Scientific Research Publishing

American Journal of Plant Sciences, 2017, 8, 1921-1952
http://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

Evaluation of a *Brassica napus* Auxin-Repressed Gene Induced by Flea Beetle Damage and *Sclerotinia sclerotiorum* Infection

Limin Wu[1,2], Min Yu[2], Jennifer Holowachuk[2], Andrew Sharpe[2,3], Derek Lydiate[2], Dwayne Hegedus[2], Margaret Gruber[2]

[1]InnoTech Alberta, Vegreville, Canada
[2]Saskatoon Research Centre, Agriculture and Agri-Food Canada, Saskatoon, Canada
[3]Global Institute for Food Security, University of Saskatchewan, Saskatoon, Canada
Email: Limin.Wu@innotechalberta.ca

How to cite this paper: Wu, L., Yu, M., Holowachuk, J., Sharpe, A., Lydiate, D., Hegedus, D. and Gruber, M. (2017) Evaluation of a *Brassica napus* Auxin-Repressed Gene Induced by Flea Beetle Damage and *Sclerotinia sclerotiorum* Infection. *American Journal of Plant Sciences*, **8**, 1921-1952.
https://doi.org/10.4236/ajps.2017.88130

Received: June 17, 2017
Accepted: July 24, 2017
Published: July 27, 2017

Abstract

Biotic stresses negatively affect canola growth and production. Flea beetle damage and *Sclerotinia sclerotiorum* (*S. sclerotiorum*) infection are two of the worst biotic stresses for canola. *Auxin Repressed Proteins* (*ARPs*) responsive to several abiotic stresses have been reported. However, information about *ARPs* induced by Flea beetle damage and *S. sclerotiorum* infection, their roles in biotic stress tolerance are still lacking in canola. ESTs for an *Auxin Repressed Protein* 1 (*BnARP*1) were highly represented (expressed) in a *Brassica napus* subtractive library developed after leaf damage by the crucifer flea beetle (*Phyllotreta cruciferae*). Expression of this gene was under different developmental control in *B. napus*, and it was co-induced in *B. napus* by flea beetle feeding, *S. sclerotiorum* infection, drought and cold. A total of 25 *BnARP* genes were represented in different *B. napus* stress and development EST libraries and indicated larger, diversified families than known earlier. Dwarf phenotypes, primary root growth inhibition, lateral root enhancement, reduced sensitivity to 2, 4-D, and reduced *PIN*1 and *LOX* expression in transgenic Arabidopsis expression lines suggest that *BnARP*1 is an auxin repressor that prevents auxin transport and supports an interaction between the auxin and jasmonate signalling pathways. And the increased survival after *S. sclerotiorum* infection in transgenic over-expression Arabidopsis suggests that *BnARP*1 could play a role in *S. sclerotiorum* tolerance through connecting auxin and jasmonate signalling pathways.

Keywords

BnARP, *Brassica napus*, Flea Beetle, *S. sclerotiorum*

1. Introduction

Plant resistance or tolerance to biotic stress consists of constitutive or induced defense mechanisms, and inducible defense is thought to be more durable than constitutive defense [1] [2]. From a molecular perspective, it is clear that a plant's response to challenge by insects and fungi is mediated by a network of cross-talking pathways and not by simple linear signal transduction cascades [2]. This network includes the jasmonic acid (JA), salicylic acid (SA), ethylene, abscisic acid (ABA) and auxin signalling pathways and presumably enables a coordinated, specific response. JA and SA function as key signaling molecules, which activate distinct sets of defense-related genes in response to herbivore damage. For example, aphid feeding induces SA-dependent transcription of *Pathogenesis-Related protein* 1 (*PR*-1) and *β*-1, 3-*glucanase* (*BGL*2), as well as *Plant Defensin Factor* 1.2 (*PDF*1.2) and *Lipoxygenase* 2 (*LOX*2), both of which are involved in the JA signaling cascade [3] [4]. JA and ethylene can be either synergistic or antagonistic hormones in terms of the expression of defensive genes in response to insect attack [5] [6] [7] [8], while both of these hormones mediate against pathogen attack (partly by defense gene induction) [9]. Curiously, plants deficient in ethylene signaling show either increased susceptibility or increased resistance, depending on the plant and pathogen [10]. ABA-dependent signaling is also well known for regulating abiotic stress-induced gene expression [11], and ABA antagonizes JA/ethylene-responsive defense gene expression and modulates disease resistance in Arabidopsis [12].

