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**Research article**

**Comparative acetylome analysis of wild-type and fuzzless-lintless mutant ovules of upland cotton (*Gossypium hirsutum* Cv. Xu142) unveils differential protein acetylation may regulate fiber development**

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**Highlights**

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This study is the first report of comparative acetylome analysis in cotton to uncover the role of acetylation in fiber development event and provides key insights for further research in the mechanism of cotton fibre development.

* •

First attempt to compare the lysine-acetylation [proteome](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/proteome) (acetylome) of upland cotton ovules in the early fiber development stage by using wild-type (Xu142) as well as its fuzzless-lintless mutant (Xu142M) with an aim to identify the role of protein acetylation in the fiber development.

* •

A total of 1696 proteins with 2754 acetylation sites were identified, of which 1358 proteins with 2104 acetylation sites were quantified by using Tandem Mass Tag (TMT) labeling and acetylation enrichment coupled with high-resolution Liquid Chromatography-Mass Spectrometry (LC-MS/MS).

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To our knowledge, this is the first report of comparative acetylome analysis in cotton to uncover the role of acetylation in fiber development event and provides key insights for further research in the mechanism of cotton fibre development.

**Abstract**

Protein [acetylation](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/acetylation" \o "Learn more about acetylation from ScienceDirect's AI-generated Topic Pages) (KAC) is a significant post-translational modification, which plays an essential role in the regulation of [growth and development](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/growth-development-and-aging). Unfortunately, related studies are inadequately available in [angiosperms](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/angiosperm), and to date, there is no report providing insight on the role of protein [acetylation](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/acetylation" \o "Learn more about acetylation from ScienceDirect's AI-generated Topic Pages) in cotton fiber development. Therefore, we first compared the lysine-acetylation [proteome](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/proteome) (acetylome) of [upland cotton](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/gossypium-hirsutum) ovules in the early fiber development stages by using wild-type as well as its fuzzless-lintless mutant to identify the role of KAC in the fiber development. A total of 1696 proteins with 2754 acetylation sites identified with the different levels of acetylation belonging to separate subcellular compartments suggesting a large number of proteins differentially acetylated in two cotton cultivars. About 80% of the sites were predicted to localize in the cytoplasm, chloroplast, and mitochondria. Seventeen significantly enriched acetylation motifs were identified. [Serine](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/serine) and [threonine](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/threonine" \o "Learn more about threonine from ScienceDirect's AI-generated Topic Pages) and cysteine located downstream and upstream to KAC sites. KEGG pathway enrichment analysis indicated [oxidative phosphorylation](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/oxidative-phosphorylation), fatty acid, ribosome and protein, and folate [biosynthesis](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/anabolism) pathways enriched significantly. To our knowledge, this is the first report of comparative [acetylome analysis](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/proteome-analysis" \o "Learn more about acetylome analysis from ScienceDirect's AI-generated Topic Pages) to compare the wild-type as well as its fuzzless-lintless mutant acetylome data to identify the differentially acetylated proteins, which may play a significant role in cotton fiber development.

* [Previous article in issue](https://www.sciencedirect.com/science/article/pii/S0981942820300875)
* [Next article in issue](https://www.sciencedirect.com/science/article/pii/S0981942820300838)

**Keywords**

Cotton fiber

Acetylome

LC-MS/MS

TMT-Labeling

**1. Introduction**

Cotton is one of the most important cash crops and grown worldwide, primarily due to its fiber1−4. The fibers are elongation product of the ovule outer epidermal cells and are the longest as well as the fastest-growing single cells in plants ([Wakelyn et al., 2010](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib86); [Ruan et al., 2001](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib73); [Lee et al., 2007a](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib43)). The fiber development process completed in unambiguous with super-impressive steps and is known to produce before or on the day of [anthesis](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/anthesis" \o "Learn more about anthesis from ScienceDirect's AI-generated Topic Pages) from ovule epidermal cells ([Wakelyn et al., 2010](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib86); [Ruan et al., 2001](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib73); [Lee et al., 2007a](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib43)). The Xuzhou142fl (Xu142M) is a fuzzless-lintless (fl) mutant of [upland cotton](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/gossypium-hirsutum) (*[Gossypium hirsutum](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/gossypium-hirsutum" \o "Learn more about Gossypium hirsutum from ScienceDirect's AI-generated Topic Pages)* Cv. Xu142) unable to develop fibers ([Nadarajan and Rangasamy, 1988](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib63); [Du et al., 2001](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib12)). Hence, the mutants with impaired [growth and development](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/growth-development-and-aging) of fiber could be used to decipher the events of fiber development. Entirely a few attempts have been employed to unravel the fiber development process to date includes characterization of mutants to identify the genes regulating the developmental process and their [position](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/position) in the genome ([Du et al., 2001](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib12)).

Furthermore, there are reports that endogenous hormones ([Beasley and Ting, 1974](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib3); [Jianyan et al., 2019](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib34); [Xiao et al., 2019](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib100)), numerous genes ([Samuel-Yang et al., 2006](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib76); [Lee et al., 2006](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib42), [2007b](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib44); [Yoo and Wendel, 2014](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib109); [Huaitong et al., 2018](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib31); [Hande et al., 2017](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib24); [Taliercio and Boykin, 2007](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib82)), and small [RNAs](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/rna), as well as micro-RNA20-21, also play a role in fiber development. Again, gel-based [proteomics](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/proteomics) is also employed to decode the fiber development process in the [cotton plant](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/cotton) by using ovules of Xu142 mutant and wild-type cultivars ([Liu et al., 2012](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib50); [Du et al., 2013](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib13)). Vividly these results indicated that fiber initiation, differentiation, and development is a complicated process and a series of well-orchestrated changes involved in the regulation of gene expression. Unfortunately, the exact mechanism and the regulatory events during the fiber development yet unexplored.

Post-translational modifications (PTMs) are known to play a very crucial role in the various growth and developmental processes ([Mann and Jensen, 2003](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib55); [Jensen, 2004](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib33); [Walsh et al., 2005](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib89); [Witze et al., 2005](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib95); [Meinnel and Giglione, 2008](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib58); [Martin and Zhang, 2007](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib56); [Ruthenburg et al., 2007](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib74)). Lysine [acetylation](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/acetylation" \o "Learn more about acetylation from ScienceDirect's AI-generated Topic Pages) (Kac/LysAc) is a ubiquitous, dynamic, and highly conserved PTM of both [histones](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/histone" \o "Learn more about histones from ScienceDirect's AI-generated Topic Pages) and non-histone proteins, which plays a significant role in various [biological processes](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/biological-phenomena-and-functions-concerning-the-entire-organism) ([Choudhary et al., 2009](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib7), [2014](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib8); [Weinert et al., 2011a](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib92), [2014](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib94); [Liu et al., 2014](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib51)). Acetylation is known to regulated by two classes of [enzymes](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/enzyme): lysine acetyltransferases (KATs) and histone deacetylases (HDACs) ([Mann and Jensen, 2003](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib55); [Jensen, 2004](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib33)). It has been reported that lysine acetyltransferases transfer acetyl group of acetyl-coenzyme A to a lysine residue of a core histone protein to make [chromatin structures](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/chromatin-structure) to [regulatory proteins](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/regulatory-protein) and promotes transcription ([Walsh et al., 2005](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib89); [Witze et al., 2005](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib95); [Meinnel and Giglione, 2008](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib58); [Martin and Zhang, 2007](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib56); [Ruthenburg et al., 2007](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib74)). However, in addition to [histones](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/histone" \o "Learn more about histones from ScienceDirect's AI-generated Topic Pages), nonhistone proteins present in subcellular [organelles](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/organelle), including cytoplasm, also undergoes [acetylation](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/acetylation" \o "Learn more about acetylation from ScienceDirect's AI-generated Topic Pages) for various cellular functions ([Choudhary et al., 2009](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib7)). With the help of a growing number of large-scale acetylation studies, it has become evident that lysine acetylation is ubiquitous as well as essential posttranslational modification ([Choudhary et al., 2014](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib8); [Weinert et al., 2011a](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib92), [2014](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib94); [Liu et al., 2011](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib49), [2014](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib51); [Mischerikow and Heck, 2011a](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib60); [Lundby et al., 2012](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib53); [Henriksen et al., 2012](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib29); [Hebert et al., 2013](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib27); [Uhrig et al., 2017](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib84); [Haberland et al., 2009](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib23); [Roth et al., 2001](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib72); [Kouzarides, 2007](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib40); [Chen and Tian, 2007](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib6); [Wu et al., 2011a](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib96), [2013a](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib98)). Therefore, the examination of lysine acetylation on non-histone proteins has gained a prominent role in PTM analysis. Identification of lysine acetylation and its role in transcriptional regulation of genes have been studied extensively ([Wu et al., 2011a](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib96); [Finkemeier et al., 2011a](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib18); [Duffy et al., 2012](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib14); [Zhao et al., 2010](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib115); [Nallamilli et al., 2014](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib64)).

Furthermore, there are several reports on the role of [protein acetylation](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/protein-acetylation) in plant response to stresses ([Kim et al., 2006a](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib36); [Kim and Yang, 2011](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib35); [Fang et al., 2015](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib16); [Weinert et al., 2011b](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib93); [Bharathi et al., 2013](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib5)). Besides this, there are reports that protein acetylation plays a very crucial role in the growth and development process in animals and fungi ([Wu et al., 2011a](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib96); [Rardin et al., 2013](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib70); [Pesaresi et al., 2003](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib67); [Linster et al., 2015](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib48); [Xu et al., 2015](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib105)). However, there are only a few studies providing insights into the role of protein acetylation in plant growth and developmental processes ([Bharathi et al., 2013](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib5); [Xu et al., 2016](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib106); [Walley et al., 2018a](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib87); [Glozak et al., 2005](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib22); [You et al., 2012](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib110)).

