

The miRNAome of *Catharanthus roseus*: identification, expression analysis, and potential roles of microRNAs in regulation of terpenoid indole alkaloid biosynthesis

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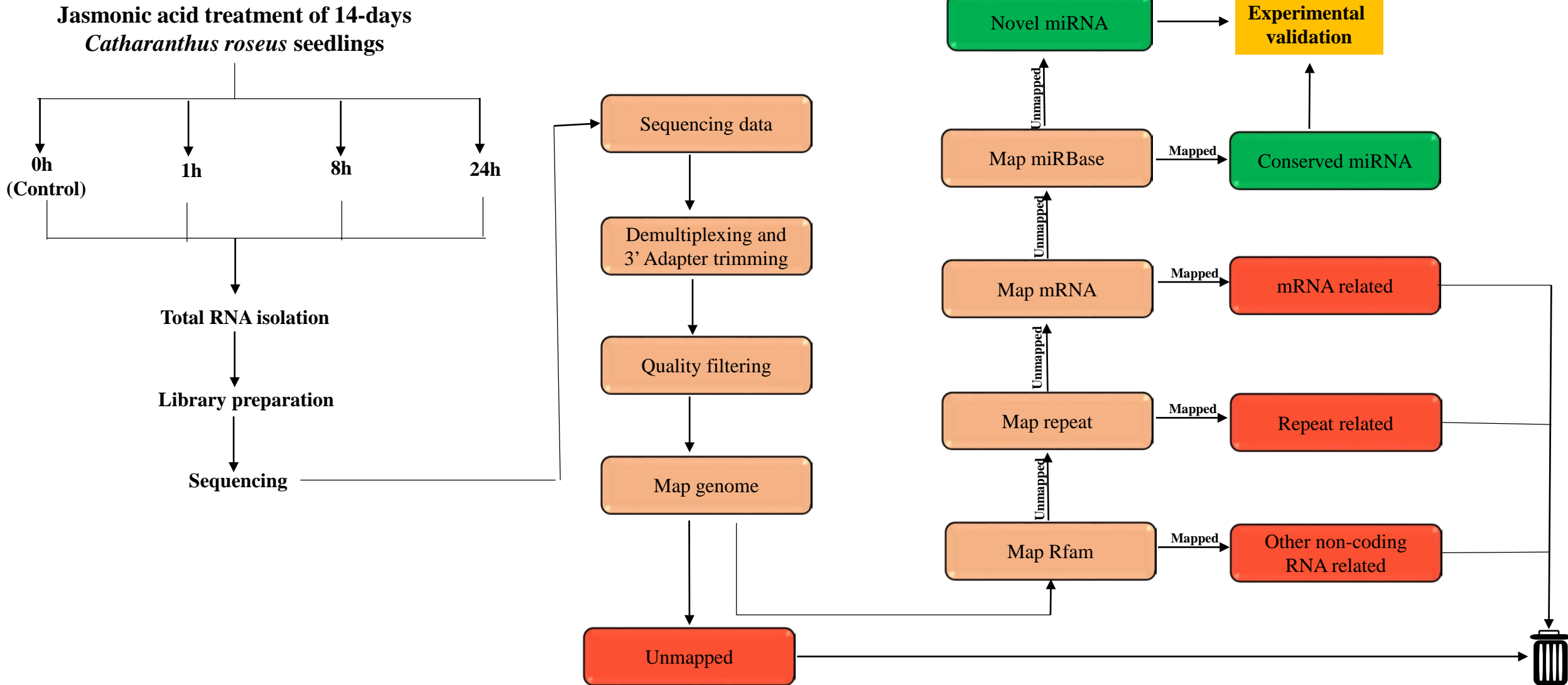


Figure S1. Schematic diagram of the experimental procedure of JA-treatment and miRNA identification in *Catharanthus roseus* seedlings.

>cro-miR399-7

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UGCAGCUAGCUAGCGCUUCUUGAGAUGCAUCAUAAAACGUUUCUAAUCUUGCUGUACAUACGUAGUUAAG
GUUUGAUUUUAAAUCUCUGGGAAAAUAUUCGGAUCAGUUUAUAUAUAUAUAUAUAUG

(((.....))).....(((.....))).....
.....)

>cro-miR408

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ACAAACAGACAUCAUAGAAUGAAAGAAAUGCUAGAAUGCUCCAUUUCAUGCUCUGUCUGUCCCGUCUG
UCCUUAACAACAUACUAGUUUCUCCUUACCUU

.(((.....))).....(((.....))).....
.....)