Auxin plays a central role in the growth and development of plants, including stem elongation, lateral branching of roots and shoots, establishment of embryonic polarity, and vascular development [13]. Recent studies in Arabidopsis have shed light on several gene families involved in the auxin signaling pathway [14] [15]. These include the Auxin/Indole-3-Acetic Acid repressor family (*Aux/IAAs*) which encodes short-lived nuclear proteins that regulate auxin responsive genes through dimerization between family members and interaction with the auxin response factor (*ARF*) family [16] [17]. *ARFs* bind to auxin response elements (*AREs*) in the promoters of auxin-inducible genes to regulate their expression [18]. *Ethylene Insensitive Root* 1 (*EIR*1) is one of the *PIN-FORMED* (*PIN*) family of auxin efflux carriers which are responsible for the active, directional transport of auxin through plant tissues [19]. A defective response to jasmonate in the auxin-signaling mutant axr1 (*AUXIN-RESISTANCE* 1 gene) provides a mechanistic link between JA and auxin-signaling pathways and integration of diverse defense pathways mediated by JA, ethylene and SA [20].

Auxin repressed proteins (*ARPs*) are also known. These include development-related *ARPs* such as the fruit repression *SAR*5 from strawberry [21], the dormancy-associated *SbDRM*1 from sorghum and pea [22] [23], a germination-related gene from tobacco [24], a hypocotyl elongation-related *RpARP* from black locust [25], the auxin-induced *EuNOD-ARP*1 gene from *Elaeagnus umbellate* root nodules [26], and the cucumber *CsGRP*1, which accumulates on the upper

side of seedlings during gravimorphogenesis [27]. Stress-responsive *ARPs* include the drought/heat-induced *AtARP*1 from Arabidopsis [28], a gene induced by salinity and cold from hot pepper [29], five genes responsive to drought/cold/high-salinity from chickpea [30], the salinity/drought-responsive *BnARP* from *B. napus* [31], and two genes responsive to chilling, heat shock and salt stress (*BrARP*1 and *BrDRM*1) from *B. rapa* [32]. However, the role of *ARPs* that mediate resistance or susceptibility to insects and fungi is unknown.

In the present study, we explored the roles of one auxin-repressed *BnARP*1 gene. *BnARP*1gene was strongly induced under several common stress conditions. Curiously, the introduction of the *BnARP*1 transgene inhibited apical dominance and primary root growth, and increased lateral root numbers when expressed in Arabidopsis. Expression in Arabidopsis of *BnARP*1 yielded plants with improved survival after infection with a fungus.

2. Materials and Methods

2.1. Plant Materials

Expression analysis, bioassays, and EST library development for *Brassica napus* were conducted using the double haploid line DH12075 (derived from a cross between the blackleg-resistant canola cultivar Cresor and the susceptible cultivar Westar, G. Seguin Schwartz and G. Rakow, formerly of AAFC Saskatoon Research Centre). Transgenic Arabidopsis over-expression (OE) lines were developed in a Columbia (Col-0) ecotype.

2.2. Methods

2.2.1. EST Subtraction Library Development, Screening, and Bioinformatics

Total RNA was isolated with guanidine hydrochloride from undamaged 8-week-old *B. napus* leaves and from flea beetle-damaged *B. napus* leaves of the same age. Leaves were damaged to 10-20% of the tissue mass after a 24 h laboratory feeding bioassay using wild *Phyllotreta cruciferae* flea beetles collected from a turnip field in Saskatoon. The two sets of RNA were treated with RQ1 RNAase-free DNAase (Promega, Madison, Wisconsin, USA) and poly (A) $^{+}$ RNA isolated using Oligotex (Qiagen, Toronto, Ontario, Canada) following the manufacturer's instructions. The cDNA synthesis and subtraction were performed according to the protocol provided in the PCR-select cDNA Subtraction Kit from Clontech Laboratories (Mountain View, CA, USA). In brief, the tester (damaged tissue) and driver (undamaged tissue) cDNA populations were digested separately with Rsa I to obtain shorter (~100 - 1200 bp), blunt-ended molecules. Tester cDNA was ligated to different adaptors to create two tester populations, which were hybridized separately with excess driver cDNAs to generate the templates required for PCR amplification of differentially expressed cDNAs. PCR subtractive amplicons were cloned in a pGEM-T easy vector (Promega, Madison, WI, USA). The subtraction library was annotated by BLAST analysis to the

Arabidopsis database (TAIR) and analyzed by bioinformatics according to Gruber *et al.* [33]. Other *B. napus* stress or development EST libraries identified in Gruber *et al.* were developed using a modified pSPORT vector and the SuperScript Plasmid System with Gateway Technology [33]. All EST libraries were deposited at http://brassicagenomics.ca. *BnARP* sequences detected in the flea beetle (FB)-damaged leaf subtractive library were assessed for representation and homologues in other EST libraries using reciprocal best hit (RBH) BLAST analysis [34]. Alignments of translated sequences for *BnARPs* were performed using the BioEdit multiple sequence alignment program (http://www.mbio.ncsu.edu/bioedit/bioedit.html).