The emergence of lysine acetylation-specific antibodies significantly improved the identification of acetylation sites ([Du et al., 2013](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib13); [Mann and Jensen, 2003](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib55); [Jensen, 2004](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib33); [Walsh et al., 2005](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib89); [Witze et al., 2005](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib95); [Meinnel and Giglione, 2008](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib58); [Martin and Zhang, 2007](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib56); [Ruthenburg et al., 2007](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib74); [Choudhary et al., 2009](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib7), [2014](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib8); [Weinert et al., 2011a](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib92), [2014](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib94)). Recently, [TMT](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/tandem-mass-tag) labeling and acetylation enrichment technology coupled with high-resolution mass spectrometry emerged as a powerful tool ([Pang and Rennert, 2013](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib66)). This quantitative [proteomics](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/proteomics) approach has not only facilitated the characterization of lysine acetylation but also has been extensively applied for global acetylation [analysis of protein](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/protein-characterization) form all domains of life ([Tapias and Wang, 2017](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib83); [Feng et al., 2016](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib17); [Li et al., 2018a](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib45); [Baeza et al., 2014](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib2); [Xiong et al., 2016a](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib103); [Svinkina et al., 2015](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib81); [Liu et al., 2016](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib52); [Zhu et al., 2016](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib117); [Wang et al., 2017](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib91); [Walley et al., 2018b](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib88); [Xu et al., 2017](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib107); [He et al., 2016](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib26)). The [acetylome](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/proteome" \o "Learn more about acetylome from ScienceDirect's AI-generated Topic Pages) analysis not only unveiled the crucial role of involvement Kac in [epigenetic](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/epigenetics) regulation of gene expression but also suggested its role in diverse cellular as well as metabolic processes ([Chen and Tian, 2007](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib6); [Zhang et al., 2016a](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib113)). In plants like [strawberry](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/strawberries) ([Finkemeier et al., 2011a](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib18)), wheat ([Schilling et al., 2012](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib77)), potato ([Corbett and Cristea, 2017](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib9); [Rauniyar et al., 2013](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib71)), [Vitis](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/vitis" \o "Learn more about Vitis from ScienceDirect's AI-generated Topic Pages) ([Marx et al., 2016](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib57)), *[Glycine max](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/glycine-max" \o "Learn more about Glycine max from ScienceDirect's AI-generated Topic Pages)* ([Xing and Poirier, 2012a](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib101)), *[Pisum](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/pisum" \o "Learn more about Pisum from ScienceDirect's AI-generated Topic Pages) sativum* ([Li et al., 2018b](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib46)), [Arabidopsis](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/arabidopsis) ([Zhang et al., 2016b](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib114); [Xing and Poirier, 2012b](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib102); [Salvato et al., 2014](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib75)), [Brachypodium](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/brachypodium" \o "Learn more about Brachypodium from ScienceDirect's AI-generated Topic Pages) ([Melo-Braga et al., 2012](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib59)), *Oryzae*45, 90−91. Lysine [acetylome](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/proteome" \o "Learn more about acetylome from ScienceDirect's AI-generated Topic Pages) has also been studied to explore the role of protein acetylation.

Nevertheless, compassion with thousands of lysine acetylation studies in mammals, fungi, and bacteria, the existing number of lysine acetylation studies in plants are limited. Significant challenges remain to elaborate lysine acetylome in plants, hindering the deep understanding of lysine acetylation studies in plant science. Furthermore, it warrants mentioning that this technique has never been applied to cotton and also to decode the role of lysine acetylation in the fiber development process, besides its enormous economic importance. Recently, [transcriptomic](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/transcriptomics" \o "Learn more about transcriptomic from ScienceDirect's AI-generated Topic Pages), proteomic, and [phosphoproteomic](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/phosphoproteomics" \o "Learn more about phosphoproteomic from ScienceDirect's AI-generated Topic Pages) approaches have been applied to decode the fiber development process, and it has been reported that the fiber development process regulated by IAA and [flavonoids](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/flavonoid" \o "Learn more about flavonoids from ScienceDirect's AI-generated Topic Pages) ([Finkemeier et al., 2011b](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib19)). Unfortunately, still, there is a significant gap between the mechanism of fiber development and the role of lysine acetylation during the process.

Taking resource to the information mentioned above, we here first applied the comparative lysine-acetylation (Kac) proteome (acetylome) analysis to identify the differentially acetylated proteins from the ovules of the Xu 142 wild-type with its fuzzless-lintless (fl) mutant (Xu142M) of upland [cotton plant](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/gossypium) (*Gossypium hirsutum* cv. Xu142) to decode the role of lysine-acetylation in fiber development. By using [TMT](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/tandem-mass-tag) labeling and acetylation enrichment technology coupled with high-resolution liquid chromatography-mass spectrometry (LC-MS/MS), a total of 2754 acetylation sites were identified in 1696 proteins, of which 2104 sites of 1358 proteins accommodated with quantitative information. Results of our study unveiled that the 101 and 250 acetylation sites belonging to 92 and 210 proteins respectively showed an increase in their abundance in Xu142M and Xu142 on 0 DPA and -1DPA. These acetylation sites show a time-dependent abundance dynamics and indicating that these acetylation sites belong to a considerable sum of non-histone proteins of various regulatory, metabolic, and signaling, transporters, categories. The increased abundance of acetylation event in these pathway proteins, on −1 DPA, and then decreased in abundance on 0 DPA from Xu142 suggestive of the role of acetylation in the fiber development event. To the best of our knowledge, it is the first comparative investigation of acetylome study in cotton and also the first most extensive dataset of comparative acetylome in plants to date. This study not only widens the understanding of comparative acetylomics but also opens the doors of lysine acetylation studies in plant developmental biology.

**2. Materials and methods**

**2.1. Plant materials, growth conditions, sample collection, and ovule isolation**

The seeds of cotton cultivar *Xu142* and it's natural isogenic fl mutant (*Xu142M*) were planted in the experimental field. The flower buds and flowers in the full-bloom stage were labeled on consecutive days. The ovules of −1 DPA (day post-anthesis, hereafter), 0 DPA, 1 DPA, and 3 DPA were collected from the labeled flowers. The ovules were then frozen in liquid nitrogen and stored at −80 °C for further experiments. Three biological replicates were obtained by using 60 plants grown at a steady growth stage. The [Electron microscopy](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/electron-microscopy) images of −1 DPA, 0 DPA, 1 DPA, and 3 DPA ovules were collected as described previously 93 by using Quanta-250 [Scanning Electron Microscope](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/scanning-electron-microscope) (Thermo Fisher Scientific).

**2.2. Protein extraction**

The isolated ovules were taken out from −80 °C, and a 500 mg of tissue sample was weighed into a precooled liquid nitrogen mortar. The samples were crushed thoroughly in liquid nitrogen into [powder](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/powder) form and then transferred to a 5 mL tube. For extraction of protein the samples were sonicated three times on ice using a high-intensity ultrasonic processor (Scientz, China) in 4 vol of [lysis buffer](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/lysis-buffer" \o "Learn more about lysis buffer from ScienceDirect's AI-generated Topic Pages) (1% Triton X-100, 10 mM [dithiothreitol](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/dithiothreitol" \o "Learn more about dithiothreitol from ScienceDirect's AI-generated Topic Pages), 1% [protease inhibitor](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/protease-inhibitor), 3 μM [Trichostatin A](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/trichostatin-a" \o "Learn more about Trichostatin A from ScienceDirect's AI-generated Topic Pages) (TSA), 50 mM [Nicotinamide](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/nicotinamide" \o "Learn more about Nicotinamide from ScienceDirect's AI-generated Topic Pages) (NAM) and 2 mM EDTA). The remaining cell debris from the samples was removed by [centrifugation](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/centrifugation) at 20,000 g at 4 °C for 10 min. The supernatant was added to a final concentration of 20% chilled TCA solution (stored at −20 °C) and placed at 4 °C for two h. After this, the samples were centrifuged at 12,000 g 4 °C for 5 min. The precipitate was washed five times with pre-cold acetone (4 °C) by discarding the supernatant. The protein pellet was reconstituted in 8 M urea, and the protein concentration was determined with the BCA [Protein Assay](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/protein-assay) Kit (ThermoFisher, USA) according to the manufacturer's instructions.

**2.3. Western blot analysis**

We performed the western-blot analysis using an antibody against acetyl-Lysine residues ([Chen and Tian, 2007](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib6)), to gain an initial overview of the extent of lysine [acetylation](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/acetylation" \o "Learn more about acetylation from ScienceDirect's AI-generated Topic Pages) on ovule isolated proteins of the [cotton plant](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/cotton) during the fiber development process. SDS-PAGE separated proteins, transferred to a PVDF (Millipore) membrane, and probed using acetylated lysine antibody (PTM Biolabs, Hangzhou, China) in a 1:1000 dilution ([Finkemeier et al., 2011a](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib18)). Secondary anti-horseradish peroxidase antibody (HuaAn Biotechnology, Hangzhou, China) was used in a 1:10,000 dilution.

**2.4. Tryptic digestion and TMT-Labeling**

For tryptic digestion, 10 mg protein from each sample was reduced with 5 mM [dithiothreitol](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/dithiothreitol" \o "Learn more about dithiothreitol from ScienceDirect's AI-generated Topic Pages) (Merck, Germany) for 30 min at 56 °C and alkylated with 11 mM [iodoacetamide](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/iodoacetamide" \o "Learn more about iodoacetamide from ScienceDirect's AI-generated Topic Pages) (Merck, Germany) for 15 min at room temperature in the dark. The protein samples were then diluted by adding 100 mM tri-ethyl-ammonium bicarbonate (TEAB) (Sigma-Aldrich, Germany) to urea (Sigma-Aldrich, Germany) concentration less than 2 M. Finally, the trypsin was added at 1:50 trypsin-to-protein mass ratio for the first digestion overnight and 1:100 trypsin-to-protein mass ratios for a second 4 h-digestion.

Trypsin-digested peptides were desalted by using the Strata X C18 SPE column (Phenomenex, UK) and vacuum-dried. ~6 mg of the peptide from each sample was reconstituted in 0.5 M TEAB, and TMT-labeling was performed as per manufacturer's protocol by using Tandem Mass Tag (TMT) kit (Thermofisher, USA). One unit of [TMT](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/tandem-mass-tag) reagent was thawed and reconstituted in acetonitrile. The peptide mixtures were incubated with the reagent at room temperature for a period of 2 h. After the labeling, the peptides were pooled out desalted and vacuum dried by centrifugation. The Sample marker information is available in the [Supplementary Data File 1](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "appsec1).

**2.5. Peptide fractionation and enrichment**

4 mg Peptides from each sample were fractionated by high pH Reverse-Phase-High-Performance Liquid Phase Chromatography (RP-HPLC) by using thermobetasil C18 (5 μm particle size, 10 mm ID, 250 mm length) column (Thermo Scientific, USA). The RP-HPLC operation was as follows: Peptide grading gradient of 8%–32% acetonitrile, pH 9.0, and time separation of 60 min, then the peptide were combined into four components, the collected elements were vacuum freeze-dried for subsequent operation. Peptide enrichment analysis was done by using PTM104 antibody resin as per [Pan et al. (2014)](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib65) and [Zhu et al. (2016)](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib117). In brief, for peptide enrichment analysis the 2 mg of peptides from each sample were lysed in [immunoprecipitation](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/immunoprecipitation" \o "Learn more about immunoprecipitation from ScienceDirect's AI-generated Topic Pages) (IP) buffer (100 mM NaCl, 1 mM EDTA, 50 mM Tris-HCl, 0.5% NP-40, pH 8.0) and the supernatant was transferred to a pre-washed antibody resin (Catalog No. PTM104, Hangzhou Jingjie Biotechnology, China). The resin transferred peptides were then placed on a rotary shaker at 4 °C for a gentle shaking and incubated overnight. After the incubation, the resin was washed four times with IP buffer solution and twice with deionized water. Finally, for the elution of peptides from the resin, a 0.1% trifluoroacetic acid (TFA, Sigma-Aldrich, Germany) was used as an eluant from the resin-bound peptide. The eluate was co-eluted three times. The eluate was collected and vacuum freeze-dried. The peptides so obtained drain dries according to RP-HPLC instructions desalination, vacuum freeze-dry extraction for [liquid chromatography](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/liquid-chromatography) analysis.

