Leaf morphology, gene analysis, and low temperature requirement for glowering of *Verbascum blattaria*

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**Table S1** Sequence of forward (F: 5’ to 3’) and reverse (R: 5’ to 3’) primer sets for nrITS and cpIS spacer and gene

|  |  |
| --- | --- |
| For nrITS: Universal ITS primer set | |
|  | F: TCCGTAGGTGAACCTGCGG |
|  | R:TCCTCCGCTTATTGATATGC |
| For *trn*L-*trn*F intergenic spacer | |
|  | F: AGAGAGGGATTCGAACCCTC |
|  | R: GAGATTTGGCAAAAAGGCTTC |
| For *trn*S-*trn*G intergenic spacer | |
|  | F: CATAATGAGATCCTAATCTCAAAAC |
|  | R: GACGACTTTGTGAGTTTCAGTAA |
| For *psb*A*-trn*Hintergenic spacer | |
|  | F: GGCGAACGACGGGAATTGAA |
|  | R: CGTAGCCGCTCATGGTTATTT |
| For maturase K (*mat*K) gene | |
|  | F: GACTGTATCGCACTATGTATCA |
|  | R: CAGAATAGTGGAAATCCCATTTTG |

**Table S2** Temperature treatment given to *Verbascum blattaria* in the a non-heated indoor before moving plants to indoor conditions and irradiance level

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| No. of days of low temperature treatment (CD) |  | Date moved  to indoor from a non-heated indoor |  | Average temperature at | | |
|  |  | 08:00 hr | 12:00 hr | 16:00 hr |
|  |  | Temperature (oC) | | |
|  |  | Maximum | Average | Minimum |
|  |  | Dec. 2, 2021 |  | 6.7 | 3.9 | 2.2 |
| 0 |  | Dec. 14, 2021 |  | 6.1 | 4.4 | 1.1 |
| 20 |  | Jan. 4, 2022 |  | 7.8 | 5.0 | 2.2 |
| 40 |  | Jan. 25, 2022 |  | 2.8 | 2.8 | 0.6 |
| 60 |  | Feb. 15, 2022 |  | 4.8 | 3.8. | 1.9 |
|  |  |  |  | Average light irradiance level (W/m2) a | | |
| Non-heated indoor |  | Dec. 2, 2021- Feb. 15, 2022 |  | 1.84 | 1.09 | 0.87 |

a Light irradiance are the average at time indicated during Dec. 14 and Mar. 8 when all temperature treatments were completed. Temperatures are the average between Dec. 14 and Jan. 3, Jan. 4 and 24, Jan. 25 and Feb. 14, and Feb. 15 and March 8.

**Table S3** Colorimetric data at various tissue z of *V. blattaria* with different color in leaves recorded on Dec. 15, 2021

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Leaf and position a | |  | L\* | |  | a\* | |  | b\* | |
|  | Brown-purple | Green |  | Brown-purple | Green |  | Brown-purple | Green |
| Leaf blade: 1 | |  | 70.2 b b | 60.3 ab |  | -8.85 b | -8.75 b |  | 16.75 de | 13.85 d |
| Leaf vein - distal end: 2 | |  | 61.55 ab | 60.4 ab |  | 7.0 e | -8.4 b |  | 7.45 c | 14.4 d |
| Leaf vein -proximal end: 3 | |  | 53.5 a | 51.9 a |  | 10.6 e | -4.25 c |  | -0.125 a | 18.75 e |
| Leaf blade: 4 | |  | 53.2 a | 51 9 a |  | -4.1 c | 0.05 d |  | 2.05 ab | 20.6 e |
| Level of significance c |  | | |  |  |  |  |  |  |  |
| Color (Co) | |  | ns |  |  | \*\*\* |  |  | \*\*\* |  |
| Position (Po) | |  | \*\*\* |  |  | \*\*\* |  |  | \*\*\* |  |
| Co × Po | |  | ns |  |  | \*\*\* |  |  | \*\*\* |  |

a Refer to the Fig. 2 for leaf position. Leaf blade close to the secondary veins (position 1), main veins as indicated at about mid-point or 1/3 point from the distal end (position 2), and also main veins close to the crown (proximal end) (position 3), and leaf B blade of the leaves of leaves formed after the leaf A (position 4)

b Mean comparison by HSD at 0.01%.

c ns, \*\*\*; non-significant and significant at *p*<0.001.