2.2.2. Transformation of Arabidopsis with *BnARP*1 Binary Vector

*BnARP*1 full-length coding regions (318 bp, GenBank accession number: KM821273) were amplified by PCR from cDNA of 8-week-old *B. napus* leaves using primers described in **Supplementary Table 1**. Amplicons were sequenced and those specifying *BnARP*1 were then cloned as XbaI-SstI fragments downstream of the *CaMV* 35*S* promoter in binary transformation vector pBI121 (Clontech, Mountain View, CA, USA). The binary plasmids were introduced into *Agrobacterium tumefaciens* by electroporation. For plant transformation, wild type Arabidopsis seeds were sown on a wet masked soil mixture in pots. Pots were placed in the dark at 4˚C for 2 days and then moved to an environmentally controlled greenhouse at 22˚C with 16 h light and 8 h dark supplemented with halogen lamps. For single binary vector transformation, flowering Arabidopsis plants were transformed with the *Agrobacterium* using the floral dip method [35].

2.2.3. Molecular Analysis

T_1 transgenic Arabidopsis plants were selected by growth on half MS plate with kanamycin and detection of the transgene fragment after PCR analysis with specific primers (**Supplementary Table 1**) using genomic DNA isolated from rosette leaves by alkaline lysis [36]. Transgenic plants and transgene insertion patterns were confirmed by Southern blot analysis using a CTAB extraction method for genomic DNA isolation [37]. Southern blotting and hybridization were performed on Hybond N nylon membranes (Roche Diagnostics GmbH, Mannheim, Germany) as previously described [38] using 10 μg DNA (per sample) digested overnight with EcoRI, Hind III or XhoI. The 318-bp *BnARP*1 coding sequence described above was labeled as probes with ^{32}P-dCTP using a random-primer labeling kit (Invitrogen, Carlsbad, CA, USA). Seed from 10 independently transformed T_1 plants selected by PCR of the transgene were used to develop T_2 homozygous plants used for bioassays.

For Northern blot analysis of transgenic or non-transgenic plants, tissue samples were collected from a range of developmental stages or stress treatments (detailed below), then frozen immediately in liquid N_2. Total RNA was extracted from tissues as described by Sambrook *et al.* [39] and separated on 1% agarose

gels (30 µg per lane for *B. napus*; 10 µg for Arabidopsis). Northern membranes were prepared and hybridized as described by Sambrook *et al.* [39]. Probes were prepared from PCR products developed using specific primers detailed in **Supplementary Table 1**, then labeled with ^{32}P-dCTP as above. Membranes were washed twice at 65˚C in 2X SSC-0.5% SDS for 15 min each and then for 30 min at 65˚C in 0.2X SSC-0.5% SDS with gentle shaking, followed by exposure to X-ray film for 3 h. The Northern blots were replicated twice using independent RNA preparations.

2.2.4. Root and Auxin Assays

Transgenic Arabidopsis seedling root growth was assayed on T_2 homozygous plants growing on Murashige and Skoog (MS) agar plates as described previously [40]. Plates containing seeds were chilled for 2 days at 4˚C before being placed in a continuously illuminated incubator at 21˚C. Primary root length of 20 seedlings per genotype was measured 4, 7 and 14 days later and the entire experiment repeated 3 times. For root sensitivity to auxin, 4-day-old seedlings were transferred to vertically oriented MS agar plates containing 2, 4-D (0.0 - 1.6 µM). The number of lateral roots of 10 seedlings per each genotype were counted after an additional 3 d of growth and the entire experiment was repeated 3 times [41].