**2.6. LC-MS/MS analysis**

The LC-MA/MS analysis was carried out as per Xiong et al. (2016). In brief, the tryptic digested peptides were separated by liquid chromatographic [mobile phase](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/mobile-phase-composition) A by using EASY-nLC 1000 Ultra-High-Performance Liquid Chromatography (UHPLC) (15-cm length, 75 μm i. d.). An aqueous solution containing 0.1% formic acid and 2% acetonitrile was used as mobile phase A and an organic phase containing 0.1% formic acid and 90% acetonitrile was used as mobile phase B. The liquid phase gradient was set as follows 0–26 min, 7–24 %B, 26–34 min, 24–38% B, 34–37 min, 38–80%B, 37–40 min, 80% with a [flow rate](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/flow-rate) of 350 nL/min. UHPLC separated peptides were injected into the Nanospray Ionisation (NSI) ion source for ionisation and analyzed by [Tandem Mass Spectrometry](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/tandem-mass-spectrometry) (MS) in Orbitrap FusionTM TribridTM (Thermo) coupled online to the UHPLC. The voltage of the ion source was set to 2.0 kV. Both the parent ion as well as secondary fragments of peptides were detected and analyzed by using High-Resolution (HR) Orbitrap. The first [MS](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/mass-spectrometry) scan range was set to 350–1550 m/z with the orbitrap scan resolution of 60,000, while the second [MS](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/mass-spectrometry) scan range is was fixed at 100 m/z with the orbitrap scan resolution of 15,000. Data-Dependent Scanning (DDA) program was used to select the precursor of the first 20 peptides of the highest signal intensity after allowing the one scan into the HCD collision cell in sequence and cleaves with 35% of the fragmentation energy, and in the same order, for mass spectrometry analysis, the data acquisition mode was used. The following parameters were set: the AGC was set to 5E4, the signal threshold to 5000 ions/s, the maximum injection time to 200 ms, and the tandem mass spectrometry scan to 15 s for dynamic exclusion repeat scanning to improve the efficiency of mass spectrometry. Mass spectrometry [quality control](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/quality-control) testing of MS data results was first examined by a mass error of all identified peptides (Panel A). The mass error was centered on 0 and centered in the range of less than 10 ppm; explain the quality error to meet out the requirements. Second, most of the peptide fragments were distributed between 8 and 20 [amino acid](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/amino-acid) residues (Panel B). This conforms to the law of [pancreatin](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/pancreatin" \o "Learn more about pancreatin from ScienceDirect's AI-generated Topic Pages) digestion peptides and indicating that the sample preparations meet the standard. Wilcoxon rank-sum test was used to evaluate if the differential expression level of acetylated proteins and P < 0.05 was considered to be statistically significant.

**2.7. Database search**

The resulting MS data were searched by using MaxQuant software (v1.5.2.8) by setting the retrieval parameter as follows: the database was set UniProt [Gossypium hirsutum](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/gossypium-hirsutum" \o "Learn more about Gossypium hirsutum from ScienceDirect's AI-generated Topic Pages) (78,387 sequences), an anti-library was added to calculate the False Discovery Rate (FDR) due to random matching, and a standard contamination library was added to the database to eliminate contamination from the identified results. Razor and unique peptides were used for quantification during our Maxquant analysis, and we also implemented the “matching between runs” option. Trypsin/P was specified as a cleavage [enzyme](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/enzyme) allowing up to four missing cleavage sites, five modifications per peptides, minimum length of peptide was set to seven [amino acid](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/amino-acids) residues, the mass the error tolerance was set to 20 ppm and 5 ppm respectively for parent precursor ions and the 0.02 Da mass error tolerance was set for fragment ions. Carbamidomethylation on Cys was specified as fixed modification and [oxidation](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/alpha-oxidation) on Met, [acetylation](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/acetylation" \o "Learn more about acetylation from ScienceDirect's AI-generated Topic Pages) of Lys, and acetylation of protein N-terminal were specified as variable modifications. FDR was adjusted to <1%, and the minimum score for modified peptides was set >40.

**2.8. Protein annotation and functional classification**

[Gene Ontology](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/gene-ontology) (GO) annotation of [proteome](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/proteome) was derived from the UniProt-GOA database (<http://www.ebi.ac.uk/GOA/>). Firstly, lysine-acetylated protein ID was converted to UniProt ID and then mapped to GO ID by protein ID. If the UniProt-GOA database did not annotate identified lysine acetylation substrates, the InterProScan soft then used to interpret protein's GO function based on [protein sequence](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/peptide-sequence) alignment method ([König et al., 2014](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib39)). Then lysine [acetylation proteins](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/protein-acetylation" \o "Learn more about acetylation proteins from ScienceDirect's AI-generated Topic Pages) were further classified by Gene Ontology annotation based on the categories of [biological process](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/biological-phenomena-and-functions-concerning-the-entire-organism) and molecular function. Kyoto Encyclopedia of Genes and Genomes (KEGG) database was used to annotate protein pathway. Firstly, KEGG online service tools KAAS was used to interpret protein's KEGG database description ([Zhen et al., 2016](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib116)). Then annotation result was mapped to the KEGG pathway database using KEGG online service tools KEGG Mapper. WoLF [PSORT](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/psort), CELLOv.2.5, and TargetP 1.1 databases were used for [subcellular localization](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/subcellular-localization" \o "Learn more about subcellular localization from ScienceDirect's AI-generated Topic Pages) prediction ([Xiong et al., 2016b](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib104); [Xue et al., 2018](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib108); [Ma et al., 2016](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib54); [Hao et al., 2012](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib25)) for better reproducibility.

**2.9. Functional enrichment analysis**

GO function enrichment analysis on three ontologies (biological process, cellular component, and molecular function), and KEGG pathway enrichment analyses were performed to gain further insights into the involved function and pathways of the acetylated proteins. Fisher's exact test was used to test for enrichment or depletion (right-tailed test) of specific annotation terms among members of resulting protein clusters. Derived p-values were further adjusted to address multiple hypotheses testing ([Dimmer et al., 2012](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib11)). Any terms having changed p-values below 0.05 in any of the groups were treated as significant ([Moriya et al., 2007](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib62)).

**2.10. Enrichment-based clustering analysis**

All the lysine acetylation subset categories (biological process, cellular component, and molecular function) were obtained after enrichment collated along with their P-values and were then filtered for those categories which were at least enriched in one of the clusters with P < 0.05. This filtered P-value matrix was transformed by the function x = −log 10 (P-value). Finally, these x-values were z-transformed for each category. These z scores were then clustered by one-way hierarchical clustering (Euclidean distance, average linkage clustering) in Genesis. Cluster membership was visualized by a [heat map](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/heat-map) using the “heatmap.2” function from the “plots” R-package ([Horton et al., 2007](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib30)).

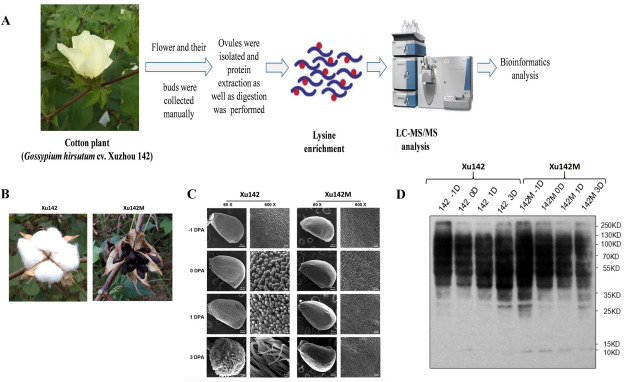
**2.11. Sequence model analysis around acetylated lysine**

Motif-x software was used to analyze the model of sequences constituted with amino acids in specific [positions](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/position) of acetyl-21-m (10 amino acids upstream and downstream of the acetylation site) in all protein sequences. All the database protein sequences were used as a background database parameter, other parameters with default ([Emanuelsson et al., 2000](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib15)).

**3. Results**

**3.1. Xu142M unable to develop fiber**

The purpose of this study was to compare the lysine acetylome data of two kinds of cotton (*Gossypium hirsutum*) cultivars *Xu142* and its natural isogenic fl mutant (*Xu142M*) to investigate the role of lysine-acetylation in fiber development event. The experimental design and workflow outlined in [Fig. 1](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "fig1)A. Briefly, the protocol contained four critical steps: (i) Cotton flowers and flower buds collection to isolate the ovule, protein extraction, and tryptic digestion, (ii) Affinity enrichment of lysine-acetylated peptides, (iii) Analysis of lysine-acetylated peptides by using nano-LC-MS/MS, (iv) Bioinformatics analysis for systematic interpretation of the identified lysine-acetylated proteins. Three biological replicates were analyzed with LC-MS/MS.



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Fig. 1. The workflow of integrated strategy applied for the comparative acetylome analysis of two cotton cultivars (i.e., Xu142 and Xu142M) **(A)**. The photographs of two different cotton cultivars used in study **(B)**, and a compassion High-resolution Scanning Electron Microscopic (HR-SEM) images of ovules of Xu142 and Xu142M on 0 DPA, -1DPA,1DPA, and 3DPA identify Xu142 unable to develop fibers **(C)**. Western blotting of cotton (Gossypium hirsutum cv. Xu142) ovule protein with pan anti-acetylation antibody **(D)**. The analysis of results of western blotting of Xu142 (Lane, 1–4) and Xu142M (Lane 5–8) on -1DPA, 0DPA, 1DPA, and 3DPA.

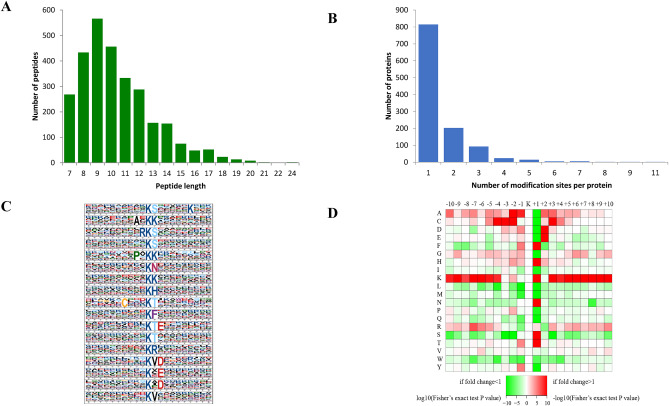
As shown in [Fig. 1](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig1)B, the Xu142M unable to develop the fiber. Furthermore, the [electron microscopy](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/electron-microscopy) images were collected from the isolated ovule, demonstrating that the development of fiber utterly absent in the mutant ([Fig. 1](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig1)C). A comparison between of Scanning Electron Microscopic (SEM) images obtained from ovule of wild-type (Xu142) and mutant (Xu142M) species on the day (0 DPA), on a day before (−1 DPA), a day after (1 DPA) and 3 days after (3 DPA) anthesis shown in [Fig. 1](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig1)C. The image analysis suggested fiber development initiated on a day before (−1 DPA) anthesis. Moreover, first of all, to detect lysine acetylome of these species, proteins prepared from the ovules were examined by [western blotting](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/western-blot) with lysine acetylation-specific pan antibodies. As a result, multiple major protein bands with molecular weight higher than [histones](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/histone" \o "Learn more about histones from ScienceDirect's AI-generated Topic Pages) were successfully detected ([Fig. 1](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig1)D), indicating that lysine acetylation not only happens to [histones](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/histone" \o "Learn more about histones from ScienceDirect's AI-generated Topic Pages) but also occurs in non-histone proteins. Furthermore, the most significant changes were observed in 0 DPA and −1 DPA. Therefore, we have selected these two-time points (−1 day and 0 days) for our analysis.

