, high mobility group (HMG) protein AT1G76110,

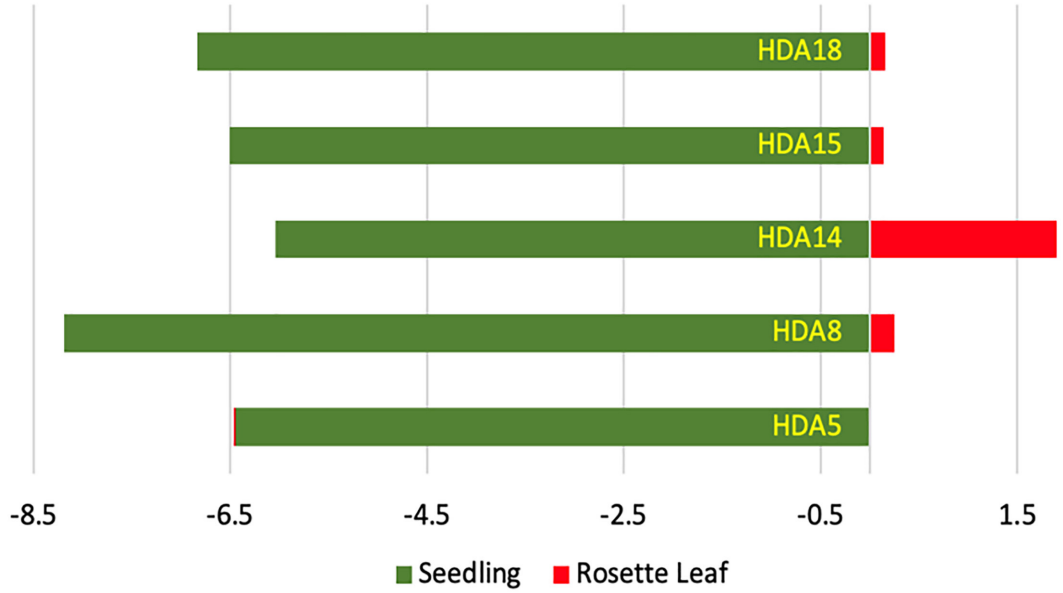

Figure 1. Gene expression of Class II HDA upon gamma irradiation and localization of HDA14. The expression of Class II HDAs was significantly reduced upon ionizing radiation treatment of seedlings indicating a global increase in its acetylation levels. However, irradiated rosette leaves of mature plants generally upregulated Class II HDAs, which was significantly pronounced in HDA14. These elevated levels suggest enhanced HDA14 activities in biological processes and molecular functions. Expression data sets from Bourbousse *et al.* (2018) and Kim and Kim (2013) were used for these analyses.

