SHORT COMMUNICATION

Trichostatin A and sodium butyrate promotes plant regeneration in common wheat

Xiao Min Bie^{a*}, Luhao Dong^{b*}, Xiao Hui Li^a, He Wang^a, Xi-Qi Gao^a, and Xing Guo Li^a

^aState Key Laboratory of Crop Biology, College of Life Sciences, Shandong Agricultural University, Tai'an, Shandong, China; ^bState Key Laboratory of Crop Biology, College of Agronomy, Shandong Agricultural University, Tai'an, Shandong, China

ABSTRACT

Histone acetylation modification plays a vital role in plant cell division and differentiation. However, the function on wheat mature embryo culture has not been reported. Here, we used the mature embryo of wheat genotypes including CB037, Fielder, and Chinese Spring (CS) as materials to analyze the effects of different concentrations of trichostatin A (TSA) and sodium butyrate (SB) on plant regeneration efficiency. The results showed that, compared with the control group, the induction rates of embryogenic callus and green shoot were significantly increased with the addition of 0.5 μ M TSA, while they were reduced under treatment of 2.5 μ M TSA on wheat mature embryo. With the respective addition of 200 μ M and 1000 μ M SB, regeneration frequency of three genotypes was enhanced, especially in Fielder, which reached significant difference compared with the control group. Unfortunately, 0.5 μ M TSA and 200 μ M SB combination had no apparent effect on wheat regeneration frequency. The results indicated that TSA and SB increase plant regeneration in common wheat. In addition, TSA had a common effect and SB had different effect among genotypes on wheat regeneration frequency. The mechanism of action needs further investigation.

ARTICLE HISTORY

Received 21 July 2020
Revised 2 September 2020
Accepted 3 September 2020

KEYWORDS

Trichostatin A; sodium butyrate; regeneration; histone deacetylases inhibitor; mature embryo; wheat

1. Introduction

Wheat (*Triticum aestivum* L.) is the most widely planted crop in the world, providing about 35% to 40% of mankind's calories. With the development of molecular biology and sequencing technology, gene function research in wheat becomes important trend, so establishment of highly efficient transformation system is necessary and important.^{1–3} Previous studies found that many factors, such as explant type, genotype and medium composition, had crucial effects on the wheat regeneration.^{4,5} At present, the frequently-used explant types in wheat genetic transformation include young embryo, mature embryo, anther and young spike.^{6–9} Mature embryo has the advantages of convenient sampling, not easily affected by environmental conditions, and the same physiological status among individuals, which becomes a convenient kind of explant for wheat genetic transformation. However, low regeneration capacity of wheat mature embryo limits its application in genetic transformation.¹⁰

The definition of epigenetics incorporates the phenomena that inheritable variation can be achieved through mitosis and/or meiosis without altering nucleotide sequences.^{11,12} These variations mainly include DNA methylation, histone modification, and noncoding RNA. In addition, histone modifications include acetylation, methylation, phosphorylation, and ubiquitination.^{13,14} Trichostatin A (TSA) and sodium butyrate (SB) are the most frequently used histone deacetylases (HDAs) inhibitors. TSA could insert the aliphatic chain into the hydrophobic cleft which is in the HDAs catalytically active domain.¹⁵

In a similar way, two SB molecules were able to occupy the hydrophobic cleft of HDAs.¹⁶

TSA specifically inhibits HDAs, which can lead to the accumulation of acetylated histones and corresponding decrease of DNA methylation in cells, induce cell differentiation, cell cycle arrest, reversal of cell morphology and apoptosis, and regulating transcription,¹⁷ and alter gene expression level on genome-wide level.¹⁸ Treatment with TSA caused decrease in the mitotic index in maize (*Zea mays* L.) root tips implying that DNA and H3K9 methylation and H4 deacetylation may play important roles in triggering mitosis.¹⁹ Wheat seedlings exposed to TSA could raise the overall level of centromere association, and affect chromatin structure and chromosome association.²⁰