**Table S4** Data collection sites at various organs of *Verbascum blattaria* flower for colorimetric measurement.

Photo was taken from flowers grown in the garden, College Station, TX

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Positions indicated in the image | Value of CIELAB CIE coordinates | | |  | Image showing positions for colorimetric measurements |
|  | L\* | a\* | b\* |  |  |
| 1; corolla – near the tip | 84.5 | -3.6 | 0.6 |  |
| 2; corolla peripheral side at the base | 54.3 | -6.9 | 25.4 |  |
| 3; corolla close to the base | 34.9 | 21.9 | 4.3 |  |
| 4; Filament | 34.4 | 13.1 | 5.2 |  |
| 5: Anther | 65.4 | 13.3 | 39.5 |  |
| 6; corolla - Center | 78.5 | -4.1 | 11.3 |  |
| 7; Adaxial side of petal - center | 79.9 | -0.7 | 1.9 |  |
| 8; Flower bud - tip | 60.8 | 2.9 | 14.5 |  |
| 9; Flower bud - base | 65.1 | -7.1 | 19.4 |  |
| 10; Calyx | 59.5 | -19.2 | 26.4 |  |
| 11; Stem | 57.9 | -21.2 | 27.2 |  |
| 12; Adaxial side of corolla petal - center | 84.6 | -3.3 | 0.7 |  |

**Table S5** Analysis of macro- and micro-elements in leaf tissues of Verbascum blattaria grown in garden in College Station, Texas and in pots in Ann Arbor, Michigan

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Source of tissue | Macro-elements (%) | | | | | |  | Micro-elements (ppm) | | | | | |
| N | P | K | Ca | Mg | S |  | Fe | Mn | B | Cu | Zn | Mo |
| Garden -Texas | 4.2 | 0.59 | 4.02 | 1.22 | 1.15 | 0.12 |  | 134 | 40.8 | 39.1 | 9.9 | 59.4 | 0.63 |
| Pot – Ann Arbor - normal a | 1.3 | 0.22 | 0.97 | 0.53 | 0.45 | 0.06 |  | 51 | 35.3 | 24.6 | 3.6 | 14.9 | 0 |
| Pot – Ann Arbor - abnormal | 1.8 | 0.26 | 1.34 | 0.42 | 0.39 | 0.05 |  | 50 | 28.9 | 27.3 | 5.0 | 18.2 | 0 |
| Suggested range b | 3.5-5.5 | 0.35-1 | 2.0-8.8 | 0.8-3 | 0.2-1.5 | 0.2-0.8 |  | 60-200 | 50-200 | 30-150 | 5-25 | 30-50 | 0.2-5 |

a Leaves with brown spots (abnormal) and without brown spots (normal) when grown in pot. Refer to Fig. 3, frame C.

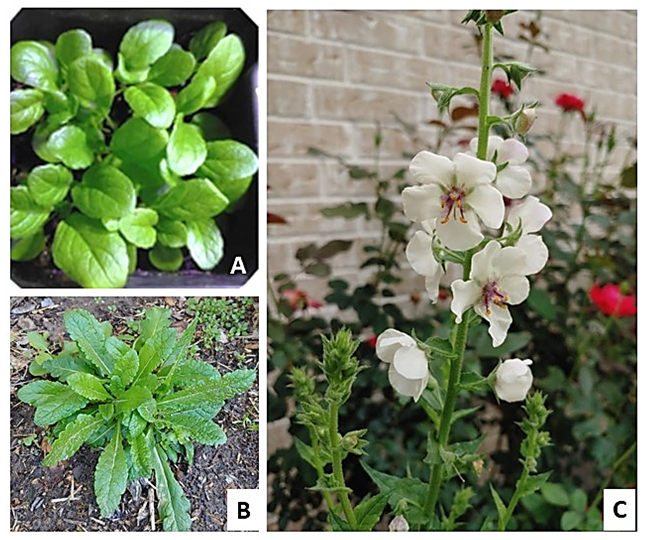
b JR Peters Inc., (Allentown, PA, USA) for general horticultural crops.