**3.2. Comparative acetylome analysis of Xu142 and Xu142M**

In work presented in our manuscript, lysine acetylation approach was applied to compare the high-throughput acetylome data of upland cotton (Xu142), with its fuzzless-lintless mutant Xu142M (hereafter, 142M) by selecting two different time points, i.e., 0 DPA and −1 DPA, with an aim to identify the role of protein lysine-acetylation in fibre development. Our present comparative acetylome data identified a total number of 2754 of unique acetyl sites from 1696 proteins. Out of these 2754 acetylation sites, 2104 from 1358, proteins possess quantitative information ([Table 1](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "tbl1A)A; [Supplementary data, File S1](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)). The length of the acetylated peptides ranged from 7 to 24 [amino acids](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/amino-acid), with 90% falling within the length range of 7–12 [amino acids](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/amino-acids) ([Fig. 2](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "fig2)A; [Supplementary data, File S1](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)). The number of KAC sites in a single protein also varied considerably among distinct proteins. In 1358 quantified acetylated proteins, more than 200 proteins possess more than one acetylation sites ([Fig. 2](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig2)B; [Supplementary data, File S1](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)). Furthermore, the acetylation sites with quantitative differences in abundance of >1.2 fold (increased in abundance) and 0.833 fold (decreased in abundance) were only considered as the differentially expressed and summarized in [Table 1](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "tbl1B)B. Interestingly we found that the 250, 31, 20, and 101 acetylation sites significantly showed increased abundance from 210, 27, 17, and 92 proteins in four comparison groups, i.e., 142-0D/142-1D, 142-0D/142M-0D, 142-1D/142M-1D, and 142M-0D/142M-1D ([Supplementary data, File 1](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)). A large number of acetylation sites severely decreased in 142M on 0 DPA and -1DPA, while their abundance in 142 is suggesting that lysine-acetylated proteins may have some role in fiber development. Hence, the result of our acetylome data indicated that a large number of proteins change their acetylation pattern during the development of fiber in two cotton cultivars.

Table 1A. The total number of identified and quantified acetylation sites.

| Empty Cell | **Identification** | **Quantified** |
| --- | --- | --- |
| **Site** | 2754 | 2104 |
| **protein** | 1696 | 1358 |



1. [Download : Download high-res image (872KB)](https://ars.els-cdn.com/content/image/1-s2.0-S0981942820300851-gr2_lrg.jpg)
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Fig. 2. Statistical analysis. **(A)**, identification of length distribution of peptides, and **(B)**, distribution of KAC sites per protein in the lysine acetylome. Motif analyses of the acetylated peptides. **(C)** Acetylation motifs and conservation of acetylation sites. The size of each letter corresponds to the frequency of corresponding amino acid residue at a given position (upstream and downstream to acetylation sites). **(D)** [Heat map](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/heat-map) of the [amino acid compositions](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/amino-acid-composition) of the acetylation sites showing the frequency of different amino acids around the K (acetylated lysine).

Table 1B. The expression statistics (p-value <0.05.) of acetylation sites.

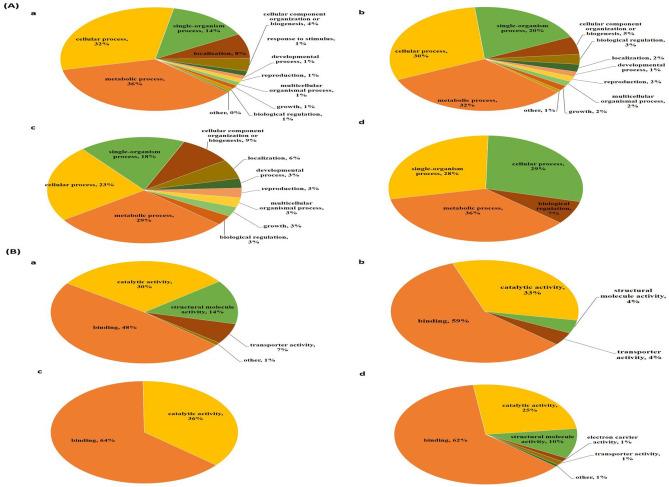
| **Compare groups** | **Increase (>1.2)** | | **Decrease (<0.833)** | |
| --- | --- | --- | --- | --- |
| **Site** | **Protein** | **Site** | **Protein** |
| 142M-0D/142M-1D | 101 | 92 | 77 | 43 |
| 142-0D/142-1D | 250 | 210 | 9 | 7 |
| 142-0D/142M-0D | 31 | 27 | 72 | 60 |
| 142-1D/142M-1D | 20 | 17 | 201 | 150 |

**3.3. Motif analysis of acetylated proteins**

To identify the possible specific [sequence motifs](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/sequence-motif) surrounding acetylated lysine residues, we generated a type of [sequence logo](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/position-weight-matrix) which computes the likelihood of amino acids being over-or under-represented at the positions surrounding the acetylation site. We identified 17 significantly enriched acetylation site motifs ([Fig. 2](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig2)C and [Supplementary data, File S2](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)). We noticed that aliphatic amino acids such as [alanine](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/alanine" \o "Learn more about alanine from ScienceDirect's AI-generated Topic Pages) (A), [valine](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/valine" \o "Learn more about valine from ScienceDirect's AI-generated Topic Pages) (V), OH group-containing amino acid [serine](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/serine) (S), sulfur-containing amino acid [cystine](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/cystine" \o "Learn more about cystine from ScienceDirect's AI-generated Topic Pages) were identified as most conserved amino acid surrounding the Kac sites. Also, most of the conserved residues are located at the ±1 or ±4 positions of the Kac sites ([Fig. 2](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig2)D). Results of motif analysis indicated that these motifs and amino acids might be conserved and essential for lysine acetylation during fiber development.

**3.4. GO-based functional classification of comparative acetylome data**

Further, to gain a better understanding of the role of lysine acetylation in cotton fiber development, the lysine-acetylated proteins were subjected to functional classification, distribution, and pathway analysis. First of all, the [GO](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/gene-ontology) analysis was done to perform the functional classification of acetylated proteins of different comparison groups. GO classified acetylated proteins into three functional categories, i.e., biological process, cellular component, and molecular function across the comparison groups ([Fig. 3](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "fig3); [Supplementary data, File S3](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)). A comparison of the biological processes between the comparison groups (142-0D/142-1D, 142M-0D/142M-1D, 142-0D/142M-0D, and 142-1D/142M-1D) showed that the metabolic, cellular and single organism processes related proteins were the major acetylated proteins, accounted for 36%, 32%, and 14%, 32%, 30%, and 20%, 29%, 23% and 18%, 36%, 28% and 29% respectively ([Fig. 3](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig3)A). The result hence suggested acetylation plays an essential role in the regulation of metabolism, cellular, and transport. Furthermore, a comparison of the cellular processes ontology between the comparison groups 142-0D/142-1D, 142M-0D/142M-1D, and 142-1D/142M-1D showed that the acetylated proteins belonged to various cellular components, the proportion of [organelle](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/organelle), macromolecular complex, and membrane-related were 37%, 27%, 25% and 11%, 47%, 21%, 14% and 14%, 36%, 36%, 18% and 5% of all acetylated proteins, respectively ([Supplementary data, File S3](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)). However, in the comparison group, 142-0D/142M-0D of the acetylated protein belonging to cellular processes mainly classified into the cell and molecular complexes accounted for 67% and 33%, respectively ([Supplementary data, File S3](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)). For the molecular function ontology, it was shown that the proteins associated with binding, catalytic, structural molecular and transporter activities in the comparison groups 142-0D/142-1D, 142M-0D/142M-1D, and 142-1D/142M-1D corresponded to nearly 48%, 30%, 14%, and 7%, 59%, 33%, 4% and 4%, 62%, 25%, 10% and 1%, respectively. However, in 142-0D/142M-0D, the acetylated protein belongs to only binding (64%) and catalytic (36%) activities ([Fig. 3](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig3)B; [Supplementary data, File S3](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)). Hence, we conclude here that the majority of the acetylated proteins of molecular function ontology belonged to binding and catalytic activity molecular annotation.

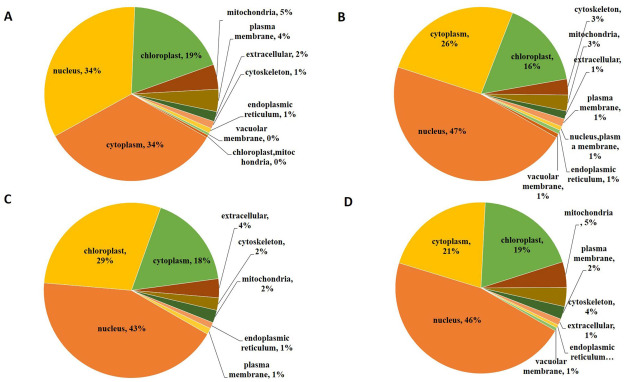


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Fig. 3. Gene Ontology analysis based functional classification and distribution of the lysine-acetylated proteins. **(A)** biological process; comparison group 142-0D/142-1D (a), 142M-0D/142M-1D (b), 142M-1D/142-1D (c) and 142M-0D/142M-1D (d). **(B)** molecular function; comparison group 142-0D/142-1D (a), 142M-0D/142M-1D (b), 142M-1D/142-1D (c) and 142M-0D/142M-1D (d).