>cro-miR477

GCUCUUUUUUGUUAAACUCUCCUCAAGGGCUUCUGGCCUCAUCUUGCUAAUCUUAUUGCCUCUUUGUCUU
AAUGGAAUAAAGAGGGCUAGAAGAACUUGAGGGAGAACUGACAAAUUGC

.....(((.....))).....(((.....))).....
.....)

>cro-miR5532

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GCAUUCUCCGGAUAAUJCAAUCGAAGCAAUUAGGUUAUCCAUCGGGCCUUAUUGACAUUGAGCGAUCAA
AGAAAUACUCCACCUUUGUCAUUAUUCUACAUCACACUAGAUAGUAUCAUAUUCUUGGAAUACGAUUC
ACUUUCAAGAUGCCUUGGUGGUGAAUAGUAGACACGCGAGACUCAAAAUCUGUCUAAAGAGCGUGAGG
UUUGAGUCCUUAAGGCAUAAUACGGAGAAUGCUCAUUGAAUGAGCAAUUAAGCGAGCUCGGAUC
GAAUCGGUAUAUA

.....(((.....))).....(((.....))).....
.....)

>cro-miR8016

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AACUUUG

.....(((.....))).....(((.....))).....
.....)

AGAGAAGUUAGAAAGA**AACUGUGUAGAGAGAGAAAGAU**AAGAAAAAAUAAUGAUUUUUUUCUCGUUAUAC
AUUCAUUAUCUCG

.((((...(((.....(((((((.....))))))))))..))))).)

>cro-novel129

UU**ACGAUCUUGCACCUGACAUGC**UAAGUGAAAGAUUUUGAGUUCAAGUCACUUAUAGAGCAGGUUAAUA
UCGUAU

.(((((((.....))))))..))))).)

>cro-novel13

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UAAGGAUAAAAUCGUCUAACUAAUUUUUAAGUCCCAAUCCAUCACUGGAAUGGGUCAAAAUGAACUUGUC
AAUGUUUUGUAUCGGCACAUAAGUCGAAUUUAUUCAAAGCAUCCAUAUGUUGAUUAGUGUUUA**AU**
UGGCUGGACAUUUU**AUCC**UUACUAAAGAUAAUUAUUUGAAUGUCAUGAAUACAAAUUGGACAGAAUCA
CCUAUCAUCGCCGUUAAGA

.(((((((.....))))))..))))).)

>cro-novel130

AAGGUCGGAUUUGGAGCCAUAAAUUUAUUCUAGAGAGUUUGGGUUUGAAUCCAUAUUUACUUAAGG
AUACGAAUCUGAAUUUGAAAUACCCAAAGAUCUA

.((((...(((.....))))))..))))).)

>cro-novel131

AAGGUUUAUUUAAGCUUGAAAGAAGUGUCCGGUUGAAGUUAAAUUUGUUGCACAUAUAUGAGAGUGU
GUU**AAAGUAACUGAUGAUCUUCUUGAAC**GUAAUUUAACUUA

.(((((((.....))))))..))))).)

>cro-novel132

CGAGUAUUGUCAGCGUUAUGGAUGCUCUGAAAUAAAAUUGUCAAGUUCUUAUUUAAUAU**ACCGUGAUA**
AUGCUGUAACACUUU

.(((((((.....))))))..))))).)

>cro-novel133

AUUGAUUAUAACUAGGAUAC**UCUGUAUUAUCUCUGAUUAUAGC**AGUAUUCUUUAUUAGAGGUUGGUUA
GAAUCCUAUAUUGAUUAGG

.((((...(((.....))))))..))))).)

>cro-novel134

AAGAAAAUCGGAUCGGACCGACCAUCAUGACUUAAGAAUCGAACU~~UUAGACCGAUCUGAUUUUCUC~~

.(((((((((((((((((((.....))))))))))))))))))..

>cro-novel135

UGAAGAAUUCUUAAGGGACCGCAUAUUCAGGUCCAUA~~CGUUGCUGUAUCUCUUAAGAUUUCUUUU~~

.(((((((((((((((((((.....))))))))))))))))..

>cro-novel136

AUAUGGGAUUGUCCACACCCCAACUCCAGGCAUGGACAUCUGAAGCGUGA~~AGUUUGUAGGACUGGACAUC
UAUGA~~

.(((((((((((((((((((.....))))))))))))))))..