Previous studies on plant regeneration mainly focused on hormone regulation^{21–24} and molecular regulation network of stem cell formation and maintenance.^{25–28} And, histone modifications play critical roles in cellular dedifferentiation and redifferentiation.^{29–32} Plant stem cells keep the competence to divide and differentiate and prevent precocious cellular differentiation, notwithstanding presence of relatively inactive chromatin, while these states are at least in part determined by recruiting HDAs.³³ Studies had shown that TSA has significant effect in inducing embryogenic callus and increasing regeneration rate in plants. Blocking HDAs activity with TSA in cultured male gametophytes of *Brassica napus* and *Arabidopsis thaliana* leads to a large increase in the proportion of cells that switch from pollen to embryogenic growth.³⁴ While embryogenic cultures of Norway spruce (*Picea abies* L. Karst.) and Scots pine (*Pinus sylvestris* L.) were treated with TSA, the

embryo maturation process was stunted and the expression levels of *HAP3A* and *VIVIPAROUS1* were maintained.³⁵

SB could inhibit the proliferation and differentiation of tumor cells, induce the senescence and apoptosis of cancer cells, and enhance immune function and disease resistance.^{36–39} As a widely used feed acidifier in livestock and poultry production, SB also has the functions of improving intestinal health, anti-inflammatory and anti-oxidation and animal performance.^{40–43} Whereas, SB is rarely used in plant tissue culture. Liang and Fang⁴⁴ found that SB preculture could significantly improve the potential of callus induction and green plantlet formation from rice (*Oryza sativa* L.) anthers.

Together, previous studies have shown that histone acetylation modification plays a vital role in plant cell division and differentiation. However, the function of TSA and SB on wheat mature embryo culture has not been elucidated. To explicit the effect of HDAs inhibitors on regeneration efficiency, wheat mature embryo of different genotypes was used as explants and treated with

different concentrations of TSA and SB, and the regeneration efficiency was evaluated.

2. Results

2.1. Low concentration of TSA improved the embryonic calli frequency and plantlet regeneration frequency of CB037, Fielder, and CS

To study the effect of TSA on wheat mature embryo regeneration, we added 0.5 μ M and 2.5 μ M TSA into the culture medium and analyzed the regeneration frequency. Results showed that 0.5 μ M TSA could improve the embryonic calli frequency and plant regeneration frequency of CB037, Fielder, and CS (Figure 1) with significant difference compared with the control group (Figure 2a,c). Treatment with 2.5 μ M TSA reduced the CB037 regeneration frequency and reached significant difference level, however, there were no significant difference between 2.5 μ M TSA and control group on CS (Figure 2). It suggested that low concentration of TSA could

Figure 1. Effect of TSA on differentiated calli frequency of wheat mature embryo. (a) CB037 was cultured without the addition of TSA (control). (b) CB037 was cultured on medium supplemented with 0.5 μ M TSA. (c) CB037 was cultured on medium supplemented with 2.5 μ M TSA. (d) Fielder was cultured without the addition of TSA (control). (e) Fielder was cultured on medium supplemented with 0.5 μ M TSA. (f) Fielder was cultured on medium supplemented with 2.5 μ M TSA. (g) CS was cultured without the addition of TSA (control). (h) CS was cultured on medium supplemented with 0.5 μ M TSA. (i) CS was cultured on medium supplemented with 2.5 μ M TSA. The 0.5 μ M and 2.5 μ M TSA were added into Adi medium, respectively.

Figure 2. Statistical analysis of TSA concentrations on embryonic calli frequency, differentiated calli frequency and plantlet regeneration frequency of CB037, Fielder, and CS. (a) Embryonic calli frequency. (b) Differentiated calli frequency. (c) Plantlet regeneration frequency. *, $p < .05$, **, $p < .01$.

improve the embryonic calli frequency and plant regeneration frequency of CB037, Fielder, and CS.

2.2. Low concentration of SB improved the regeneration efficiency of CB037, Fielder, and CS

To uncover the effect of SB on wheat regeneration, different concentrations of SB were added into the culture medium and the regeneration frequency was analyzed. The result of the

experiments showed that addition of SB could increase the embryonic calli frequency, differentiated calli frequency and plantlet regeneration frequency on CB037, Fielder, and CS (Figures 3 and 4). Compared with the control, embryonic calli frequency and differentiated calli frequency had the extremely significant difference and plantlet regeneration frequency had significant difference for the Fielder genotype (Figure 4). For the effect on regeneration efficiency, 200 μM SB was more effective compared with 1000 μM SB among the three

Figure 3. Effect of SB on differentiated calli frequency of wheat mature embryo. (a) CB037 was cultured without the addition of SB (control). (b) CB037 was cultured on medium supplemented with 200 μM SB. (c) CB037 was cultured on medium supplemented with 1000 μM SB. (d) Fielder was cultured without the addition of TSA (control). (e) Fielder was cultured on medium supplemented with 200 μM SB. (f) Fielder was cultured on medium supplemented with 1000 μM SB. (g) CS was cultured without the addition of TSA (control). (h) CS was cultured on medium supplemented with 200 μM SB. (i) CS was cultured on medium supplemented with 1000 μM SB. The 200 μM and 1000 μM SB were added into Adi medium, respectively.