**3.5. Sub-cellular localization analysis of acetylated proteins**

The distribution within the subcellular localization analysis of acetylproteome data was performed by using three independent prediction databases such as WoLF [PSORT](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/psort), CELLO v.2.5 and TargetP 1.1. Although WoLF PSORT is a well-annotated database it has biases, therefore for subcellular localization predictions of acetylated proteins, three programs were used in this study. Furthermore, the results obtained by using three programs were not alike. Therefore we have taken only the similar results obtained with acetylated proteins. The results of subcellular localization prediction indicated that the majority of identified Kac proteins were localized in the cytoplasm, nucleus, chloroplast, and mitochondria ([Fig. 4](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "fig4); [Supplementary data, File 3](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)), suggesting the important roles of lysine acetylation in these compartments. In the comparison group 142-0D/142-1D, 142M-0D/142M-1D, 142-1D/142M-1D and 142-0D/142M-0D the identified Kac proteins were predicted to localized in chloroplast, cytoplasm, nucleus and mitochondria 19%, 34%, 34%, and 16%, 26%, 47% and 3%, 29%, 18%, 43% and 2%, 19%, 21%, 46% and 5% ([Fig. 4](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig4)). Moreover, a significant proportion (58%) of KAC protein in the cytoplasm, mitochondria, and chloroplast were sub-cellularly localized in Xu142; however, in Xu142M, KAC proteins were abundant in the nucleus (47%) ([Fig. 4](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig4)A–B). Hence, the results of sub-cellular analysis suggesting a significant portion of proteins localized in chloroplasts, mitochondria, and cytoplasm (58%) were acetylated in Xu142 during fiber development in comparison to the nucleus (34%). However, in Xu142M most of the proteins undergo acetylation were belonging to the nucleus (47%).

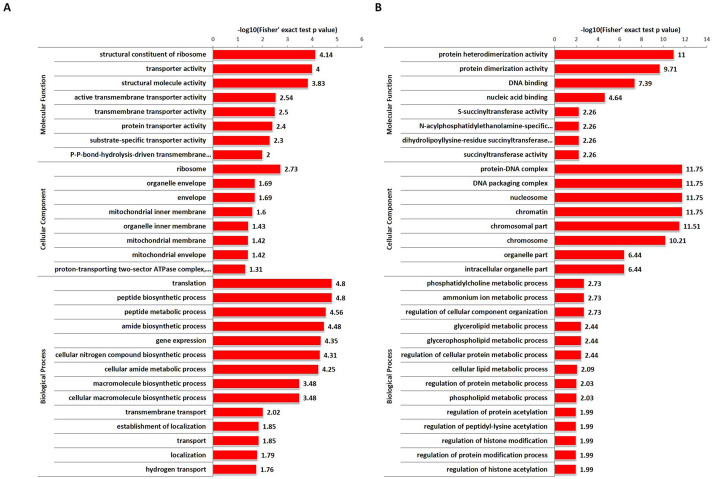


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Fig. 4. Subcellular localization of the lysine-acetylated proteins analyzed by WolfpSort database. **(A)** Comparison group 142-0D/142-1D, **(B)** Comparison group 142M-0D/142M-1D, **(C)** Comparison group 142M-1D/142-1D and **(D)**142M-0D/142-0D comparison group.

**3.6. Enrichment analysis of acetylated proteins**

Enrichment analysis was done to determine the preferred target proteins for lysine acetylation in different comparison groups ([Supplementary data, File 4](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)). The results of the functional enrichment analysis of comparison groups 142-0D/142-1D and 142M-0D/142M-1D were shown in [Fig. 5](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "fig5). The biological process ontology in comparison group 142-0D/142-1D significantly enriched with macromolecular [biosynthesis](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/anabolism) and transport such as translation, peptide and amide biosynthetic process, gene expression, cellular nitrogen compound biosynthesis, cellular amide metabolic process, [macromolecule](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/macromolecule) and cellular macromolecule biosynthesis, transmembrane transport, establishment of localization, transport localization, [hydrogen transport](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/proton-transport) and [DNA binding](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/dna-binding) ([Fig. 5](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig5)A). However, in comparison 142M-0D/142M-1D the biological process ontology mainly enriched with various regulatory processes such as regulation of cellular component organization, cellular [protein metabolism](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/protein-metabolism), [protein metabolism](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/protein-metabolism), macromolecular complex assembly, cellular macromolecular complex assembly, cellular component assembly, macromolecular complex subunit organization, regulation of histone, protein and chromatin modification, regulation of protein and peptidyl-lysine acetylation ([Fig. 5](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig5)B).

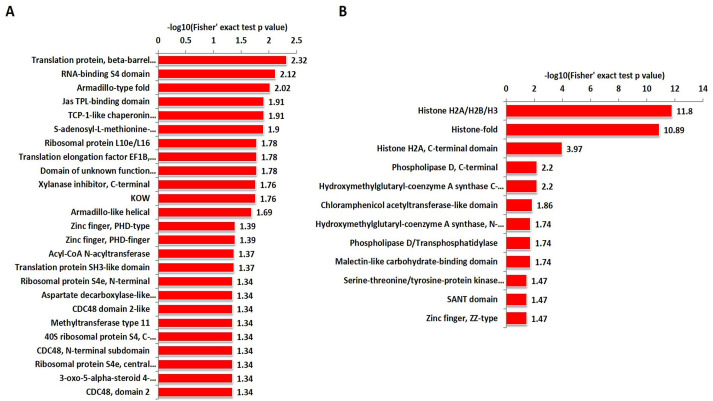


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Fig. 5. GO-based functional enrichment analysis of comparison group **(A)**, 142-0D/142-1D, and **(B)**, 142M-0D/142M-1D. The abscissa value is taken as a negative logarithmic conversion of significant p-values (p < 0.05).

Similarly the 142-1D/142M-1D compassion group the biological process term enriched mainly with negative regulation of biological process, negative regulators of gene expression, metabolism, macromolecule metabolism, cellular metabolism, cellular processes, macromolecule biosynthesis, cellular macromolecule biosynthesis, cellular biosynthesis, nitrogen compound metabolic processes and [chromosome organization](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/chromosome-organization) ([Supplementary data, File 4](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)). However, the biological process term in 142-0D/142M-0D enriched mainly with [mRNA processing](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/rna-processing) and metabolism, RNA processing, nucleotide-excision repair, proteasomal [protein catabolism](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/protein-catabolism), proteasome-mediated ubiquitin-dependent protein catabolism, [fatty acid biosynthesis](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/fatty-acid-synthesis), organic acid biosynthesis, carboxylic acid biosynthesis, [small molecule](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/small-molecule) biosynthesis, monocarboxylic acid biosynthesis, [fatty acid metabolism](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/fatty-acid-metabolism), cellular response to stimulus, modification-dependent macromolecule and protein catabolism, ubiquitin-dependent protein catabolism and lipid biosynthesis-related terms ([Supplementary data, File 4](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)). Therefore, based on the GO enrichment analysis of lysine-acetylated proteins suggested protein lysine acetylation affects the functioning of proteins belonged to various metabolic processes such as assembly and disassembly of macromolecular complexes in Xu142.

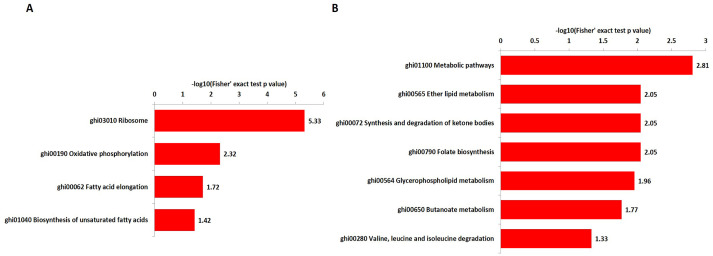
To discover further possible functions of these acetylated proteins in the ovule of the cotton plant, we performed [protein domain](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/protein-domain) enrichment analysis ([Supplementary data, File 4](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)). The domain enrichment analysis results of 142-0D/142-1D and 142-1D/142M-1D comparison groups were shown in [Fig. 6](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "fig6). The domain enrichment analysis of 142-0D/142-1D identified that 26 domains families were enriched, mainly including protein or macromolecular biosynthesis, histones modifying and stress-responsive proteins such as [translation protein](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/translation-protein-synthesis), beta-barrel domain, RNA-binding S4 domain, S-adenosyl-L-methionine-dependent [methyltransferase](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/methyltransferase" \o "Learn more about methyltransferase from ScienceDirect's AI-generated Topic Pages), TCP-1-like [chaperonin](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/chaperonin" \o "Learn more about chaperonin from ScienceDirect's AI-generated Topic Pages) intermediate domain, [ribosomal protein](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/ribosomal-protein) L10e/L16, translation [elongation factor](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/elongation-factor) EF1B, gamma chain, translation protein SH3-like domain, [ribosomal protein](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/ribosomal-protein) S4e, N-terminal aspartate decarboxylase-like domain, CDC48 domain 2-like, [methyltransferase](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/methyltransferase" \o "Learn more about methyltransferase from ScienceDirect's AI-generated Topic Pages) type 11, 40S ribosomal protein S4 C-terminal domain, CDC48 N-terminal subdomain, ribosomal protein S4e central region, CDC48 domain 2, NET domain, [peptidase](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/peptidase) family A1 domain, N-terminal [mitochondrial carrier](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/mitochondrial-carrier) domain ([Fig. 6](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig6)A; [Supplementary data, File 4](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)). It is notable that three-domain families, including the translation protein and elongation factors, ribosomal proteins, and chaperones family, were significantly enriched, suggesting that KAC affects different processes in developing fiber. The domain enrichment analysis of comparison group 142M-0D/142M-1D indicated that 15 domain families were enriched, mainly including histones and regulatory response proteins ([Fig. 6](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig6)B; [Supplementary data, File 4](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)). Therefore the enriched domains belonged to macromolecule biosynthesis (protein, amino acids, [lipids](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/lipid), etc.) as well as [regulatory proteins](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/regulatory-protein) in 142-0D/142-1D; however, in 142M-0D/142M-1D most of the enriched domains belonged to mainly histone and regulatory proteins ([Fig. 6](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig6); [Supplementary data, File 4](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)). Again, in comparison group 142-1D/142M-1D, eight enriched domains primarily belonged to regulatory proteins such as histone (H2A/H2B/H3), histone-fold, [histone H2A](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/histone-h2a" \o "Learn more about histone H2A from ScienceDirect's AI-generated Topic Pages) C-terminal domain, zinc finger, RING-type ([Supplementary data, File 4](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)). While in 142-0D/142M-0D comparison group the enriched domains were histone-fold, histone (H2A/H2B/H3), [polyketide synthase](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/polyketide-synthase" \o "Learn more about polyketide synthase from ScienceDirect's AI-generated Topic Pages), beta-ketoacyl [synthase](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/synthase" \o "Learn more about synthase from ScienceDirect's AI-generated Topic Pages), XPC-binding and heat shock chaperonin-binding ([Supplementary data, File 4](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)). Hence, a comparison of domain enrichment term between 142-1D/142M-1D and 142-0D/142M-0D comparison group identified that in Xu142 at 0DPA most of the domain of the acetylated protein was belong to proteins of macromolecular and fatty acid biosynthesis pathway, while in Xu142M were [histone acetylation](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/histone-acetylation" \o "Learn more about histone acetylation from ScienceDirect's AI-generated Topic Pages) and [pentose phosphate pathway](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/pentose-phosphate-pathway) ([Fig. 6](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig6)). The domain enrichment analysis hence infers that the acetylation of macromolecular and fatty acid metabolism-related proteins in Xu142 may be attributed to the requirement of this molecule for the development of cotton fiber.