>cro-novel137

AGCCUCUUGUGUCUUUCUGUAGGGUUAACUUCUGUUAUUUUU~~UCAGUCUGAUAGUGAGAUUCUAC
UGAGCGGCA~~

.(((((((((((((((((((.....))))))))))))))))..

>cro-novel138

UGGUUUUAUUCUUCUUAACACUUAUACACCCUGUUAUUGAUUAUAAAGAGAAGAACAUGAAGAGUA
AUAUGACUUAUUAUGCCACA~~UAUCUAUGAGUGAAGACAUGAAUCU~~

.(((((((((((((((((((((((.....))))))))))))))))..

>cro-novel139

CAUUGAAAUGGUGACAAGUAGGAAUCACAUGCAUCAUUCUGUAAUGAAUAU~~UACCUUGCUCGUCUUCU
AUUJCAUU~~

.(((((((((((((((((((((((.....))))))))))))))))..

>cro-novel14

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AUCAUGGCAAUAGAGGCAUUAUUAUUCUGCGUCGGCAGUUUGUCACUUGGCUACG~~UUGGGCUCCUU
CCUUUCAUGUUAUGCAACACACCGA~~

.(((.....))))))))))))))))))))))))))))))))..

>cro-novel140

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CUGUGAGCGCGAAAUGUAA

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>cro-novel141

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.((((((.....(((((.....(((((((.....(((((((.....(((((((.....(((((((.....(((((((.....)))))).....)))))).....)))))).....)))))).....))))).

>cro-novel142

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GUUUCUGAAGAUG

.((((((((((((.....((((((((.....((((((((.....((((((((.....((((((((.....)))))).....)))))).....)))))).....)))))).....))))).

>cro-novel143

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GUAACAAUACAUGC

.((((.....((((.....((((.....((((.....((((.....((((.....)))))).....)))))).....))))).)

>cro-novel144

CUUAAUCUAUAAUUAUAGUCAUGGGUAUGGAGCCAACUUCUUCACAUGCGUACUGUUGUCCAAACUUCUGAC
AUAAUUGGUUGGA

.((((.....((((.....((((.....((((.....((((.....)))))).....)))))).....))))).

>cro-novel145

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.((((.....((((.....((((.....((((.....)))))).....)))))).....))))).

>cro-novel146

UCAAGUAUGAACCGACGAGUAUUC AUGGCACAAAGAGUAAGUUGUAAGUACCGUUAGACUCAUGAGUUG
C

.((((.....((((.....((((.....((((.....)))))).....)))))).....))))).

>cro-novel147

AUGCAUGAUGUGAUUCGUGACUCCUCACUAAAAGAGUAAGAUGUUGUGAGUUUGAGUCAUGUAC

.((((.....((((.....((((.....((((.....)))))).....)))))).....))))).

>cro-novel148

AGGGUCUGGAUUUGGAUCUAAUUUCACUAUAAGGUUCUAAGUUUUGGAAUAUUCGGAGGGUUUAGAUGCA

.((((.....((((.....((((.....((((.....)))))).....)))))).....))))).

>cro-novel149

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.((((((((((((((...(((.....)))))))))...)))))))).

>cro-novel164

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.(((.(.(((.....(.....))))))..)).

>cro-novel165

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.((((....((((.....(.....))))))..)).

>cro-novel166

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UUACAAAUAAUUUAUA

.((((((((((((.....(.....(.....)))))))))...)))))))).

>cro-novel167

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.(((.....((((.....(.....)))))).....)))))))).

>cro-novel168

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.(((((((.....(.....(.....))))))..)).

>cro-novel169

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CAGUUC

.((((....((((.....(.....)))))).....)))))))).

>cro-novel17

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UAAUUUAAGGGUAAAAGACAAAAGACGACUUUGGGAUUAUAGCGAUUUUAAGUAAA

.((((((((((((.....(.....(.....)))))))))...)))))))).

>cro-novel170

GUAUAUAAUAAGAUCUACUUGCAUACUGUACUCAUCGUUAUUGAAGUAAAUUGAUUAAUUGUUAG

.(((((((.....)))))))).

>cro-novel171

AUGCAUCUGUUAUUCGUCUUGGAACAAACCACCACUGGUUAUAAAUUUAAAUGAGAAAAACAAGUCCAC

.(((((((.....)))))))).