Figure 4. Statistical analysis of SB concentrations on embryonic calli frequency, differentiated calli frequency and plantlet regeneration frequency of CB037, Fielder, and CS. (a) Embryonic calli frequency. (b) Differentiated calli frequency. (c) Plantlet regeneration frequency. *, $p < .05$, **, $p < .01$.

genotypes. Appropriate addition of SB could increase the regeneration efficiency (Figure 4).

2.3. Combination of 0.5 μM TSA and 200 μM SB had no apparent effect on wheat regeneration efficiency

According to the above experiment data, we found that 0.5 μM TSA increased the embryonic calli frequency and plantlet regeneration frequency; Addition of 200 μM SB increased the regeneration efficiency on CB037, Fielder, and CS to some extent. Therefore, both 0.5 μM TSA and 200 μM SB was added into culture medium. However, our results indicated that regeneration efficiency of CB037, Fielder and CS had no significant difference under this combined concentration (Figures 5 and 6).

3. Discussion

Histone acetylation modification is an important form of epigenetic inheritance and a key regulatory scheme in regulating overall gene-expression level.^{13,14,18} Multiple signaling pathways of leaf-to-callus transition in *A. thaliana* were controlled by means of histone modification during cell dedifferentiation.^{45–47} TSA and SB can inhibit HDAs which lead to the accumulation of acetylated histones. The male gametophyte of *A. thaliana* genotype with recalcitrant haploid embryo development could form embryogenic cell clusters after TSA treatment, which is associated with the hyperacetylation of histones H3 and H4.³⁴ Application of 0.5 μM TSA stimulated barely (*Hordeum vulgare* L.) pollen embryogenesis with a gametophytic style chromatin pattern.⁴⁸ Supplement of 0.1 μM TSA led to a significant increase of haploid plants derived from wheat microspore culture which was validated in both cultivated varieties and F1 hybrids, where TSA showed a positive effect on both embryogenesis and regeneration of green plantlets.⁴⁹

Increasing knowledges of wheat mature embryo *in vitro* culture makes it more and more popular as explants. In this work, 0.5 μM TSA improved the embryonic calli induction and plantlet regeneration frequency on CB037, Fielder, and CS, compared with control group, reached to the significant level. When the concentration of TSA increased to 2.5 μM, the regeneration efficiency was lower than the control

group (Figure 2). Addition of SB could increase the regeneration efficiency of CB037, Fielder, and CS. Interestingly, embryonic calli frequency, differentiated calli frequency and plantlet regeneration frequency of Fielder were significantly increased compared with the control group, while regeneration efficiency of CB037 and CS was slightly improved (Figure 4). Therefore, the function of SB on wheat mature embryo regeneration improvement may be genotype-dependent, while TSA universal. The difference may be caused by different mechanisms, because SB is a noncompetitive inhibitor of HDAs, which does not associate with the substrate-binding region.¹⁶ Our results showed that 0.5 μM TSA and 200 μM SB could enhance wheat regeneration efficiency respectively. Unfortunately, combination of 0.5 μM TSA and 200 μM SB had no apparent effect (Figure 6). When TSA and SB were simultaneously added into the medium, HDAs level of wheat mature embryo callus maybe not suitable for regeneration. It is possible that histone acetylation and deacetylation were maintained at a certain level to promote plant regeneration. Histone acetylation levels were globally elevated on a genomic scale during cellular dedifferentiation, but the histone deacetylation process might also be required for suppression of shoot identity in the process of callus formation.⁴⁶

In general, TSA induces changes in cell wall, auxin, and cell division pathways.³⁴ Auxin-mediated transcription is postulated to be repressed by histone deacetylases, which convert chromatin into a highly packed inactive state.⁵⁰ In Arabidopsis, auxin, cytokinin, ethylene, and abscisic acid response signals gradually form a mutually exclusive pattern during *de novo* shoot regeneration,^{51,52} and thioredoxin-mediated ROS effects the embryogenic program of development.⁵³ In the somatic cells of Arabidopsis, TSA triggers an embryogenic transition through an auxin-mediated pathway.⁵⁴