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Fig. 6. GO-based domain enrichment analysis of the comparison group. **(A)**, 142-0D/142-1D, and **(B)**, 142M-0D/142M-1D. The abscissa value is taken as a negative logarithmic conversion of significant p-values (p < 0.05).

Furthermore, the KEGG pathway enrichment analysis was subsequently performed by the acetylated proteins ([Fig. 7](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "fig7); [Supplementary data, File 4](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)). The pathway enrichment analysis indicated that a significant proportion of acetylated proteins in comparison group 142-0D/142-1D associated with the ribosome, [oxidative phosphorylation](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/oxidative-phosphorylation), fatty acid elongation, and biosynthesis of [unsaturated fatty acids](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/unsaturated-fatty-acid) ([Fig. 7](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig7)A). However, the acetylated proteins in comparison group 142M-0D/142M-1D belong to metabolic pathways, [ether lipid](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/ether-lipid) metabolism, synthesis, and degradation of [ketone bodies](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/ketone-bodies" \o "Learn more about ketone bodies from ScienceDirect's AI-generated Topic Pages), folate biosynthesis, [glycerophospholipid](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/glycerophospholipid" \o "Learn more about glycerophospholipid from ScienceDirect's AI-generated Topic Pages) metabolism, butanoate metabolism, and amino acid degradation pathways ([Fig. 7](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig7)B). Hence pathway enrichment analysis also unveiled that in Xu142 a considerable number of acetylated proteins belonging to fatty acid biosynthesis, unsaturated fatty acid biosynthesis pathway, glycolysis, and ribosome biosynthesis pathways, maybe playing some crucial role in fiber development process.

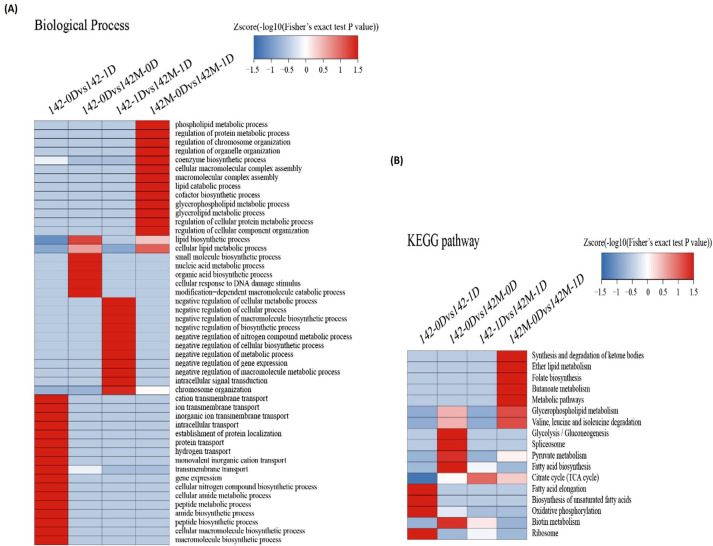


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Fig. 7. GO-based pathway enrichment analysis of comparison group **(A)**, 142-0D/142-1D, and **(B)**, 142M-0D/142M-1D. The abscissa value is taken as a negative logarithmic conversion of significant p-values (p < 0.05).

**3.7. Enrichment-based clustering analysis**

To identify the preferred target substrates for acetylation, enrichment-based clustering analysis was done with all lysine-acetylated proteins, and the top significantly enriched GO terms ([Supplementary data, File 5](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)). The results of the biological process and KEGG pathway-based clustering analysis of lysine-acetylated proteins were shown in [Fig. 8](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "fig8). The functional enrichment-based clustering analysis of the biological process in the 142-0D/142-1D comparison group enriched mainly with the macromolecular biosynthesis pathways, peptide, and amide biosynthesis pathway, fatty acid biosynthesis, [transmembrane transporters](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/transmembrane-transporters" \o "Learn more about transmembrane transporters from ScienceDirect's AI-generated Topic Pages), [intracellular transport](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/intracellular-transport), gene expression, hydrogen transport-related terms ([Fig. 8](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig8)A). However, in comparison group 142M-0/142M-1D, the offer said term mostly enriched with [lipid catabolism](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/lipolysis), [glycerophospholipid](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/glycerophospholipid" \o "Learn more about glycerophospholipid from ScienceDirect's AI-generated Topic Pages) metabolism, [phospholipid metabolism](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/phospholipid-metabolism" \o "Learn more about phospholipid metabolism from ScienceDirect's AI-generated Topic Pages), co-enzyme biosynthesis, macromolecular complex assembly pathways related terms ([Fig. 8](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig8)A). Furthermore, in comparison group 142-1D/142M-1D, the term mainly enriched with negative regulation cellular, macromolecular biosynthesis, gene expression, [chromosomal organization](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/chromosome-structure), [signal transduction](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/signal-transduction), and metabolic processes. Moreover, in comparison group 142-0D/142M-0D, the biological process term mainly enriched with small-molecule biosynthesis, nucleic and organic acid biosynthesis, cellular [lipid biosynthesis](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/lipogenesis), cellular [response to DNA damages](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/dna-damage-response) ([Fig. 8](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig8)A). The result of biological process-based clustering analysis revealed that in Xu142 the macromolecular (protein, peptide, amino acids, and fatty acid) biosynthesis, transmembrane, and energy-related proteins were significant acetylated proteins, however, in Xu142M the process was mainly enriched with the various regulatory pathways. The result of molecular function-based clustering analysis of 142-0D/142-1D enriched mostly with substrate-specific, protein transporter activity, P–P bond [hydrolysis](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/enzymatic-hydrolysis) based transmembrane transporter activity, active transmembrane transporter activity, inorganic diphosphatase activity, [protein transmembrane](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/transmembrane-protein) transporter activity, [translation elongation](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/translation-elongation) factor and ribosomes structural constituents ([Supplementary data, File 5](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)). However, in the comparison group 142M-0D/142-1D, the process was mainly enriched with succinyltransferase, dihydro lipoyl lysine-residue succinyltransferase, S-succinyl [transferase](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/transferase" \o "Learn more about transferase from ScienceDirect's AI-generated Topic Pages), N-acylphosphatidylethanolamine-specific [phospholipase](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/phospholipase" \o "Learn more about phospholipase from ScienceDirect's AI-generated Topic Pages) D, transcription coactivator, phosphoric diester [hydrolase](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/hydrolase" \o "Learn more about hydrolase from ScienceDirect's AI-generated Topic Pages), [lipase](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/lipase), and [phospholipase](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/phospholipase" \o "Learn more about phospholipase from ScienceDirect's AI-generated Topic Pages) D activities related terms ([Supplementary data, File 5](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)). The results of molecular function term better augmented the biological process term and suggesting that Xu142 enriched with macromolecular, fatty acid, protein biosynthesis, ribosomes, and energy-related pathway. Moreover, cellular process term in comparison 142-0D/142-1D significantly enriched with the ribosome, organelle envelope, proton-transporting two-sector [ATPase](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/atpase" \o "Learn more about ATPase from ScienceDirect's AI-generated Topic Pages) complex and [catalytic domain](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/enzyme-active-site), inner organelle membrane, [mitochondrial inner membrane](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/inner-mitochondrial-membrane), envelope and membrane-related processes ([Supplementary data, File 5](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)). However, in comparison group 142M-0D/142M-1D, mainly enriched with an intracellular non-membrane-bounded organelle, chromosome, protein-DNA, and [DNA](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/dna) packaging complex and nucleosome processes ([Supplementary data, File 5](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)).



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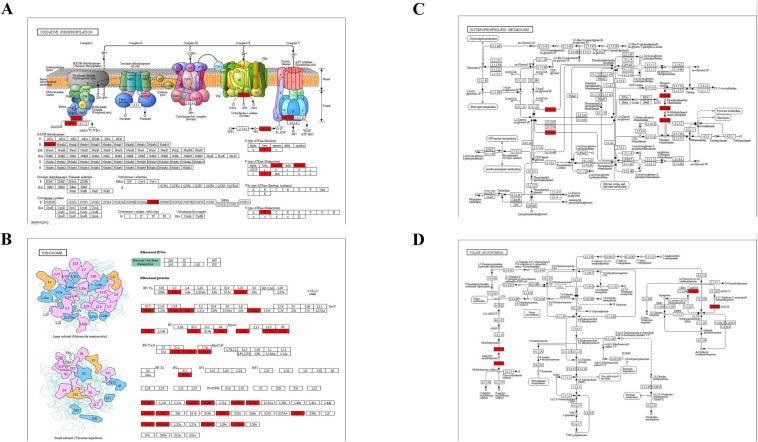
Fig. 8. Enrichment analysis based clustering analysis of all lysine-acetylated proteins. **(A)** A comparison of biological process functional ontologies of different comparison groups, and **(B)** KEGG pathway enrichment based clustering analysis.

The protein domain-based clustering analysis revealed that in comparison group 142-0D/142-1D, RNA-binding S4, TPL-binding, TCP-1-like [chaperonin](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/chaperonin" \o "Learn more about chaperonin from ScienceDirect's AI-generated Topic Pages) intermediate domain, translation protein SH3-like, Acyl-CoA N-acyltransferase, Zinc finger, PHD-type, NET, translation protein, beta-barrel, methyltransferase, translation elongation factor EF1B gamma chain, conserved ribosomal protein, [peptidase](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/peptidase) family A1, N-terminal [mitochondrial carrier](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/mitochondrial-carrier), 40S ribosomal protein S4 C-terminal domain, methyltransferase type 11, C-terminal ribosomal protein S4e, and [mitochondrial transport](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/mitochondrial-transport) like domains were significantly enriched. However, in the comparison group, 142M-0D/142M-1D, the enriched domains were phospholipase, helicases, and [argonaute](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/argonaute" \o "Learn more about argonaute from ScienceDirect's AI-generated Topic Pages) linkers ([Supplementary data, File 5](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)). Moreover, a comparison of 142-1D/142M-1D unveiled that proteins with [dehydrogenase](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/dehydrogenase" \o "Learn more about dehydrogenase from ScienceDirect's AI-generated Topic Pages), zinc-finger, histone protein, and [RNA binding](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/rna-binding) domains were enriched considerably. However, the comparison group 142-0D/142M-0D identifies that proteins with ketoacyl synthase, [polyketide synthase](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/polyketide-synthase" \o "Learn more about polyketide synthase from ScienceDirect's AI-generated Topic Pages), ubiquitin and chaperonin domains were enriched significantly ([Supplementary data, File 5](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)).