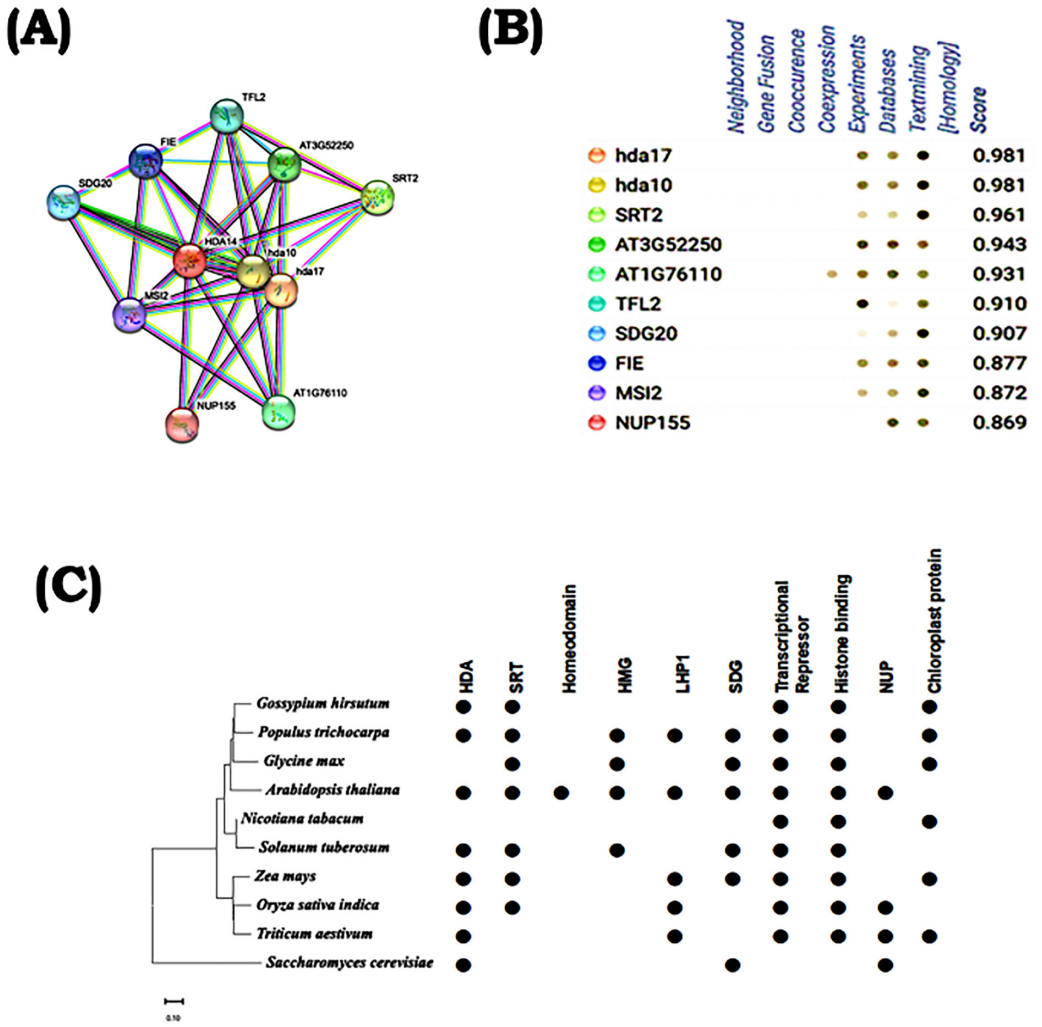

Figure 2. HDA14 protein interacting network and phylogenetic co-occurrence of interacting proteins. [A] Interacting protein network of HDA14 was predicted using STRING-DB. [B] The corresponding names of predicted functional partners of HDA14 are listed based on its binding score using different methods. [C] Phylogenetic co-occurrence pattern of interacting proteins was conserved in its corresponding orthologs from other plant model systems.

heterochromatin component TERMINAL FLOWER 2 (TFL2), histone methyltransferase SET Domain Group 20 (SDG20), endosperm regulator FERTILIZATION-INDEPENDENT ENDOSPERM (FIE), core histone-binding subunit MULTICOPY SUPPRESSOR OF IRA1 2 (MSI2), and nuclear pore complex protein nucleoporin 155 (NUP155). Based on these predicted interactions, it is plausible that AtHDA14 forms a complex with HDA17/HDA10 deacetylating lysine residues on histone tails, thereby leading to transcriptional repression, whereas its interaction with SRT2 regulates plant defense (Wang *et al.* 2010). Moreover, AtHDA14 may play regulatory roles in conjunction with other proteins such as AT1G76110, which is significant in female gametophyte development, whereas its interaction with TFL2 suggests involvement in repressing floral homeotic genes (Larsson *et al.* 1998). The interaction with AT3G52250 implicates a role in mRNA splicing while its interaction with FIE indicates a regulatory role in establishing the endosperm polar axis (Ye *et al.* 2022; Mosquna *et al.* 2004). SDG20, a histone methyltransferase, may require the prior removal of acetyl groups by AtHDA14 before transferring methyl groups (Satish *et al.* 2018). Additionally, AtHDA14 may serve as one of the chromatin remodeling factors recruited by MSI2 for gene silencing. The interaction with NUP155, involved in nucleocytoplasmic transport, suggests that AtHDA14 may shuttle in and out of the nucleus given the proper signaling cues (Kehat *et al.* 2011; Radu *et al.* 1993). Interestingly, as shown in Figure 2C, the identification of conserved phylogenetic co-occurrence patterns among interacting proteins in AtHDA14 orthologs from various plant systems