Interestingly, the effect of TSA on callus formation indicated that the dual mode of HDAs actions was exist among different origin of the plant tissue.⁴⁶ When TSA was administered to embryogenic barely pollen, the chromatin markers reversed toward a gametophytic profile, embryogenesis was halted and all pollen invariably died.⁴⁸ The effect of TSA and SB treatment on early plant development was not limited to the modulation of histone acetylation levels, because *hda6* mutants did not exhibit any obvious phenotypic defects and the phenotypes of seedlings

Figure 5. Effect of 0.5 μM TSA and 200 μM SB combination concentrations on differentiated calli frequency of wheat mature embryo. (a) CB037 was cultured without the addition of TSA and SB (control). (b) CB037 was cultured on medium supplemented with 0.5 μM TSA and 200 μM SB. (c) Fielder was cultured without the addition of TSA and SB (control). (d) Fielder was cultured on medium supplemented with 0.5 μM TSA and 200 μM SB. (e) CS was cultured without the addition of TSA and SB (control). (f) CS was cultured on medium supplemented with 0.5 μM TSA and 200 μM SB. The 0.5 μM TSA and 200 μM SB were added into Adi medium at the same time.

were markedly affected.⁵⁵ Histone demethylase may precisely regulate the expression of related genes in coordination with different interaction factors in different cellular microenvironments, and this regulation mechanism may be universal.⁵⁶ The mechanism of TSA and SB action on wheat regeneration efficiency needs further investigated.

4. Materials and methods

4.1. Plant materials

Three wheat genotypes, CB037, Fielder, and CS, were used as plant materials in this work. CB037 was kindly provided by Prof. Xiao Chen at the Institute of Crop Science of the Chinese

Figure 6. Statistical analysis of 0.5 μM TSA and 200 μM SB combination concentrations on embryonic calli frequency, differentiated calli frequency and plantlet regeneration frequency of CB037, Fielder, and CS. (a) Embryonic calli frequency. (b) Differentiated calli frequency. (c) Plantlet regeneration frequency. *, $p < .05$, **, $p < .01$.

Academy of Agricultural Sciences in Beijing, China. Fielder was kindly provided by Japan Tobacco Company. CS was provided by the laboratory of plant development molecular biology at the College of Life Sciences of the Shandong Agricultural University (SDAU) in Tai'an, Shandong province, China. The experimental materials were sowed in the 25th February and harvested in the 15th June of 2018 at the Experimental Station of SDAU.

4.2. Wheat mature embryo culture

Mature wheat seeds were selected and surface sterilized using 70% ethyl alcohol for 10 min and 25% sodium hypochlorite for 25 min. After washed three times with sterile water, seeds were kept in sterile water in darkness overnight. The next morning, wheat seeds were surface sterilized in 25% sodium hypochlorite for 15 min and then washed with sterile water for four times.

The scraped mature embryos from the sterilized seeds were cultured on Adi medium for 7 d and then were transferred to XCSX medium for 14 d in darkness at 25°C. Finally, embryonic calli were cultured on XCFH medium for 14 d at 25°C and a 16/8 h photoperiod. About 120 mature embryos were scraped for each treatment of three genotypes in three replications.⁹

4.3. Culture medium

Adi medium: MS basal medium (without MS vitamins) + 0.75 g/L MgCl₂ + 15.0 g/L mannitol + 15.0 g/L sorbitol + 12.0 g/L glucose + 0.5 g/L casein hydrolyte + 10 mg/L VB₁ + 1.0 mg/L VB₃ + 1.0 mg/L VB₆ + 2.0 mg/L glycine + 5.0 mg/L glutamine + 39.0 mg/L acetosyringone + 2.0 mg/L dicamba + 4.0 mg/L AgNO₃ + 40.0 mg/L cysteine + 100.0 mg/L ascorbic acid + 8 g/L agar, pH 5.8; XCSX medium: MS basal medium + MS vitamins + 3.0% sucrose + 2.0 mg/L dicamba + 4.0 mg/L AgNO₃ + 2.4 g/L phytagel, pH 5.8; XCFH medium: MS basal medium (without MS vitamins) + 10 mg/L VB₁ + 1.0 mg/L VB₃ + 1.0 mg/L VB₆ + 2.0 mg/L glycine + 5.0 mg/L glutamine + 2% sucrose + 0.2 mg/L IAA + 2.4 g/L phytagel, pH 5.8. The 0.5 μM and 2.5 μM TSA (V900931, Sigma-Aldrich, USA) were added into Adi medium respectively. The 200 μM and 1000 μM SB (B5887, Sigma-Aldrich, USA) were added into Adi medium respectively. Both 0.5 μM TSA and 200 μM SB combination were added into Adi medium. The same medium without TSA and SB addition was the control group. Media were autoclaved at 1.1 Mpa and 121°C for 16 min.