**Table S6** Nucleotide blast identification finding regions of similarity between biological sequences with greater than 95% identification with nrITS of *Verbascum blattaria*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Accession |  | Identification |  | [Maximum score](https://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Get&ADV_VIEW=yes&ADV_VIEW=on&ALIGNMENTS=100&ALIGNMENT_VIEW=Pairwise&CONFIG_DESCR=Ds,ClustMemNbr,ClustTaxNbr,ClustComn,Sc,Ms,Ts,Cov,Eval,Idnt,AccLen,Acc&DATABASE_SORT=0&DESCRIPTIONS=100&DYNAMIC_FORMAT=on&FIRST_QUERY_NUM=0&FORMAT_NUM_ORG=1&FORMAT_OBJECT=Alignment&FORMAT_PAGE_TARGET=&FORMAT_TYPE=HTML&GET_SEQUENCE=yes&I_THRESH=&LINE_LENGTH=60&MASK_CHAR=2&MASK_COLOR=1&NUM_OVERVIEW=100&PAGE=MegaBlast&QUERY_INDEX=0&QUERY_NUMBER=0&RESULTS_PAGE_TARGET=&RID=5KUATKDG013&SHOW_LINKOUT=yes&SHOW_OVERVIEW=yes&STEP_NUMBER=&ADV_VIEW=on&DISPLAY_SORT=1&HSP_SORT=1) | [Percent ident](https://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Get&ADV_VIEW=yes&ADV_VIEW=on&ALIGNMENTS=100&ALIGNMENT_VIEW=Pairwise&CONFIG_DESCR=Ds,ClustMemNbr,ClustTaxNbr,ClustComn,Sc,Ms,Ts,Cov,Eval,Idnt,AccLen,Acc&DATABASE_SORT=0&DESCRIPTIONS=100&DYNAMIC_FORMAT=on&FIRST_QUERY_NUM=0&FORMAT_NUM_ORG=1&FORMAT_OBJECT=Alignment&FORMAT_PAGE_TARGET=&FORMAT_TYPE=HTML&GET_SEQUENCE=yes&I_THRESH=&LINE_LENGTH=60&MASK_CHAR=2&MASK_COLOR=1&NUM_OVERVIEW=100&PAGE=MegaBlast&QUERY_INDEX=0&QUERY_NUMBER=0&RESULTS_PAGE_TARGET=&RID=5KUATKDG013&SHOW_LINKOUT=yes&SHOW_OVERVIEW=yes&STEP_NUMBER=&ADV_VIEW=on&DISPLAY_SORT=3&HSP_SORT=3)ification | [Accession len](https://blast.ncbi.nlm.nih.gov/Blast.cgi)gth (bp) |
|  |  |
| [OM996045.1](https://www.ncbi.nlm.nih.gov/nucleotide/OM996045.1?report=genbank&log$=nucltop&blast_rank=1&RID=5KUATKDG013) |  | *V. blattaria* |  | 1190 | 100% | 644 |
| [KP738147.1](https://www.ncbi.nlm.nih.gov/nucleotide/KP738147.1?report=genbank&log$=nucltop&blast_rank=2&RID=5KUATKDG013) |  | *V. cheiranthifolium* |  | 1086 | 97% | 779 |
| [KP738150.1](https://www.ncbi.nlm.nih.gov/nucleotide/KP738150.1?report=genbank&log$=nucltop&blast_rank=3&RID=5KUATKDG013) |  | *Verbascum gossypinum;* |  | 1081 | 97% | 692 |
| [MW546364.1](https://www.ncbi.nlm.nih.gov/nucleotide/MW546364.1?report=genbank&log$=nucltop&blast_rank=4&RID=5KUATKDG013) |  | *V. virgatum* |  | 1081 | 99% | 656 |
| [JQ801746.1](https://www.ncbi.nlm.nih.gov/nucleotide/JQ801746.1?report=genbank&log$=nucltop&blast_rank=5&RID=5KUATKDG013) |  | *V. thapsus* |  | 1050 | 97% | 689 |
| [KX640974.1](https://www.ncbi.nlm.nih.gov/nucleotide/KX640974.1?report=genbank&log$=nucltop&blast_rank=6&RID=5KUATKDG013) |  | *V. thapsus* |  | 1035 | 97% | 667 |
| [KP738153.1](https://www.ncbi.nlm.nih.gov/nucleotide/KP738153.1?report=genbank&log$=nucltop&blast_rank=7&RID=5KUATKDG013) |  | *V. kermanense* |  | 1035 | 96% | 806 |
| [HE602435.1](https://www.ncbi.nlm.nih.gov/nucleotide/HE602435.1?report=genbank&log$=nucltop&blast_rank=8&RID=5KUATKDG013) |  | *V. sinuatum* |  | 1035 | 97% | 667 |
| [KP738156.1](https://www.ncbi.nlm.nih.gov/nucleotide/KP738156.1?report=genbank&log$=nucltop&blast_rank=9&RID=5KUATKDG013) |  | *V. phlomoides* |  | 1020 | 95% | 864 |
| [HE602436.1](https://www.ncbi.nlm.nih.gov/nucleotide/HE602436.1?report=genbank&log$=nucltop&blast_rank=10&RID=5KUATKDG013) |  | *V. dentifolium* |  | 1020 | 97% | 669 |
| [AJ550576.1](https://www.ncbi.nlm.nih.gov/nucleotide/AJ550576.1?report=genbank&log$=nucltop&blast_rank=11&RID=5KUATKDG013) |  | *B. madagascariensis* |  | 1013 | 95% | 806 |