2.2.5. Flea Beetle Feeding Bioassays

Adult *Phyllotreta cruciferae* flea beetles (from a spring population) were collected and maintained in white-walled cabinets (housed in clear plastic cages) on cabbage and water for up to one week at 20˚C, 16 h photoperiod, 100 µmol $m^{-2}{\cdot}s^{-1}$, then starved for 24 h prior to using them. To obtain FB damaged leaves (for subtraction library development and Northern blots) and cotyledons (for Northern blots), *B. napus* plants were grown in a soil-less mixture in 10 cm pots in a greenhouse supplemented with halogen lamps for 8 weeks (leaves). To test *B. napus* seedling damage by FB, one week-old seedlings were grown in small cylinders (for cotyledons). Plants and seedlings were pre-selected for uniformity and then evenly spaced in a foam-based arena placed inside a 50 × 50 × 50 cm clear plastic cage and exposed to 400 flea beetles per cage for 0 - 24 h in an uniformly lit, white-walled, controlled environment chamber (20˚C, 16 h photoperiod, 100 µmol·$m^{-2}{\cdot}s^{-1}$; 65% relative humidity). Damage on leaves or cotyledons was scored using a rating scale from 0 (denoting no damage) to 10 (denoting the entire tissue destroyed) as described in Palaniswamy *et al.* [42]. Thus, a rating of 1 was assigned if approximately 10% of the area of the tissue was damaged. Leaf and cotyledon tissues were collected and frozen in liquid N_2, then stored at −80˚C. For bioassays on transgenic Arabidopsis expression lines and non-transgenic lines, eight (8) T_2 seedlings per transgenic line, plus WT and empty vector control lines, were grown in randomized rows for 12 days in the same white-walled growth cabinet in 96-well micro-titre plate arenas; then bio-assayed with 50 FBs per arena fitted with a modified top and scored after 24 h for damage as described in Hallett *et al.* [43]. Each bioassay experiment was repeated 3 times.

2.2.6. Cold and Freezing Bioassays

One-week-old transgenic and non-transgenic Arabidopsis seedlings grown in MS were placed in a 4˚C chamber with a 16 h photoperiod (approx. 100 μmol m^{-2}·sec^{-1}) for 24 h. Non-acclimated 7-day-old T_2 transgenic Arabidopsis seedlings and cold-acclimated transgenic plants were grown in petri dishes with MS. Plants were placed at −2˚C in the dark in controlled temperature freezing chamber for 3 h, after which freezing of the plates was nucleated with ice chips as previously described [44]. The plants were then incubated an additional 21 h at −2˚C, followed by −5˚C for 24 h. The temperature was then raised to 4˚C for 24 h and plants allowed to recover at 24˚C for a further 48 h in continuous light (approx. 100 μmol·m^{-2}·sec^{-1}). The entire experiment was repeated 3 times in a randomized design and then scored for survival.

2.2.7. Mechanical Wounding Assay

Seeds of *B. napus* were sown in potting medium and grown in greenhouse pots at 22˚C with 16 h light and 8 h dark supplemented with halogen lamps for 4 weeks. Fully expanded leaves of the plants were punctured with a sterile forceps and post-wounded plants incubated for one hour.

2.2.8. Drought Tolerance Bioassay

Transgenic and non-transgenic Arabidopsis plants were grown in a soil-less potting mixture (one plant per pot; 40 pots per flat) in a greenhouse at 22˚C with 16 h light and 8 h dark supplemented with halogen lamps. After 3 weeks of growth with one watering per day (until draining), water was withheld for 9 days and then all pots were re-watered daily (until draining) and plant re-growth scored 4 days later. The bioassay was laid out in a randomised design, with twenty plants per line and four biological replicates, means (± standard error) were separated using t-tests at $p < 0.05$.

2.2.9. *S. sclerotiorum* Infection Test

A modified *S. sclerotiorum* infection method was used based on a spray bioassay previously described by Pedras and Ahiahinu [45]. Transgenic and control Arabidopsis seedlings were grown in a potting mixture (5 seedlings in one pot representing one line) with pots placed in a randomized design in 50 cm × 25 cm trays (4 pots per line; total of 50 pots per tray). *S. sclerotiorum* was obtained from Dr. Fengqun Yu at the Saskatoon Research Centre, and liquid cultures were initiated with five sclerotia per 100 ml of potato dextrose broth (PDB) media and shaken (110 rpm) at 20˚C for 7 days. Trays of three-week-old Arabidopsis plants were uniformly sprayed for 1 min with the fresh *S. sclerotiorum* inoculum (500 ml per tray) using a hand sprayer. The pots were then incubated in a growth chamber at 24˚C with 16 h light and 8 h dark supplemented with halogen lamps for 7 days and examined visually for disease symptoms and survival.