4.4. Statistical analysis

Number of embryonic calli, differentiated calli, and green bud were counted after culturing for 14 d on XCFH medium. Experimental data were analyzed with ANOVA using Microsoft Excel, version 2010 (Microsoft Corp. Redmond, Washington, USA).

Embryonic calli frequency (%) = the number of embryonic calli / the total number of mature embryos incubated × 100%.

Differentiated calli frequency (%) = the number of differentiated calli / the total number of mature embryos incubated × 100%.

Plantlet regeneration frequency (%) = the number of plantlets / the total number of mature embryos incubated × 100%.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

This research was financially supported by the Agricultural Variety Improvement Project of Shandong Province (2019LZGC010).

Author contributions

X.M.B. and X.G.L. planned and designed the study. L.D., X.H.L., and H.W. performed the experiments. X.M.B. and L.D. analyzed the data and wrote the manuscript. X.M.B., X.G., and X.G.L. revised and approved the final version of the manuscript. All authors have read and agreed to the published version of the manuscript.

References

- Bie XM, Wang K, She MY, Du LP, Zhang SX, Li JR, Gao X, Lin ZS, Ye XG. Combinational transformation of three wheat genes encoding fructan biosynthesis enzymes confers increased fructan content and tolerance to abiotic stresses in tobacco. *Plant Cell Rep.* 2012;31:2229–2238. doi:10.1007/s00299-012-1332-y.
- Gao XQ, Wang N, Wang XL, Zhang XS. Architecture of wheat inflorescence: insights from rice. *Trends Plant Sci.* 2019;24:802–809. doi:10.1016/j.tplants.2019.06.002.
- Zhao B, Wu TT, Ma SS, Jiang DJ, Bie XM, Sui N, Zhang XS, Wang F. *TaD27-B* gene controls the tiller number in hexaploid wheat. *Plant Biotechnol J.* 2020;18:513–525. doi:10.1111/pbi.13220.