The pathway enrichment-based clustering analysis was performed to gain a better understanding of our acetylome data ([Fig. 8](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig8)B). The study suggested in comparison group 142-0D/142-1D fatty acid metabolism, biosynthesis of [polyunsaturated fatty acids](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/polyunsaturated-fatty-acid), TCA cycle, ribosome, and [oxidative phosphorylation](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/oxidative-phosphorylation) pathways were enriched, however in 142M-0D/142M-1D, synthesis, and degradation of [ketone bodies](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/ketone-bodies" \o "Learn more about ketone bodies from ScienceDirect's AI-generated Topic Pages), glycerophospholipid metabolism, ether [lipid metabolism](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/lipid-metabolism), and folate biosynthesis, [valine](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/valine" \o "Learn more about valine from ScienceDirect's AI-generated Topic Pages), [leucine](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/leucine" \o "Learn more about leucine from ScienceDirect's AI-generated Topic Pages), and [isoleucine](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/isoleucine" \o "Learn more about isoleucine from ScienceDirect's AI-generated Topic Pages) degradation pathways were significantly enriched ([Fig. 8](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig8)B). Furthermore, in comparison group 142-1D/142M-1D, TCA cycle, [biotin](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/biotin) metabolism, metabolites biosynthesis, and [carbon metabolism](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/carbon-metabolism) were enriched considerably. However, in 142-0D/142M-0D fatty acid biosynthesis, pyruvate metabolism, [spliceosome](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/spliceosome" \o "Learn more about spliceosome from ScienceDirect's AI-generated Topic Pages), glycolysis/gluconeogenesis, valine, [leucine](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/leucine" \o "Learn more about leucine from ScienceDirect's AI-generated Topic Pages), and [isoleucine](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/isoleucine" \o "Learn more about isoleucine from ScienceDirect's AI-generated Topic Pages) degradation, glycerophospholipid metabolism, [biotin](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/biotin) metabolism mainly enriched ([Fig. 8](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig8)B). Therefore, the enrichment clustering analysis based on domain, function, and pathway indicated a complete metabolic rearrangement in Xu142 on -1DPA operated, while in Xu142M this rearrangement absent.

**3.8. Comparative analysis of enriched pathways**

The pathway enrichment analysis was done with acetylated to pinpoint the enriched pathways ([Supplementary data, File 6](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)). The primary representative enriched pathways of comparison group 142-0D/142-1D shown in [Fig. 9](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "fig9). Surprisingly, almost all the core parts of oxidative phosphorylation NADH and succinate, cytochrome C-oxidase, and various ATPases were lysines acetylated in their multiple subunits ([Fig. 9](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig9)A). Besides this, many ribosomal proteins such as Ef-Tu, RpoA, and B, IF3, Ef-TuG, and Ef-TuS ([Fig. 9](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig9)B).



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Fig. 9. Representative significantly enriched related KEGG pathways of the 142-0D/142-1D comparison group. **(A)**, Oxidative phosphorylation pathway, **(B)** ribosome related enriched pathways, **(C)** glycerophospholipid metabolism, and **(D)**, folate biosynthesis pathway with significant enrichment of the corresponding protein at the different modification sites. In the figure, red indicates the acetylated proteins.

Moreover, the proteins of long-chain and polyunsaturated fatty acid biosynthesis and fatty acid elongation pathways were also significantly acetylated ([Supplementary data, File 6](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)). These results further proved that many lysine acetylations occur exclusively in the proteins of the respiration system, protein biosynthesis, fatty acid, and polyunsaturated fatty acid biosynthesis processes in Xu142. While, in comparison group 142M-0D/142M-1D, the majority of acetylated proteins belong to glycerophospholipid metabolism, and folate biosynthesis was shown in [Fig. 9](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig9). Besides this ether-lipid metabolism, butanoate metabolism, synthesis, and degradation of ketone bodies, and amino acid biosynthesis pathways were mainly enriched significantly ([Supplementary data, File 6](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)). Furthermore, in comparison group 142-1D/142M-1D, the proteins of carbon and TCA cycle metabolisms were enriched considerably, while ribosome biosynthesis and biotin metabolisms pathway proteins were showed decreased in their acetylation ([Supplementary data, File 6](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)). Moreover, in 142-0D/142M-0D comparison group reduced acetylation of proteins of the [spliceosome](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/spliceosome" \o "Learn more about spliceosome from ScienceDirect's AI-generated Topic Pages), glycolysis, fatty acid biosynthesis, fatty acid and biotin metabolism pathways ([Supplementary data, File 6](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)). Hence, the pathway enrichment analysis of acetylated proteins demonstrated lysine acetylation of protein in cotton plants leads to complete metabolic rearrangement.

**4. Discussion**

Cotton is a globally cultivated cash crop because it is an indispensable source of [natural fiber](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/natural-fibre) ([Gao et al., 2017](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib21)). Unfortunately, the mechanism underlying cotton fiber development mostly unexplored. Lysine acetylation (KAC) is a conserved post-translational modification with diverse [biological functions](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/biological-functions) in various organisms ([Du et al., 2013](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib13); [Mann and Jensen, 2003](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib55); [Jensen, 2004](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib33); [Walsh et al., 2005](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib89); [Witze et al., 2005](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib95); [Meinnel and Giglione, 2008](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib58); [Martin and Zhang, 2007](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib56); [Ruthenburg et al., 2007](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib74); [Choudhary et al., 2009](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib7), [2014](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib8); [Weinert et al., 2011a](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib92), [2014](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib94); [Liu et al., 2014](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib51)). Despite the fact, KAC has been shown to affect the growth and developmental processes in various organisms, related studies are still limited in [angiosperms](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/angiosperm), especially proteomic-based dissections of different developmental stages. In this paper, comparative lysine acetylation proteomics (i.e., acetylome) was applied in wild-type upland cotton (i.e., *Gossypium hirsutum* cv. Xu142) and Xu142M fuzzless-lintless mutant of Xu142 to understand the role of protein lysine acetylation in cotton fiber development ([Fig. 1](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig1)A). The EM images of mutant are suggesting that Xu142M unable to develop fiber ([Fig. 1](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig1)C). Hence, the mutants with impaired [growth and development](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/growth-development-and-aging) of fiber Xu142M and wild-type Xu142 were used in our study to decipher the events of fiber development.

Furthermore, to gain an initial overview of the extent of lysine acetylation in these two cultivars, w[Fig. 5](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig5), [Fig. 6](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig6), [Fig. 7](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig7)estern blot analysis was done by using anti-lysine acetylation antibody ([Chen and Tian, 2007](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib6)). Western blot analysis results identified multiple major protein bands with molecular weight higher than histones ([Supplementary Fig. 1](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)), revealed that both histones, as well as nonhistones, undergo acetylation, which is consistent with a previous report ([Chen and Tian, 2007](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib6)). Hence, protein lysine-acetylation of histone, as well as nonhistone proteins, may be involved in the fiber development process.

In order to investigate the role of KAC in cotton fibre development, we performed Tandem Mass Tag (TMT) labeling and acetylation enrichment technology coupled with high-resolution Liquid Chromatography-Mass Spectrometry (LC-MS/MS) ([Fig. 1](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig1)A) and quantified a total of 2104 acetylation sites from 1358 proteins ([Table 1A](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#tbl1A), [Table 1B](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#tbl1B); [Supplementary data, File 1](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)). The [mass spectrometry](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/mass-spectrometry) proteomics data have been deposited to the ProteomeXchange Consortium ([Yu et al., 2004](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib111)) via the PRIDE partner repository with the dataset identifier PXD009052. To the best of our knowledge, the paper constitutes the first report on the quantification of more than 2000 lysine acetylation sites and also the most extensive comparative proteomics dataset of lysine acetylation in plants to date. Furthermore, we first applied comparative acetylome analysis to dissect the role of lysine acetylation in cotton fiber development. In comparison group 142-0D/142-1D, 250 (210 proteins) and 9 (7 proteins) quantified acetylation sites, increased and decreased respectively. However, in the comparison group, 142M-0D/142M-1D, 101 (92 proteins), and 77 (43 proteins) acetylation sites increased and decreased ([Table 1](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#tbl1B)B; [Supplementary data, File 1](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)). Thus, a considerable number of proteins were detected with multiple lysine acetylation sites may be playing a potential role in cotton fiber development, although it is not clear that these numerous acetylation sites occur on a single [polypeptide](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/polypeptide) simultaneously. To date, although studies have identified some lysine-acetylated proteins in plants were primarily focused on the dissection of regulatory pathways ([Zhu et al., 2016](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib117); [Wang et al., 2017](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib91); [Walley et al., 2018b](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib88); [Xu et al., 2017](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib107); [He et al., 2016](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib26); [Zhang et al., 2016a](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib113), [2016b](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib114); [Mischerikow and Heck, 2011b](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib61); [Schilling et al., 2012](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib77); [Corbett and Cristea, 2017](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib9); [Rauniyar et al., 2013](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib71); [Marx et al., 2016](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib57); [Xing and Poirier, 2012a](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib101), [2012b](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib102); [Li et al., 2018b](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib46); [Salvato et al., 2014](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib75); [Melo-Braga et al., 2012](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib59); [Smith-Hammond et al., 2014a](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib78), [2014b](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib79); [Finkemeier et al., 2011b](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib19); [Wu et al., 2011b](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib97); [König et al., 2014](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib39); [Zhen et al., 2016](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib116); [Xiong et al., 2016b](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib104); [Xue et al., 2018](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib108); [Ma et al., 2016](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib54); [Hao et al., 2012](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib25); [Dimmer et al., 2012](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib11); [Moriya et al., 2007](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib62); [Horton et al., 2007](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib30); [Emanuelsson et al., 2000](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib15)), with only a few in relation plant growth and development ([Walley et al., 2018a](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib87)). Here we first speculate that the lysine acetylation may also play an essential role in cotton fiber development. Surrounding to acetylation site preferential amino acids is present in all life forms 105-106. To determine conserved motifs around the acetylation sites ([Fig. 2](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig2)CD). Two amino acids were found to be more common across the acetylated-lysine motifs. The first type is [threonine](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/threonine" \o "Learn more about threonine from ScienceDirect's AI-generated Topic Pages) (T), [serine](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/serine) (S) or aspartate (D) downstream to Kac sites, and the second type is [cytosine](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/cytosine) (C) or [alanine](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/alanine" \o "Learn more about alanine from ScienceDirect's AI-generated Topic Pages) (A) or [proline](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/proline" \o "Learn more about proline from ScienceDirect's AI-generated Topic Pages) (P) in the upstream of Kac sites ([Fig. 2](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig2)C and [Supplementary data, File S2](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)). T and S are highly conserved amino acids next to the Kac sites ([Fig. 2](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig2)CD). T and S are [essential amino acid](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/essential-amino-acid) and is a component of serine-threonine signaling system suggesting that during fiber development besides acetylation there is also phosphorylation of serine-threonine system to bring some changes ([Benjamini and Hochberg, 1995](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib4)).