>cro-novel172

UGUAUUUUUGCGUAUCCUCUUAUUUUUGAAAAAUGGAAACUUAGGUAAGUGCUUUAGAAUAUA

.(((((((.....)))))))).

>cro-novel173

AUAAGAUCAAUUAUAUGUAAGACUGAAUAUAUGAACUGUUUAUAAAUGAUUCGAACUAA

.(((((((.....)))))))).

>cro-novel18

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UGAGGAUACCCUACUCGCGCAAAGACGUGGCUAAGUGUAUAUUGGUGUAUUUUU

.(((((((.....)))))))).

>cro-novel19

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GAUGAUAGAGAUUGUAUUUGUUGAUGCGUCCUUUAGUUAUGUAGCUGUAUUUUAAUAAGGAAUAAU
GUGCAGCAUACCUAAAUAUAUUAAAAUUAUUGCGUUGAGUAAAUUUUGUCGUACC

.(((((((.....)))))))).

>cro-novel2

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UUUAUUUUUCGAGUUCGGGUCGAUAAAGAUAAUCUGGAAUUCUAUAAGGAUAACUCGAAAACUUUAU
AUGUCUGGUGUUGCCUGAAGAUCCUUGCCUCUGUUAACAACCCCUUCUUGAAACUGCAGUCACGAACGA
ACGAUGGAAGA

.(((((((.....)))))))).

>cro-novel20

>cro-novel30

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UGGGCUUGUCGGGAUUGGUUCUAAAUGGAUGAAAUAUUUUUUGACG

.(((((((((((((((((((((.....))))))))))))))))).))))).))))).))))).))))).))))).

>cro-novel31

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UGAAUGUUAACAAGAGUGACAUAAGGAAUGUUACAGAAAUCCA

.(((.(.(((((((((((((((.....))))))))))))).))))).))))).))))).))))).))))).))))).

>cro-novel32

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.(((((((((((((((((((((.....))))))))))))).))))).))))).))))).))))).))))).

>cro-novel33

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CAUUCACACCGCUCUCUUAU

.(((((((((((((((((((((.....))))))))))))).))))).))))).))))).))))).))))).

>cro-novel34

AGAGGUUCAGUACGCAGGAGAGAUGGCGCCGUCGACUAGUUCAGUCAGUUUCUACAGAGAUUUUAGACUG
GAAAGUAAGACUUGUCGAUGGCAUCAUCUCUUGCGGACUGAACCUUG

.(((((((((((((((((((((.....))))))))))))).))))).))))).))))).))))).))))).

>cro-novel35

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AGCAAGAAGUCUAUGAGUAGAGAUACGGAUCUGUUCUAUAAAAGAAUUUC

.(((((((((((((((((((((.....))))))))))))).))))).))))).))))).))))).))))).

>cro-novel36

CGAGACAUGAACCUUGAACUUAUUAACCCACUUCAUUCAUGUGGUUAAUACAUUAUCUUUUUUAUUAU
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.(((.(((.....(((.....)))))))))))))

>cro-novel37

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GAGAAUUUGACUGUUCGGAUCUUUUAACAUAUUCUGUCAUCUCUUGUAUAUUC

.(((.....)))))))))

>cro-novel38

AUGGCAUAUCCGGAAAAUAUCACGCUCAAGUUAUGUGGUCACCAUAACAAAUCAGCCAAAGACAAU
UGCAUUGUCCGGUGAGCACGUGACUUGGGCAUGGUUUAUUAAGCUGGAUUCGCCAC

.(((.....)))))))))

>cro-novel39

UUGAAAAUUAUGUUAUGAAUCUGAUUCACAUCCUCAUUCUGUAUCGUGUAGUUUAUUAUAAUAAUAG
CACAUCACCUCCGAUUGUUGAUUAUGUCUUCAAUUAUGGAGAUACGGCUUAAAUAAGACCAAUGAUUUA
UGAGGGGGACUUGGAUCAAGUUC AUGACAGAAUUUUUCAC

.(((.....)))))))))

>cro-novel4

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UGCAUAAUUAUAAUACAAGAGAGAAUGAGAGAGAAGUAGAGAUAGUACUUCGAGGUGUGUCCAACUUAU
GAAGA

.(((.....)))))))))