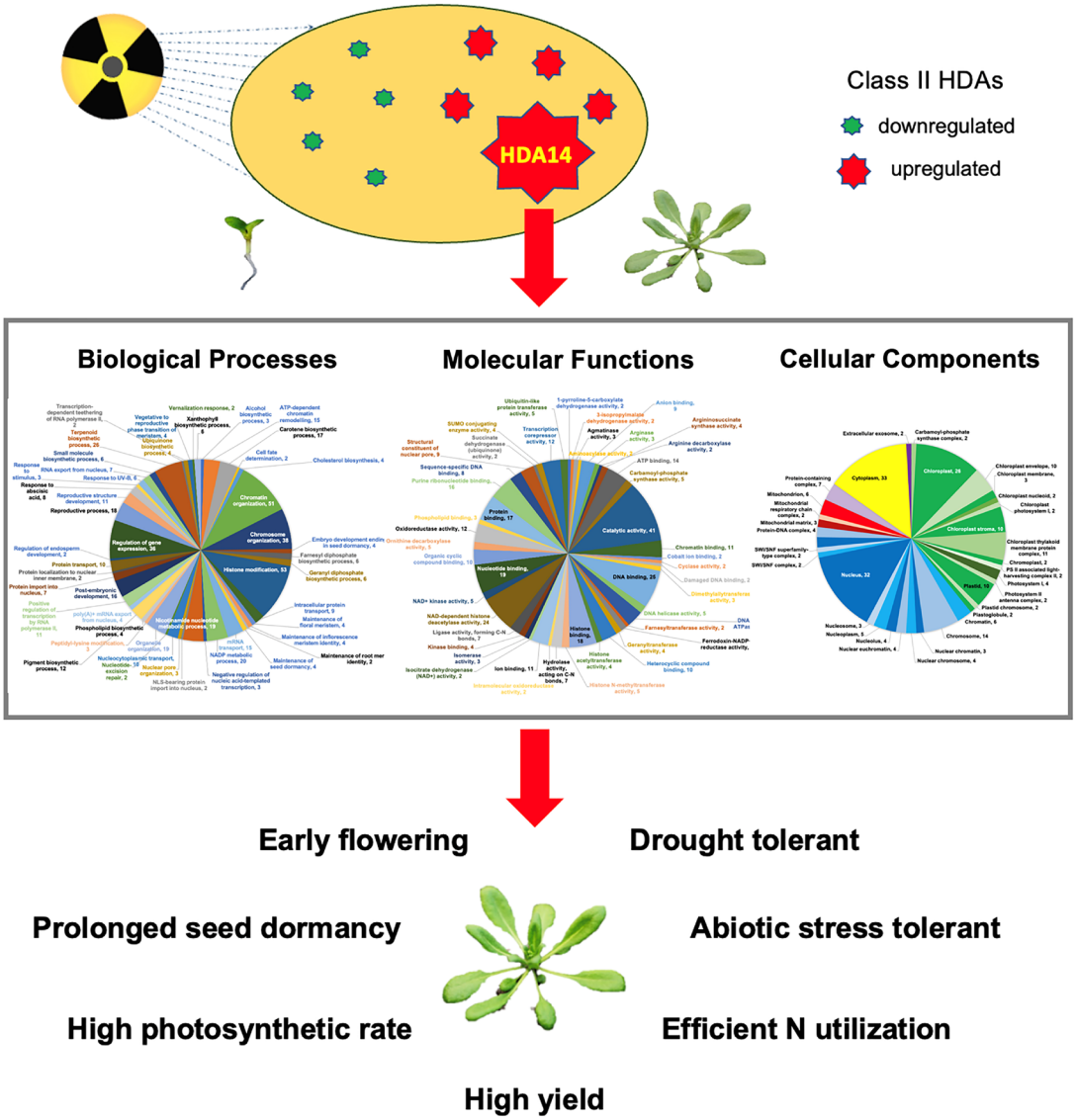

Figure 3. A concept of rapid radiation-aided plant breeding without genetic mutation. With radiation epigenesis, ionizing radiation acts as a stress signal to repress or upregulate epigenetic regulators, *i.e.* Class II HDAs. In mature rosette leaves, gamma radiation significantly enhanced HDA14 expression stimulating a plethora of biological processes and molecular functions that can produce transgenerational epigenetic variants. These "radiation epivariants" are linked with enhanced plant resilience to stress (*e.g.* drought tolerant, abiotic stress tolerant) and improved crop performance (*e.g.* efficient N utilization, high photosynthetic rate, high yield) (see Appendix Tables I–III).