4. Bie XM, Wang K, Liu C, Liu YW, Du LP, Mao XG, Ye XG. Effects of soil drought stress on plant regeneration efficiency and endogenous hormone levels of immature embryos in wheat (*Triticum aestivum* L.). *Pak J Bot.* 2017;49:1673–1679.
5. Zhang W, Yin MQ, Zhao P, Wang K, Du LP, Ye XG. Regeneration capacity evaluation of some largely popularized wheat varieties in China. *Zuo Wu Xue Bao.* 2018;44:208–217.
6. Shariatpanahi ME, Belogradova K, Hessamvaziri L, Heberle-Bors E, Touraev A. Efficient embryogenesis and regeneration in freshly isolated and cultured wheat (*Triticum aestivum* L.) microspores without stress pretreatment. *Plant Cell Rep.* 2006;25:1294–1299. doi:10.1007/s00299-006-0205-7.
7. Hakam N, Udupa SM, Rabha A, Ibriz M, Iraqi D. Efficient callus induction and plantlets regeneration in bread wheat using immature and mature embryos. *Int J Biotechnol Res.* 2015;3:1–9.
8. Zhao XY, Hong P, Wu JY, Chen XB, Ye XG, Pan YY, Wang J, Zhang XS. The ta-miR408-mediated control of *TaTOC1* gene transcription is required for the regulation of heading time in wheat. *Plant Physiol.* 2016;170:1578–1594. doi:10.1104/pp.15.01216.
9. Dong LH, Yao FQ, Zhan Y, Li XG, Bie XM. Optimization of callus induction and regeneration system of mature embryo in spring wheats. *Mol Plant Breed.* 2020;18:1244–1249.
10. Ye XG, Xu HJ, Du LP, He GY, Wang K, Lin ZS. Establishment and application of large-scale transformation systems in wheat. *Zhongguo Nong Ye Ke Xue.* 2014;47:4155–4171.
11. Holliday R. The inheritance of epigenetic defects. *Science.* 1987;238:163–170. doi:10.1126/science.3310230.
12. Holliday R. Epigenetics: an overview. *Dev Genet.* 1994;15:453–457. doi:10.1002/dvg.1020150602.
13. Ronemus MJ, Galbiati M, Ticknor C, Chen J, Dellaporta SL. Demethylation induced developmental pleiotropy in *Arabidopsis*. *Science.* 1996;27:654–657.
14. Jones PA, Takai D. The role of DNA methylation in mammalian epigenetics. *Science.* 2001;293:1068–1070. doi:10.1126/science.1063852.
15. Finnin MS, Donigian JR, Cohen A, Richon VM, Rifkind RA, Marks PA, Breslow R, Pavletich NP. Structures of a histone deacetylase homologue bound to the TSA and SAHA inhibitors. *Nature.* 1999;401:188–193. doi:10.1038/43710.
16. Cousens LS, Gallwitz D, Alberts BM. Different accessibilities in chromatin to histone acetylase. *J Biol Chem.* 1979;254:1716–1723.
17. Marks PA, Richon VM, Rifkind RA. Histone deacetylase inhibitors: inducers of differentiation or apoptosis of transformed cells. *J Natl Cancer Inst.* 2020;92:1210–1216. doi:10.1093/jnci/92.15.1210.
18. Chang S, Pikaard CS. Transcript profiling in *Arabidopsis* reveals complex responses to global inhibition of DNA methylation and histone deacetylation. *J Biol Chem.* 2005;280:796–804. doi:10.1074/jbc.M409053200.
19. Yang F, Zhang L, Li J, Huang J, Wen R, Ma L, Zhou D, Li L. Trichostatin A and 5-azacytidine both cause an increase in global histone H4 acetylation and a decrease in global DNA and H3K9 methylation during mitosis in maize. *BMC Plant Biol.* 2010;10:178. doi:10.1186/1471-2229-10-178.
20. Vorontsova M, Shaw P, Reader S, Moore G. Effect of 5-azacytidine and trichostatin A on somatic centromere association in wheat. *Genome.* 2004;47:399–403.
21. Su YH, Su YX, Liu YG, Zhang XS. Absciscic acid is required for somatic embryo initiation through mediating spatial auxin response in *Arabidopsis*. *Plant Growth Regul.* 2012;69:167–176. doi:10.1007/s10725-012-9759-2.
22. Bai B, Su YH, Yuan J, Zhang XS. Induction of somatic embryos in *Arabidopsis* requires local auxin biosynthesis mediated by the down-regulation of ethylene biosynthesis. *Mol Plant.* 2013;6:1247–1260.
23. Cheng TJ, Wang L, Sun W, Zhang Y, Zhou C, Su YH, Li W, Sun TT, Zhao XY, Li XG, et al. Pattern of auxin and cytokinin responses for shoot meristem induction results from the regulation of cytokinin biosynthesis by AUXIN RESPONSE FACTOR3. *Plant Physiol.* 2013;161:240–251. doi:10.1104/pp.112.203166.