2.2.10. Statistical Analysis

Analysis of variance was conducted using LSD tests in SAS ver 9.0 [46]. Means (±standard error) were separated using *t*-tests at $p < 0.05$.

3. Results and Analysis

3.1. *BnARPs* Represent Differently in Tissue-Specific or Stress-Responsive EST Libraries

A survey was conducted of genes induced in a FB damaged leaf subtractive EST library developed from eight-week-old *B. napus* leaves damaged by crucifer flea beetle feeding. ESTs coding for an *Auxin Repressed Protein BnARP*1 were much more strongly represented in this library than in a wide range of other *B. napus* tissue-specific or stress-responsive EST libraries (Table 1). In fact, *BnARP*1 was represented by 17 ESTs (1.32%) of a total of 1292 ESTs in the FB leaf damaged library. *BnARPs* from the FB damaged leaf subtraction library were present in low abundance in the etiolated seedling library (0.02%), a flea beetle damaged cotyledon library (0.03%), a *S. sclerotiorum* infected stem library (0.09%), an early anther library (0.03%), and moderately expressed in a senescent leaf library (0.28%). We predicted that the *BnARP*1 is the major representative of *BnARPs* in the FB damaged leaf subtraction library and may function mainly in flea beetle-host plant interactions.

3.2. *ARP* Comprises Gene Families in *Brassica napus*

The *B. napus* flea beetle damaged leaf subtractive library included a total of 31 *ARP* ESTs. These ESTs were classified into distinct genes using the criteria that ESTs represented the same gene if sequences were ≥90% identical (out of a total of 200 bp) [47]. The FB leaf subtractive library contained four *ARP* proteins

Table 1. *ARP* representation within Saskatoon *Brassica napus* EST Librarie.

Tissue or Development Stage												
Tissue Source	AM	R	S	FB	MFB	MF	VEA	EA	E	C	YL	SF
[a]Individual *ARP* ESTs	12(2), 23(1), 24(1)	ND	14(2)	5(2)	ND	21(1), 22(1)	25(1)	1(1), 9(1), 10(1)	8(1), 11(1), 12(1), 13(1)	ND	ND	1(3)
Total ESTs	4844	11250	2798	6014	3051	6711	3680	3263	5498	3838	4763	1055
ARP Representation	0.08%	0.00%	0.07%	0.03%	0.00%	0.03%	0.03%	0.09%	0.07%	0.00%	0.00%	0.28%
Stress												
Tissue Source	Es1	Es2	Cald	Call	DR	DL	M-wls	Fb-dls	Fb-dc	S-is		
[a]Individual *ARP* ESTs	1(1), 13(1), 14(3), 15(4), 16(1), 17(1) 18(1)	2(1), 5(1), 7(1), 8(1), 14(2), 15(3), 19 (2), 20 (1)	6(1)	ND	ND	ND	ND	1(17), 2(11), 3(2), 4(1)	4(1), 7(1), 8(1)	2(1)		
Total ESTs	5551	5116	6012	7914	6577	5941	933	1292	3762	1106		
ARP Representation	0.22%	0.23%	0.02%	0.00%	0.00%	0.00%	0.00%	2.40%	0.08%	0.09%		

[a]Unbracketed numbers 1 through 25 indicates 25 unique genes (out of 81 ESTs in total for all the libraries). Bracketed number ( ) shows the frequency of each specific *ARP* within each EST library (http://brassica.ca and http://brassicagenomics.ca). The coloured highlights indicate the *ARPs* from the flea beetle damaged leaf subtraction library. ND, not detected. AM: Apical Meristem, R: Root, S: Stem, FB: Flower Bud, MFB: Mature Flower Bud, MF: Mature Flower, VEA: Very Early Anther, EA: Early Anther, E: Embryo, C: Cotyledon, YL: Young Leaf, SF: Senescent Leaf, Es1: Etiolated seedling (vector 1), Es2: Etiolated seedling (vector 2), Cald: Cold-acclimation-leaf (dark), Call: Cold-acclimation-leaf (light), DR: Drought (Root), DL: Drought (Leaf), M-wls: Mechanical-wound leaf subtraction, Fb-dls: Flea beetle-damaged leaf subtraction, Fb-dc: Flea beetle-damaged cotyledon, S-is: *S. sclerotiorum*-infected stem.