highlights their functional importance across diverse plant species, thereof suggesting co-evolutionary relationships.

To further validate the biological significance of the predicted interactions involving AtHDA14, we performed a comprehensive Gene Ontology analysis (Thomas *et al.* 2022; Ashburner *et al.* 2000) using the annotation tools provided by the STRING database and UniProtKB (The UniProt Consortium 2023). HDA14 generally localizes in the nucleus, cytoplasm, chloroplast, and mitochondria (Tran *et al.* 2012; Alinsug *et al.* 2012; Hartl *et al.* 2017). A detailed list of AtHDA14 localization in various subcellular compartments is presented in Appendix Table I. Furthermore, the analysis of AtHDA14 interactome revealed its involvement in a wide range of crucial biological processes and molecular functions, including response to abscisic acid (ABA), reproductive-to-vegetative phase transition of meristems, reproductive structure development, maintenance of floral meristem, vernalization response, maintenance of seed dormancy, embryo development ending in seed dormancy, and several others (Figure 3; Appendix Table II). Furthermore, AtHDA14 exhibited molecular functions such as

ferredoxin-NADP reductase activity, photosynthesis and antenna proteins, cholesterol/terpene/carotene/xanthophyll biosynthetic processes, carbamoyl phosphate synthase activity, UV-B response, NADP biosynthetic and metabolic processes, and nicotinamide biosynthesis. The localization of AtHDA14 in the chloroplasts aligns with the findings of Hartl *et al.* (2017), where a significant portion of its protein targets play essential roles in photosynthesis.

With these identified functional pathways, it can be surmised that enhancing the expression of AtHDA14 may hold the potential to prime plant responses to various conditions that contribute to drought tolerance, regulation of flowering time, maintenance of seed dormancy, elevated photosynthetic rates, enhanced cholesterol biosynthesis, increased efficiency in nitrogen (N) utilization, UV-B responses, and improved NADP biosynthetic processes. Additionally, these molecular and physiological changes can enhance plant resilience to stress, including drought and abiotic stresses, and ultimately lead to improved crop performance with higher N utilization efficiency, elevated photosynthetic rates, and increased yield (Appendix Table III).

The functional prospects of AtHDA14 in terms of improved crop performance align with the findings of acetylome studies conducted on *hda14* mutants by Hartl *et al.* (2017). The study identified multiple protein targets of HDA14 involved in various metabolic processes, including photosynthesis, redox regulation, melatonin biosynthesis, protein synthesis and degradation, transcriptional regulation, tetrapyrrole synthesis, nucleotide metabolism, cell division, ABC transport, secondary metabolism, and amino acid metabolism. Notably, one of the most compelling targets of HDA14 is ribulose-1,5-bisphosphate carboxylase/oxygenase highlighting its critical role in the Calvin-Benson cycle. Although HDA14 is primarily localized in the chloroplast, cytoplasm, and mitochondria – upon specific signaling cues – it can translocate to the nucleus and form complexes with its prospective nuclear partners for transcriptional repression (Alinsug *et al.* 2012).

However, the specific impact of gamma irradiation on the deacetylation of AtHDA14 target proteins remains unknown. Further investigations are needed to comprehensively understand the regulatory effects of gamma irradiation on AtHDA14 and its associated proteins. Lysine acetylation sites can act as molecular switches, and the upregulation and activation of epigenetic regulators like AtHDA14 in response to gamma irradiation may modulate cell signaling cascades, gene expression, and the activities of metabolic enzymes. This discovery sheds new light on the potential role of gamma irradiation in regulating plant metabolic processes – where AtHDA14 is known to play critical roles – such as photosynthesis, flowering time regulation, root development, redox regulation, salt stress tolerance, ABA signaling, and anthocyanin biosynthesis (Li *et al.* 2020; Gu *et al.* 2017).