**Table S7** Nucleotide blast identification finding regions of similarity between biological sequences with greater than 95% identification with cpIS of *Verbascum blattaria*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Accession  Sequence ID |  | Identification |  | [Maximum score](https://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Get&ADV_VIEW=yes&ADV_VIEW=on&ALIGNMENTS=100&ALIGNMENT_VIEW=Pairwise&CONFIG_DESCR=Ds,ClustMemNbr,ClustTaxNbr,ClustComn,Sc,Ms,Ts,Cov,Eval,Idnt,AccLen,Acc&DATABASE_SORT=0&DESCRIPTIONS=100&DYNAMIC_FORMAT=on&FIRST_QUERY_NUM=0&FORMAT_NUM_ORG=1&FORMAT_OBJECT=Alignment&FORMAT_PAGE_TARGET=&FORMAT_TYPE=HTML&GET_SEQUENCE=yes&I_THRESH=&LINE_LENGTH=60&MASK_CHAR=2&MASK_COLOR=1&NUM_OVERVIEW=100&PAGE=MegaBlast&QUERY_INDEX=0&QUERY_NUMBER=0&RESULTS_PAGE_TARGET=&RID=5KUATKDG013&SHOW_LINKOUT=yes&SHOW_OVERVIEW=yes&STEP_NUMBER=&ADV_VIEW=on&DISPLAY_SORT=1&HSP_SORT=1) | Range 1 | Per. Ident. |
|  |  |
| *trn*S-*trn*G intergenic spacer | | |  |  |  |  |
| [NC\_053691.1](https://www.ncbi.nlm.nih.gov/nucleotide/NC_053691.1?report=genbank&log$=nuclalign&blast_rank=1&RID=5M2YW35X016) |  | *V. thapsus* chloroplast, complete genome; *V. thapsus* chloroplast *trn*S-*trn*G intergenic spacer region |  | 1441 | 8221 to 9000 | 100 |
| [NC\_051533.1](https://www.ncbi.nlm.nih.gov/nucleotide/NC_051533.1?report=genbank&log$=nuclalign&blast_rank=2&RID=5M2YW35X016) |  | *V. chinense* voucher 1907005 chloroplast, complete genome |  | 1373 | 8318 to 9095 | 99 |
| [MT610040.1](https://www.ncbi.nlm.nih.gov/nucleotide/MT610040.1?report=genbank&log$=nuclalign&blast_rank=3&RID=5M2YW35X016) |  | *V. chinense* chloroplast, complete genome |  | 1373 | 3 to 663 | 99 |
| [KP738133.1](https://www.ncbi.nlm.nih.gov/nucleotide/KP738133.1?report=genbank&log$=nuclalign&blast_rank=4&RID=5M2YW35X016) |  | *V. armenum* *trn*S-*trn*G intergenic spacer, partial sequence; chloroplast |  | 1179 | 3 to 663 | 98.8 |
| [MW618406.1](https://www.ncbi.nlm.nih.gov/nucleotide/MW618406.1?report=genbank&log$=nuclalign&blast_rank=5&RID=5M2YW35X016) |  | *V. virgatum* isolate IS724 *trn*S-*trn*G intergenic spacer region, partial sequence; chloroplast |  | 1162 | 129 to 766 | 99 |
| *trn*L-*trn*F intergenic spacer | | |  |  |  |  |
| KM385520.1 |  | *Verbascum thapsus* chloroplast *trn*L-*trn*F intergenic spacer |  | 1123 | 8337 to 8976 | 98 |
| [NC\_051533.1](https://www.ncbi.nlm.nih.gov/nucleotide/NC_051533.1?report=genbank&log$=nuclalign&blast_rank=3&RID=5M41R3G9013) |  | *V.*  *chinense* |  |  |  |  |
| *trn*H-*psb*A intergenic spacer | | |  |  |  |  |
| NC 053691.1 |  | *V. thapsus* chloroplast, complete genome |  | 1552 | 1 to 840 | 100 |
| [HQ596879.1](https://www.ncbi.nlm.nih.gov/nucleotide/HQ596879.1?report=genbank&log$=nuclalign&blast_rank=11&RID=5M4APTTD016) |  | *V.* thapsus intergenic spacer |  |  |  | 97 |
| maturase K (*mat*K) gene | | |  |  |  |  |
| [NC\_053691.1](https://www.ncbi.nlm.nih.gov/nucleotide/NC_053691.1?report=genbank&log$=nuclalign&blast_rank=1&RID=5M4T4GUN01R) |  | *V. thapsus* chloroplast, complete genome |  | 3214 | 1981 to 3720 | 100 |
| KT176609.1 |  | *V. thapsus* plastid maturase K (*mat*K) gene |  | 2826 | 1 to 1530 | 100 |



**Fig. S1.** *Verbascum blattaria* grown at the home garden, College Station, Texas, USA. Three to five seedlings in a clump (frame A) were transplanted on Nov. 7, 2021, and became rosette (frame B) between Mar. 4, and flowered (frame C) on May 7, 2022. Plant heights varied between 90 and 120 cm.