(Table 1) that clustered into one sub-group within 5 major groups comprising all the *BnARP* proteins identified by this study (Figure 1(a)). The 25 *BnARPs* match with orthologs of 8 *BrARPs* (A genome *ARPs* of *Brassica rapa*), 8 *BoARPs* (C genome *ARPs* of *Brassica oleracea*) and 4 Arabidopsis *ARPs* (Figure 1(a), Figure S1, Supplementary Table 2). *BnARP*1 (with the most highly represented

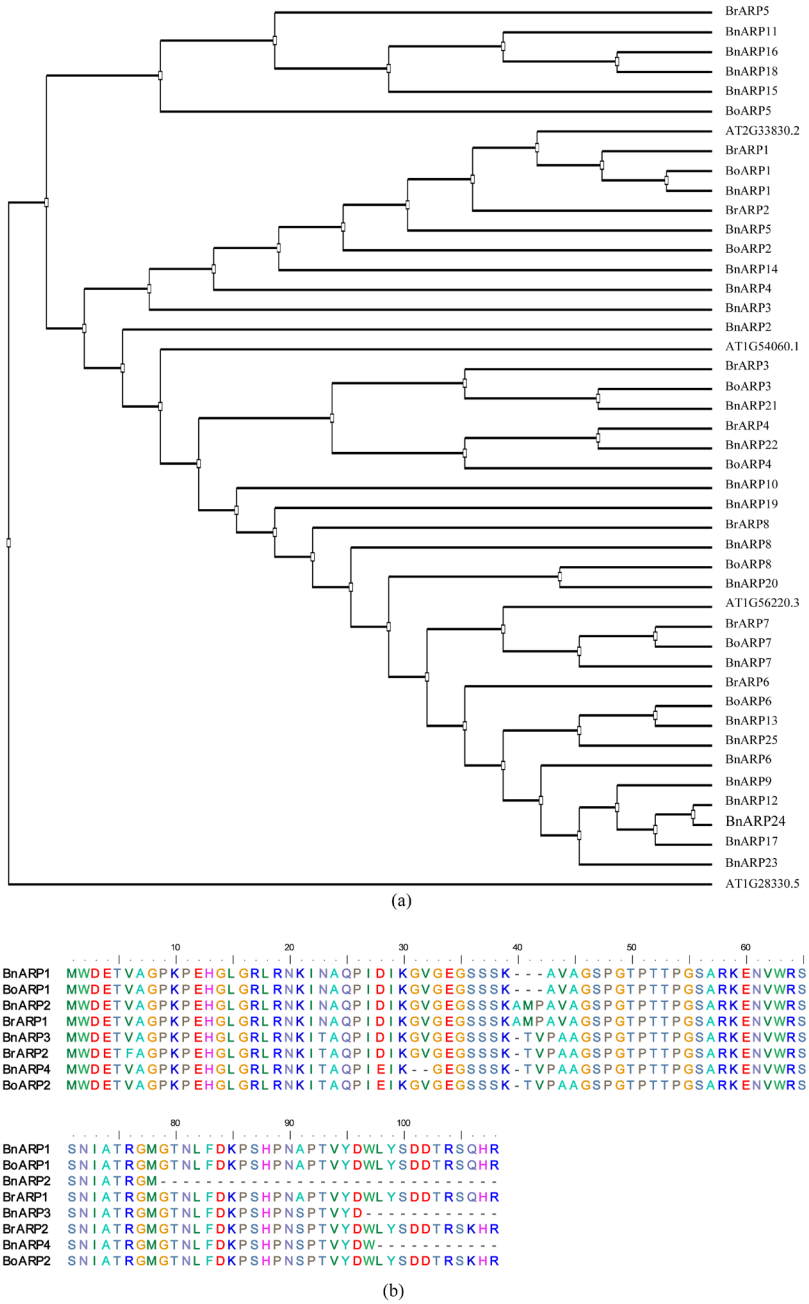


Figure 1. (a) Phylogenetic amino acid analysis of *BnARP* gene family (putative from ESTs, all are part of the proteins, except *BnARP*1) and orthologues (intact proteins) from Arabidopsis, *B. rapa* and *B. oleracea*. *ARP* (auxin repressed protein). Family members used in these phylogenetic trees are shown in the alignment in Figure S1 (in the on-line version); (b) Amino acid sequences alignment of *BnARP*1 -4 and their closest orthologues from *B. rapa* and *B. oleracea*.

set of ESTs) showed 100% identities with *BoARP*1, a C genome ancestor protein from *Brassica oleracea* (Bol036995; http://brassicadb.org/brad/); *BnARP*2 appeared equally similar to *BrARP*1, an A genome proteins of *Brassica rapa* (Bra022955; http://brassicadb.org/brad/); *BnARP*3 appeared closest to another A genome protein *BrARP*2 (Bra005469; http://brassicadb.org/brad/) and *BnARP*4 appeared closest to another C genome protein *BoARP*2 (Bol027300; http://brassicadb.org/brad/) (**Figure 1(b)**). In total, 81 *ARP* ESTs representing a family of 25 proteins (using the above criterion) were recovered with diverse amino acid sequences from the 12 tissue-specific and 10 stress-responsive *B. napus* libraries (**Table 1**; **Figure 1**; **Figure S1**; **Supplementary Table 2**).