>cro-novel40

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CUUUAUAACAUAUUAUUUGGUGUAUUUAUUAUAUAAAACCUACCAAUAAAAGAUAAACGCUAAAGAAA
AAAGAUAAACCUAAGGGAGAAAACAUUAUUAUAAAAGGUAUGUUAACAGCAAUUGUUAUCAAUGGAUCUUGA
GUGUCAUCAAUGGGCCUCCUUAUGAGUUUAUGUUUUUUUAGC

.(((.....)))))))))

>cro-novel41

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AUGAUUAUAAAAGUAAUUAACAUGAUUCGAACCGAACUAGUAAAUAUGCAUAAACGAGUUUCAUGUGCA
UGGUAAGGUGAUAGCUCACUACUAAAAACGUAUGUUUAUUAAGUUACAGUUCUGGAUUCGAAUCACAUCU
CAUCCUCAAUAUUA

.((((...(((.....((((((((((((.....(((((((.....)))))))))((((...(((((((.....))))))))).....)))))).....))))))

>cro-novel95

AUUUAUAAUACUCAAGAUCGUCUAAUACAUCUGUAAUACACUCUCUACUUAUCUCCUUUUAAUUUAGAUUU
UAUUUUUUAUUCUAUAAGAUUUUUUAUUAAUAAAAAUUUUAUAAAUAAGUAUUUAAAAAAAAUAUA
AUAGAAGUACUAGACGAUCCGGCCAAAAUUAUAAA

.((((...(((.....((((((((((((.....(((((((.....)))))))))((((...(((((((.....))))))))).....)))))).....))))))

>cro-novel96

ACAUCUUCACCUGAGCGUUGGACAGUCUGGUGGGAAUUUCGCUUCUUAUAUAAAAAAUAAUCUGAUCU
GUGGAUCAUUGUUUAAAUUCUGACUGGAAAUUUAACCUUUCAAUAUGAUAGGUGG

.((((...(((.....((((((((((((.....(((((((.....)))))))))((((...(((((((.....))))))))).....)))))).....))))))

>cro-novel97

UCGUUUGCCAGUGAGUCGACUCGAGCUCGGUCAAUAGUAAACUCAAAACCAAGUUUUGACCGAGCAACUGU
GAUUGCUCACGAGCAACUCGC

.((((...(((.....((((((((((((.....(((((((.....)))))))))((((...(((((((.....))))))))).....)))))).....))))))

>cro-novel98

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GAUUUGGAUAAUGUUUAGAUAAAGACUUGACUGGUUUACUAUCCCUAUUAAU

.((((...(((.....((((((((((((.....(((((((.....)))))))))((((...(((((((.....))))))))).....)))))).....))))))

>cro-novel99

AUAUAAUUAUAAAAUUAUUAAUUAACACUCUCUAGUAUACACUAUUUAUAGUAAUUUUUAUUAAUAAAAU
CGUAUAGAAUAAAAGAUAAAUCUAAACUUAUAAAAAAUAAAACCCUAAGAAAAAAUAAUUAUCAAAUAAA
UGUACUAAAUAGUGUACUAGAGAAUGUAUUAGACGAUCUUGAGUGCAUAUAU

.((((...(((.....((((((((((((.....(((((((.....)))))))))((((...(((((((.....))))))))).....)))))).....))))))

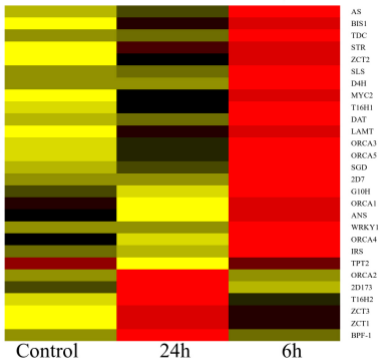


Figure S4. Heatmap of terpenoid indole alkaloid (TIA) biosynthesis pathway genes in response to different Jasmonic acid treatment regimes. The color scale represents the values of reads per kilobase of transcript per million fragments mapped (RPKM).

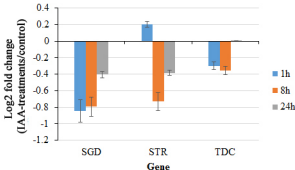


Figure S5. Quantitative real-time RT-PCR analysis of selected terpenoid indole alkaloid (TIA) biosynthetic pathway genes. The relative abundance of individual gene is presented as the ratio of auxin-treated and control samples. The mean expression value of target gene was normalized against the expression of endogenous control RPS9. The normalized expression ($2^{-\Delta CT}$) in IAA-treated sample was divided by the normalized expression in control sample and \log_2 transformed. The data represent the mean values \pm SD of three replicates.