It is important to note that among the 18 known RPD3-HDA1-like HDAs, Class II HDAs – namely, HDA5, HDA8, HDA14, HDA15, and HDA18 – possess unique characteristics exhibiting nucleocytoplasmic shuttling and deacetylating both histones and non-histone proteins (Alinsug *et al.* 2020). Extensive research has revealed their critical roles in regulating gene expression, protein activity, and signaling networks involved in various developmental processes such as seed germination, root development, photomorphogenesis, flowering, ABA signaling, and heat tolerance (Alinsug and Deocaris 2023; Alinsug *et al.* 2020; Liu *et al.* 2013; Xu *et al.* 2005). In addition, this study highlights the specific and context-dependent nature of the response of Class II HDAs to ionizing radiation in *Arabidopsis*.

We acknowledge that the scope of the current study is limited to a gamma treatment dose of 200 Gy. Given the dose-dependent nature of radiation, it is reasonable to speculate that lower doses of ionizing radiation may elicit distinct responses in the expression of other Class II HDAs in mature plant rosette leaves. Further investigation is warranted to elucidate the dose-dependent responses of Class II HDAs to ionizing radiation, which will provide a more comprehensive understanding of their role in plant radiation response.

Exploring the intricacies of epigenetic diversity, coupled with a thorough understanding of the molecular mechanisms and functional roles of epigenetic regulators in response to radiation, represents a compelling avenue for advancing crop improvement. Epigenetic modifications serve as a wellspring of variation, offering a means to optimize crop traits without resorting to genetic mutations. This not only enhances the adaptability of crops but also fortifies their resilience against environmental stresses. The contemporary focus on unraveling and harnessing epigenetic variation, underscored by numerous studies (Alfalahi *et al.* 2022; Lieberman-Lazarovich *et al*. 2022; Kakoulidou *et al*. 2021; Samantara *et al*. 2021; Hou and Wan 2021; Gahlaut *et al.* 2020; Springer and Schmitz 2017), aligns seamlessly with the overarching objective of mitigating the challenges posed by climate change in agriculture. Thus, this research trajectory not only holds substantial promise for sustainable agricultural practices but also represents a concerted effort to address and surmount the evolving complexities of climate-related adversities in the agricultural landscape.

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APPENDICES

Table I. Localization of HDA14 interactome in cellular compartments.

Subcellular compartment	Count
Carbamoyl-phosphate synthase complex	2
Chloroplast	26
Chloroplast envelope	10
Chloroplast membrane	3
Chloroplast nucleoid	2
Chloroplast photosystem I	2
Chloroplast stroma	10
Chloroplast thylakoid membrane protein complex	11
Chromatin	6
Chromoplast	2
Chromosome	14
Cytoplasm	33
Extracellular exosome	2
FACT complex	4
Mitochondrial matrix	3
Mitochondrial respiratory chain complex	2
Mitochondrion	6
Nuclear chromatin	3
Nuclear chromosome	4
Nuclear euchromatin	4
Nucleolus	4
Nucleoplasm	5
Nucleosome	3
Nucleus	32
Photosystem I	4
Photosystem II antenna complex	2
Plastid	10
Plastid chromosome	2
Plastoglobule	2
Protein-containing complex	7
Protein-DNA complex	4
PS II associated light-harvesting complex II	2
SWI/SNF complex	2
SWI/SNF superfamily-type complex	2

Table II. Biological processes affected by protein interactions of HDA14 categorized by Gene Ontology.