3.3. *BnARPs* Are Differentially Regulated during Development and Stress

The flea beetle damaged subtractive leaf library was most enriched in *BnARP*1 (17 ESTs) and *BnARP*2 (11 ESTs) compared with other members of the *ARP* families. Hence, we were curious to find out how the most particular *BnARP*1 gene responded to other forms of stress. Northern blot analysis showed that transcripts detected by the *BnARP*1 probe were present already in undamaged mature *B. napus* rosette leaves (**Figure 2**), although they had not been represented in the young leaf EST library (**Table 1**). Transcripts detected by the *BnARP*1 probe were also strongly induced in leaves damaged by flea beetle feeding, *S. sclerotiorum* infection, dehydration, and by 7 h of cold shock, although *BnARP*6 was the only *ARP* EST recovered in a cold acclimated (dark) library and *BnARP*2 was the only member found in the *S. sclerotiorum*-infected stem library (1 EST). Transcripts detected by the *BnARP*1 probe were completely repressed by mechanical wounding (**Figure 2**); and it did not appear in the flea beetle damaged cotyledon library, instead, *BnARP*4, 7 and 8 appeared in the damaged cotyledon library (**Table 1**). *BnARP*3 was exclusively expressed in the flea beetle-damaged leaf library in addition to *BnARP*1, *BnARP*4 and *BnARP*2. Since cotyledons are critical tissues impacted by flea beetle damage to the canola crop [48], we also conducted additional laboratory bioassays and Northern blots

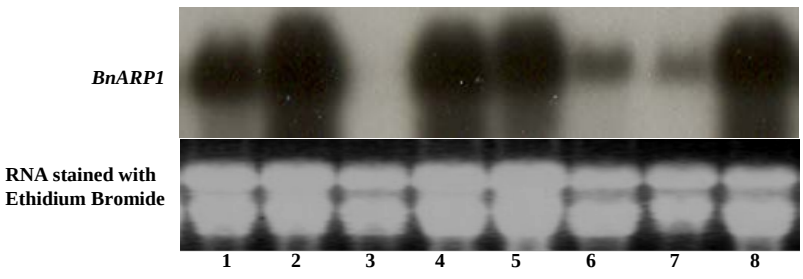


Figure 2. Representative Northern blot analysis of *BnARP*1 in *Brassica napus* leaves after different stress applications. Bottom Panel: Ethidium bromide stained rRNA shown as a gel loading control. 1) No stress, 2) Crucifer flea beetle feeding, 3) Mechanical wounding, 4) *S. sclerotiorum* infection, 5) Dehydration, 6) Cold shock 1 h, 7) Cold shock 2 h, 8) Cold shock 7 h. The probe was hybridized with membrane overnight and membrane was exposed to film for 3 h.

on cotyledons fed upon by flea beetles. Here, the *BnARP*1 gene was only transiently induced at 8 h and 16 h after flea beetle feeding on cotyledons, and expression was no longer detectable by 24 h of feeding (Figure 3).

To confirm whether *BnARP*1 was also subject to tissue or development constraints, Northern blots were conducted on a range of tissues. *BnARP*1 was moderately detected in 8-week-old fully expanded leaves and weakly detected in mature (8-week) vegetative stem and petioles, open flowers, and seed pods, barely detected in seedling leaves, stem, or roots, and not at all detected in undamaged cotyledons (Figure 4). Curiously, undamaged young leaf and undamaged cotyledons were the only tissues without any *ARP* genes. This was consistent with representation in the development EST libraries, although *BnARP*1 ESTs did appear at very low frequencies (1 & 3 ESTs) in early anther and senescent leaf EST libraries (Table 1).