24. Su YH, Liu YB, Bai B, Zhang XS. Establishment of embryonic shoot-root axis is involved in auxin and cytokinin response during *Arabidopsis* somatic embryogenesis. *Front Plant Sci.* 2015;5:792. doi:10.3389/fpls.2014.00792.
25. Su YH, Liu YB, Zhou C, Li XM, Zhang XS. The microRNA167 controls somatic embryogenesis in *Arabidopsis* through regulating its target genes *ARF6* and *ARF8*. *Plant Cell Tissue Organ Cult.* 2016;124:405–417. doi:10.1007/s11240-015-0903-3.
26. Meng WJ, Cheng ZJ, Sang YL, Zhang MM, Rong XF, Tang YY, Zhang XS. Type-B ARABIDOPSIS RESPONSE REGULATORS specify the shoot stem cell niche by dual regulation of *WUSCHEL*. *Plant Cell.* 2017;29:1357–1372. doi:10.1105/tpc.16.00640.
27. Tang LP, Li XM, Dong YX, Zhang XS, Su YH. Microfilament depolymerization is a pre-requisite for stem cell formation during *in vitro* shoot regeneration in *Arabidopsis*. *Front Plant Sci.* 2017;8:158. doi:10.3389/fpls.2017.00158.
28. Zhang QQ, Li Y, Fu ZY, Liu XB, Yuan K, Fang Y, Liu Y, Li G, Zhang XS, Chong K, et al. Intact *Arabidopsis* RPB1 functions in stem cell niches maintenance and cell cycling control. *Plant J.* 2018;95:150–167. doi:10.1111/tpj.13939.
29. Li W, Liu H, Cheng ZJ, Su YH, Han HN, Zhang Y, Zhang XS. DNA methylation and histone modifications regulate *de novo* shoot regeneration in *Arabidopsis* by modulating *WUSCHEL* expression and auxin signaling. *PLoS Genet.* 2011;7:e1002243. doi:10.1371/journal.pgen.1002243.
30. Ikeuchi M, Iwase A, Sugimoto K. Control of plant cell differentiation by histone modification and DNA methylation. *Curr Opin Plant Biol.* 2015;28:60–67. doi:10.1016/j.pbi.2015.09.004.
31. Sang YL, Cheng ZJ, Zhang XS. iPSCs: A comparison between animals and plants. *Trends Plant Sci.* 2018;23:660–666. doi:10.1016/j.tplants.2018.05.008.
32. Sang YL, Cheng ZJ, Zhang XS. Plant stem cells and *de novo* organogenesis. *New Phytol.* 2018;218:1334–1339. doi:10.1111/nph.15106.
33. Rosa S, Ntoukakis V, Ohmido N, Pendle A, Abranches R, Shaw P. Cell differentiation and development in *Arabidopsis* are associated with changes in histone dynamics at the single-cell level. *Plant Cell.* 2014;26:4821–4833. doi:10.1105/tpc.114.133793.
34. Li H, Soriano M, Cordewener J, Muino JM, Riksen T, Fukuoka H, Angenent GC, Boutilier K. The histone deacetylase inhibitor trichostatin A promotes totipotency in the male gametophyte. *Plant Cell.* 2014;26:195–209. doi:10.1105/tpc.113.116491.
35. Uddenberg D, Valladares S, Abrahamsson M, Sundström JF, Sundås-Larsson A, von Arnold S. Embryogenic potential and expression of embryogenesis-related genes in conifers are affected by treatment with a histone deacetylase inhibitor. *Planta.* 2011;234:527–539. doi:10.1007/s00425-011-1418-8.
36. Chen G, Ran X, Li B, Li Y, He D, Huang B, Fu S, Liu J, Wang W. Sodium butyrate inhibits inflammation and maintains epithelium barrier integrity in a TNBS-induced inflammatory bowel disease mice model. *EBioMedicine.* 2018;30:317–325. doi:10.1016/j.ebiom.2018.03.030.
37. Schwarz A, Bruhs A, Schwarz T. The short-chain fatty acid sodium butyrate functions as a regulator of the skin immune system. *J Invest Dermatol.* 2017;137:855–864. doi:10.1016/j.jid.2016.11.014.
38. Zhang J, Yi M, Zha L, Chen S, Li Z, Li C, Gong M, Deng H, Chu X, Chen J, et al. Sodium butyrate induces endoplasmic reticulum stress and autophagy in colorectal cells: implications for apoptosis. *PLoS One.* 2016;11:e0147218. doi:10.1371/journal.pone.0147218.
39. Zhou D, Pan Q, Xin FZ, Zhang RN, He CX, Chen GY, Liu C, Chen YW, Fan JG. Sodium butyrate attenuates high-fat diet-induced steatohepatitis in mice by improving gut microbiota and gastrointestinal barrier. *World J Gastroenterol.* 2017;23:60–75. doi:10.3748/wjg.v23.i1.60.
40. Kowalski ZM, Górka P, Flaga J, Barteczko A, Burakowska K, Oprządek J, Zabielski R. Effect of microencapsulated sodium butyrate in the close-up diet on performance of dairy cows in the early