The GO-based functional classification and distribution of acetylated proteins were done to gain a better understanding of our acetylome data ([Fig. 3](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig3); [Supplementary data, File 3](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)). The GO-based functional classification suggested that nearly 70% of the acetylated proteins belonging to metabolic and cellular processes under [biological ontology](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/biological-ontology), implying that protein lysine acetylation not only involved in regulation of regulatory pathways but also associated with the various metabolic processes ([Fig. 3](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig3)A). Furthermore, the molecular function ontology classifies nearly 90% of all acetylation sites related to catalytic activity and binding ([Fig. 3](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig3)B). It is well-documented that the enzymes modulate the various pathways by bindings with specific substrates ([Wu et al., 2013b](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib99); [Pan et al., 2014](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib65)). Hence, it could be attributed that the protein lysine acetylation in the cotton plant may require acting regulator of different metabolic enzymes. It has been reported that the lysine-acetylation acts as a reversible regulator of metabolic pathway enzymes ([Pang and Rennert, 2013](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib66)). Based on our functional classification data, we infer that the majority of acetylated proteins were enzymes belonging to various metabolic as well as cellular processes. The subcellular localization indicated ~55% and 34% of Kac proteins localized in the cytoplasm, as well as chloroplast and nucleus in Xu142, contrary to this in Xu142M, 42% proteins, belong to cytoplasm and chloroplast and 47% belongs to nucleus ([Fig. 4](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig4); [Supplementary data, File 2](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)), suggesting the frequency lysine acetylation in cytoplasm and chloroplast is higher in Xu142, while in Xu142M these compartments are less enriched with KAC proteins. In plants, one of the most important metabolic processes is [photosynthesis](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/photosystem), and it operates in the chloroplast, while the [protein synthesis](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/protein-synthesis), [fatty acid biosynthesis](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/fatty-acid-synthesis), respiration (glycolysis) operates in the cytoplasm, the increased acetylation of these compartments proteins in Xu142 may be attributed with the enhancement of these metabolic processes for the fiber development. While in Xu142M, KAC proteins mainly localized into the nucleus may be associated with the routine cellular processes. Consistent with the previous studies, interestingly, our acetylome results suggested enhanced acetylation of carbon and acid [metabolism proteins](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/protein-metabolism), which mainly operates in these compartments ([Hebert et al., 2013](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib27); [Uhrig et al., 2017](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib84)).

Moreover, the enrichment analysis was performed to determine the preferred targets of protein types for lysine acetylation ([Fig. 5](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig5), [Fig. 6](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig6), [Fig. 7](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig7); [Supplementary data, File 3](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)). The quantified lysine-acetylated proteins of different comparison groups subjected to functional enrichment ([Fig. 5](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig5)), domain ([Fig. 6](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig6)), and pathway-based enrichment ([Fig. 7](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig7)) analysis to identify the top significantly enriched GO terms and pathways. On comparing the functional enrichment process, the biological ontology identified that several GO terms related to carbon and [fatty acid metabolisms](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/fatty-acid-metabolism) such as macromolecular [biosynthesis](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/anabolism) (polyunsaturated fatty acid biosynthesis), [response to DNA damage](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/dna-damage-response) and ROS defense, transporter related, organic acid biosynthesis, [lipid biosynthesis](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/lipogenesis), glycolysis, and TCA cycle were enriched significantly in Xu142 ([Fig. 5](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig5)A). Respiration is a vital process including glycolysis, and [citrate cycle](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/citric-acid-cycle), known to plays a very crucial role in plant growth and development ([Vizcaíno et al., 2014](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib85)). An enhanced abundance of acetylation of these proteins in Xu142 may be attributed with an important regulatory mechanism to meet out enhanced energy demand for the biosynthesis of [macromolecules](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/macromolecule), and also most of the intermediates of these pathways used in the biosynthesis of [secondary metabolites](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/secondary-metabolite) as well as hormones for the fiber development. Furthermore, the acetylation of fatty acid and [hormones biosynthesis](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/hormone-synthesis) pathways related proteins may be associated with the fiber development process ([Finkemeier et al., 2011b](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib19)). Furthermore, the enrichment-based clustering analysis was performed to get the preferred target substrates of lysine-acetylated proteins in different subcellular compartments. As our data are shown in [Fig. 8](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig8)A, in the biological process term, macromolecular, peptides and amino acid biosynthesis pathway, [transmembrane transporters](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/transmembrane-transporters" \o "Learn more about transmembrane transporters from ScienceDirect's AI-generated Topic Pages), respiration were enriched significantly, and most of these processes operate in the cytoplasm. The result hence, suggested that the proteins related to these pathways are the preferred target substrates of lysine acetylation. In agreement with this observation, the analysis by cellular component indicated that macromolecular biosynthesis, nucleus, chloroplast, and transporter related GO terms such as TCA cycle, ribosome, [ATPase](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/atpase" \o "Learn more about ATPase from ScienceDirect's AI-generated Topic Pages) complexes, dihydrolipoyl [dehydrogenase](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/dehydrogenase" \o "Learn more about dehydrogenase from ScienceDirect's AI-generated Topic Pages), including [mitochondrial membrane](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/mitochondrial-membrane), [photosystem](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/photosystem" \o "Learn more about photosystem from ScienceDirect's AI-generated Topic Pages), nucleosomal and chromosome, and fatty acid biosynthesis enriched significantly. The synthesis of fatty acid occurred in the cytoplasm by using acetyl-CoA and NADPH as a substrate by an enzyme [fatty acid synthases](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/fatty-acid-synthase). Furthermore, most of the acetyl-CoA which is converted into fatty acids are derived from carbohydrates or glycolytic pathways. Therefore, an enhanced enrichment of these terms associated with cytoplasm. Besides this, the chromosome terms, DNA-protein, nucleosome are mainly located in the nucleus ([Henriksen et al., 2012](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib29); [Zhao et al., 2010](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#bib115)). It is well-known that histones participate in [DNA](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/dna) and [chromatin assembly](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/chromatin-remodeling) and organization. Therefore, many DNA and chromatin assembly and organization related terms were enriched significantly in the nucleus. Furthermore, the KEGG pathway enrichment analysis indicated that several terms related to ribosomes, fatty acid, and polyunsaturated fatty acids biosynthesis, as well as elongation, respiration, folate biosynthesis, and [glycerophospholipid](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/glycerophospholipid" \o "Learn more about glycerophospholipid from ScienceDirect's AI-generated Topic Pages) metabolism-related pathways, were enriched ([Fig. 8](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig8); [Supplementary data, File 5](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#appsec1)). Surprisingly, almost all the core parts of [oxidative phosphorylation](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/oxidative-phosphorylation) complexes such as NADH and [succinate dehydrogenase](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/fumarate-reductase" \o "Learn more about succinate dehydrogenase from ScienceDirect's AI-generated Topic Pages), [cytochrome oxidase](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/cytochrome-oxidase" \o "Learn more about cytochrome oxidase from ScienceDirect's AI-generated Topic Pages), and various [ATPase](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/atpase" \o "Learn more about ATPase from ScienceDirect's AI-generated Topic Pages) indicating the proteins of the [respiratory chain](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/electron-transport-chain) are also the preferred target of lysine acetylation. Besides, many fatty acids, [biotin](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/biotin) metabolism, and ribosome biosynthesis-related proteins are also lysine-acetylated in Xu142. While in Xu142M the proteins of folate biosynthesis, butanoate metabolism, ether and [lipid metabolism](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/lipid-metabolism), [glycerophospholipid](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/glycerophospholipid" \o "Learn more about glycerophospholipid from ScienceDirect's AI-generated Topic Pages) metabolism, and degradation and synthesis of [ketone bodies](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/ketone-bodies" \o "Learn more about ketone bodies from ScienceDirect's AI-generated Topic Pages) are preferred targets for lysine acetylation. These results further proved that lysine acetylations occur mainly in the fatty acid biosynthesis, ribosome, and energy-related substrates in Xu142, while in [Xu142M proteins](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/mutant-protein) of these pathways are not acetylated ([Fig. 8](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub#fig8)). Biotin is an essential cofactor for a large number of enzymes, which catalyzes the [carboxylation](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/carboxylation" \o "Learn more about carboxylation from ScienceDirect's AI-generated Topic Pages), decarboxylation, and [transcarboxylation](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/carboxylation" \o "Learn more about transcarboxylation from ScienceDirect's AI-generated Topic Pages) reactions in some important metabolic processes ([Kim et al., 2006b](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib37)). Therefore the enhanced acetylation of proteins of [biotin](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/biotin) metabolism may be associated with the requirement of the high amount of biotin co-factors for various metabolic processes during the fiber development in Xu142. Furthermore, enhanced acetylation of proteins of glycolysis and TCA cycle in Xu142 may be due to requirements of intermediates for biosynthesis and elongation of fatty acid and also to meet energy demand for macromolecular biosynthesis. Therefore, a complete metabolic reprogramming operates in Xu142 due to acetylation of proteins involved in the biosynthesis of macromolecules and ribosomes, respiration, membrane and transporters, biotin metabolism, fatty acid, and [polyunsaturated fatty acid](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/polyunsaturated-fatty-acid) biosynthesis, TCA cycle and glycolysis for fiber development. The acetylation of antioxidative defense and [chaperone protein](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/chaperone-protein) may associate with ROS accumulation, which may acts as a signaling molecule in response to plant hormones known to be associated with the fiber development ([Huaitong et al., 2018](https://www.sciencedirect.com/science/article/pii/S0981942820300851?via%3Dihub" \l "bib31)). Although, the fiber initiation, as well as differentiation, is a complex process structured by various pathways and protein interaction networks. The investigation of comparative acetylome data identified differential acetylation of various metabolic pathway proteins in Xu142 in a time-dependent fashion may operate for fiber development.

In conclusion, an enriched acetylation of (i) TCA cycle and glycolysis, (ii) fatty acid and polyunsaturated fatty acid (PUFA) biosynthesis and elongation, (iii) amino acid as well as protein biosynthesis pathway proteins, [ribosomal proteins](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/ribosomal-protein) and translation [elongation factors](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/elongation-factor) in addition to chaperons, (iv) transporters as well as ATPases, (v) [cellular defence](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/cellular-immunity) strategies such as antioxidative defence system, [protein folding](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/protein-folding) and modification and protection of DNA damages, and (vi) chromosome and nucleosome assembly proteins could be responsible for a complete metabolic reprogramming for the fibre development event in Xu142. To summarize, our results provide the first comparative acetylome proteomics in cotton plants and also the first most extensive dataset of comparative acetylome in plants to date. Our findings underpin that lysine acetylation plays a very crucial role, maybe by adjusting the diverse aspects of the metabolic process, especially in fatty acid biosynthesis, respiration, and protein biosynthesis during the fiber development process. Besides this, the study also opens the door to target the pathways and even proteins involved in the fiber development process. This study broadens the range of operations regulated by lysine acetylation including the development and delivers a precious resource that could be applied to scrutinize the plant's developmental processes. We are working to explore the role of differentially acetylated proteins to unlock the fiber developmental events.

**Author contributions**

PKS, CPS, and WG analyzed and interpreted data and wrote the manuscript. PL and XF collected the samples and performed the ovule isolation experiment. YL performed the electron microscopy imaging experiments. PKS, WG, MN, and LL isolated proteins and completed all the experiments. PKS and CPS designed the study and supervised all of the work. All authors read and approved the final manuscript.

**Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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