Biological process	Count
Alcohol biosynthetic process	3
ATP-dependent chromatin remodeling	15
Carotene biosynthetic process	6
Carotenoid biosynthetic process	11
Cell fate determination	2
Cholesterol biosynthetic process	4
Chromatin organization	37
Chromatin silencing	14
Chromosome organization	38
Embryo development ending in seed dormancy	4
Farnesyl diphosphate biosynthetic process	6
Geranyl diphosphate biosynthetic process	6
Histone acetylation	2
Histone deacetylation	15
Histone lysine methylation	4
Histone methylation	2
Histone modification	30
Intracellular protein transport	9
Maintenance of floral meristem identity	4
Maintenance of inflorescence meristem identity	4
Maintenance of root meristem identity	2
Maintenance of seed dormancy	4
mRNA export from nucleus	5
mRNA transport	10
NAD metabolic process	6
NADP biosynthetic process	7
NADP metabolic process	7
Negative regulation of nucleic acid-templated transcription	3
Nicotinamide nucleotide biosynthesis process	9
Nicotinamide nucleotide metabolic process	10
NLS-bearing protein import into nucleus	2
Nuclear pore organization	3
Nucleocytoplasmic transport	10
Nucleotide-excision repair	2
Organ boundary specification between lateral organs	4
Organelle organization	15
Peptidyl-lysine modification	3
Phospholipid biosynthetic process	4
Pigment biosynthetic process	12
poly(A)+ mRNA export from nucleus	4
Positive regulation of transcription by RNA polymerase II	11
Post-embryonic development	16
Protein import into nucleus	7

Table II. Cont.

Protein localization to nuclear inner membrane	2
Protein transport	10
Regulation of endosperm development	2
Regulation of gene expression by genetic imprinting	18
Regulation of gene expression, epigenetic	18
Reproductive process	18
Reproductive structure development	11
Response to abscisic acid	8
Response to stimulus	3
Response to UV-B	6
RNA export from nucleus	7
Small molecule biosynthetic process	6
Terpene biosynthetic process	7
Terpenoid biosynthetic process	19
Transcription-dependent tethering of RNA polymerase II	2
Ubiquinone biosynthetic process	4
Vegetative to reproductive phase transition of meristem	4
Vernalization response	2
Xanthophyll biosynthetic process	6

Table III. Molecular functions affected by protein interactions of HDA14 categorized by Gene Ontology.

Molecular functions	Count
1-pyrroline-5-carboxylate dehydrogenase activity	2
3-isopropylmalate dehydrogenase activity	2
Agmatinase activity	3
Aminoacylase activity	2
Anion binding	9
Arginase activity	3
Arginine decarboxylase activity	2
Argininosuccinate synthase activity	4
ATP binding	14
Carbamoyl-phosphate synthase activity	5
Catalytic activity on a protein:	7
Catalytic activity	34
Chromatin binding	11
Cobalt ion binding	2
Cyclase activity	2
Damaged DNA binding	2
Dimethylallytransferase activity	3
DNA binding	25
DNA helicase activity	5
DNA-dependent ATPase activity	11
Farnesyltransferase activity	2

Table III. Cont.

Ferrodoxin-NADP+ reductase activity	4
Geranyltransferase activity	4
Heterocyclic compound binding	10
Histone acetyltransferase activity	4
Histone binding	18
Histone-lysine N-methyltransferase activity	5
Hydrolase activity, acting on carbon-nitrogen bonds	7
Intramolecular oxidoreductase activity	2
Ion binding	11
Isocitrate dehydrogenase (NAD+) activity	2
Isomerase activity	3
Kinase binding	4
Ligase activity, forming carbon-nitrogen bonds	7
NAD-dependent histone deacetylase activity	20
NAD-dependent protein deacetylase activity	4
NAD+ kinase activity	5
Nucleosome binding	4
Nucleotide binding	15
Organic cyclic compound binding	10
Ornithine decarboxylase activity	5
Oxidoreductase activity	12
Phospholipid binding	3
Protein binding	6
Protein dimerization activity	4
Protein domain-specific binding	4
Protein heterodimerization activity	3
Purine ribonucleoside triphosphate binding	8
Purine ribonucleotide binding	8
Sequence-specific DNA binding	8
Structural constituent of nuclear pore	9
Succinate dehydrogenase (ubiquinone) activity	2
SUMO conjugating enzyme activity	4
Transcription corepressor activity	12
Ubiquitin-like protein transferase activity	5