- lactation period. *J Dairy Sci.* **2015**;98:3284–3291. doi:[10.3168/jds.2014-8688](https://doi.org/10.3168/jds.2014-8688).
41. Jaworska J, Zalewska T, Sypecka J, Ziemka-Nalecz M. Effect of the HDAC inhibitor, sodium butyrate, on neurogenesis in a rat model of neonatal hypoxia-ischemia: potential mechanism of action. *Mol Neurobiol.* **2019**;56:6341–6370.
 42. Wang CC, Wu H, Lin FH, Gong R, Xie F, Peng Y, Feng J, Hu CH. Sodium butyrate enhances intestinal integrity, inhibits mast cell activation, inflammatory mediator production and JNK signaling pathway in weaned pigs. *Innate Immun.* **2018**;24:40–46. doi:[10.1177/1753425917741970](https://doi.org/10.1177/1753425917741970).
 43. Zhang WH, Gao F, Zhu QF, Li C, Jiang Y, Dai SF, Zhou GH. Dietary sodium butyrate alleviates the oxidative stress induced by corticosterone exposure and improves meat quality in broiler chickens. *J Poult Sci.* **2011**;90:2592–2599. doi:[10.3382/ps.2011-01446](https://doi.org/10.3382/ps.2011-01446).
 44. Liang H, Fang G. Effect of sodium butyrate preculture on formation of green plantlets from rice anthers in vitro. *Zhejiang Da Xue Xue Bao Li Xue Ban.* **1986**;13:475–481.
 45. Zhao XY, Liang GT, Li XG, Zhang XS. Hormones regulate in vitro organ regeneration from leaf-derived explants in *Arabidopsis*. *Am J Plant Sci.* **2014**;5:3535–3550. doi:[10.4236/ajps.2014.524370](https://doi.org/10.4236/ajps.2014.524370).
 46. Lee K, Park OS, Jung SJ, Seo PJ. Histone deacetylation-mediated cellular dedifferentiation in *Arabidopsis*. *J Plant Physiol.* **2016**;191:95–100.
 47. Liu H, Ma X, Han HN, Hao YJ, Zhang XS. AtPRMT5 regulates shoot regeneration through mediating histone H4R3 dimethylation on *KRPs* and pre-mRNA splicing of *RKP* in *Arabidopsis*. *Mol Plant.* **2016**;9:1634–1646. doi:[10.1016/j.molp.2016.10.010](https://doi.org/10.1016/j.molp.2016.10.010).
 48. Pandey P, Daghma DS, Houben A, Kumlehn J, Melzer M, Rutten T. Dynamics of post-translationally modified histones during barley pollen embryogenesis in the presence or absence of the epi-drug trichostatin A. *Plant Reprod.* **2017**;30:95–105. doi:[10.1007/s00497-017-0302-5](https://doi.org/10.1007/s00497-017-0302-5).
 49. Jiang F, Ryabova D, Diedhiou J, Hucl P, Randhawa H, Marillia EF, Foroud NA, Eudes F, Kathiria P. Trichostatin A increases embryo and green plant regeneration in wheat. *Plant Cell Rep.* **2017**;36:1701–1706.
 50. Weiste C, The D-LW. *Arabidopsis* transcription factor bZIP11 activates auxin-mediated transcription by recruiting the histone acetylation machinery. *Nat Commun.* **2014**;5:3883. doi:[10.1038/ncomms4883](https://doi.org/10.1038/ncomms4883).
 51. Su YH, Liu YB, Zhang XS. Auxin-cytokinin interaction regulates meristem development. *Mol Plant.* **2011**;4:616–625. doi:[10.1093/mp/ssr007](https://doi.org/10.1093/mp/ssr007).
 52. Su YH, Zhang XS. The Hormonal control of regeneration in plants. *Curr Top Dev Biol.* **2014**;108:35–69.
 53. Zhang H, Zhang TT, Liu H, Shi DY, Wang M, Bie XM, Li XG, Zhang XS. Thioredoxin-mediated ROS homeostasis explains natural variation in plant regeneration. *Plant Physiol.* **2018**;176:2231–2250. doi:[10.1104/pp.17.00633](https://doi.org/10.1104/pp.17.00633).
 54. Wójcikowska B, Malwina B, Morończyk J, Wójcik AM, Nodzyński T, Jagna K, Gaj MD. Trichostatin A triggers an embryogenic transition in *Arabidopsis* explants via an auxin-related pathway. *Front Plant Sci.* **2018**;9:1353. doi:[10.3389/fpls.2018.01353](https://doi.org/10.3389/fpls.2018.01353).
 55. Lochmanová G, Ihnatová I, Kuchaříková H, Brabencová S, Zachová D, Fajkus J, Zdráhal Z, Fojtová M. Different modes of action of genetic and chemical downregulation of histone deacetylases with respect to plant development and histone modifications. *Int J Mol Sci.* **2019**;20:5093.
 56. Wang J, Yu CY, Zhang SB, Ye JY, Dai H, Wang HK, Huang JY, Cao XF, Ma JB, Ma H, et al. Cell-type-dependent histone demethylase specificity promotes meiotic chromosome condensation in *Arabidopsis*. *Nat Plants.* **2020**. doi:[10.1038/s41477-020-0697-0](https://doi.org/10.1038/s41477-020-0697-0).