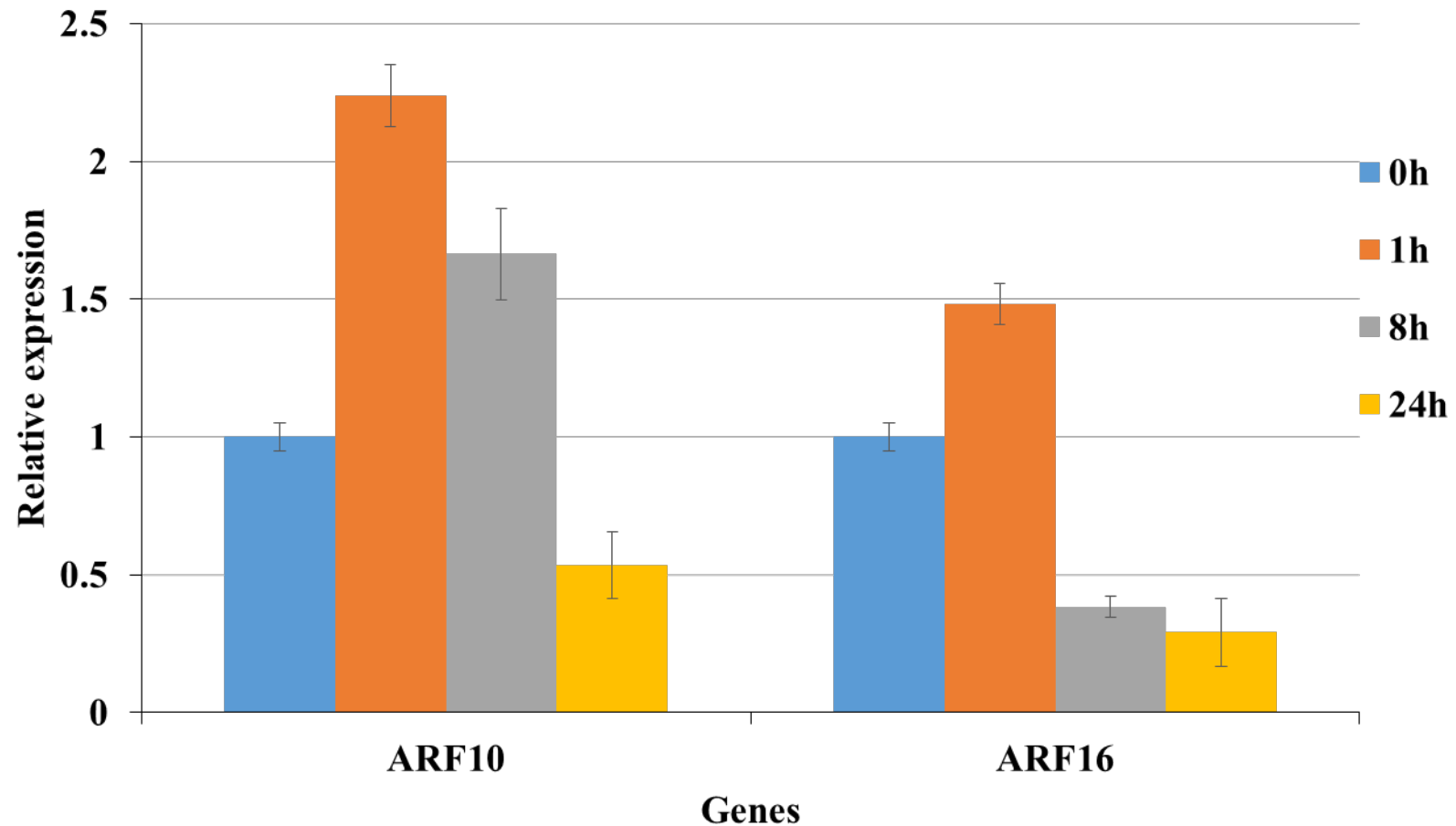


Figure S6. Relative expression of *ARF10* and *ARF16* in the control and IAA-treated *C. roseus* seedlings as measured by quantitative RT-PCR. The expression values of target genes were normalized against that of the endogenous control, RPS9. The data represent the mean \pm SD of two biological replicates.