3.4. Expression of *BnARP*1 in Transgenic Arabidopsis and Impact on Flea Beetle Feeding, Dehydration, and Cold

Since *BnARP*1 was most highly induced by flea beetle-feeding and moderately induced by drought and cold temperatures, we tested whether this gene can impact plant responses to these three types of stress when expressed in transgenic Arabidopsis. Therefore, 99 *BnARP*1 over-expression lines (*BnARP*1-*OE*) were developed by transfection of Arabidopsis with *A. tumefaciens* binary vector.

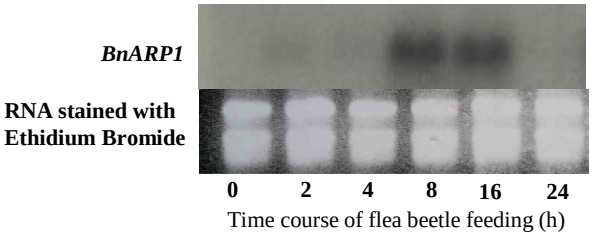


Figure 3. Representative Northern blot analysis of *BnARP*1 in *Brassica napus* cotyledons fed upon by crucifer flea beetles. Bottom panels show ethidium bromide stained rRNA shown as a gel loading control. The probe was hybridized with membrane overnight and membrane was exposed to film for 3 h.

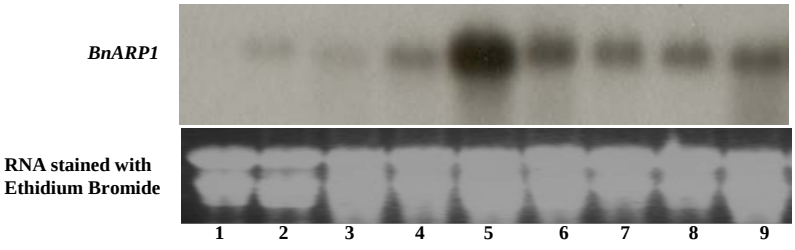


Figure 4. Representative Northern blot analysis of *BnARP*1 expression in specific *B. napus* tissues and developmental stages. Bottom Panel: Ethidium bromide stained rRNA shown as a gel loading control. 1) 7-day cotyledon, 2) 14-day seedling leaf, 3) 14-day seedling stem, 4) 14-day seedling root, 5) 8-week fully-expanded adult leaf, 6) 8-week adult stem, 7) fully-opened flower, 8) seed pod at 20 days-after-pollination, 9) 8-week adult petiole. The probe was hybridized with membrane overnight and membrane was exposed to film for 3 h.

These plants showed diverse growth phenotypes and were confirmed by PCR using primers that amplified the transgene fragment (a small number of representative lines shown in Figure S2(A); Supplementary Table 3). A second band (a 519 bp AT2G33830 genomic sequence from ATG to TGA) was occasionally amplified because of similar sequences occurring between *BnARP*1 and AT2G33830 when using *BnARP*1*F* and *BnARP*1*R* primers (Figure S2(A), **S3**). We manipulated this model plant rather than *B. napus*, since Arabidopsis is responsive to the crucifer flea beetle [43], genetically close to *B. napus* [49], and grows more quickly than the crop *Brassicas*. A range of transgenic lines were then verified as having strong Southern blot signals when tested for transgene copy number, although this probe also picked up very weak signals from the native Arabidopsis gene in WT Arabidopsis plants (Figure S2(B)). Representative Northern blots showed that the expression of transgene in 10 selected single transgene *BnARP*1-*OE* expression lines varied strongly (Figure 5). However, FB feeding, drought, and freezing bioassays on these lines showed no statistical significant differences compared with WT control Arabidopsis or transgenic control Arabidopsis expressing an empty vector binary vector (data not shown).

3.5. Phenotypes of *BnARP*1-*OE* Lines

Nearly half of the independently generated T_1 *BnARP*1-*OE* Arabidopsis seedlings (49/99) showed obvious inhibition of primary root and shoot elongation and stimulation of lateral root formation while growing on kanamycin-selective MS medium (Supplementary Table 3). Out of 10 representative T_2 homozygous lines selected for further analysis, eight lines continued this absence of root apical dominance and dwarf vegetation phenotypes after being transferred into kanamycin-free soil-less mixture for at least 7 days (Figure 6(a)-(c)), while the remaining two *BnARP*1-*OE* lines (41 and 52) showed earlier flowering than WT. This dwarf vegetation phenotype began to recover by 14 days in the potting mixture without kanamycin, such that the eight transgenic lines appeared to be growing at a WT growth rate after 3 weeks. However, they were delayed in bolting and their seeds ripened from one-to-four weeks later than WT because of the initial growth inhibition.

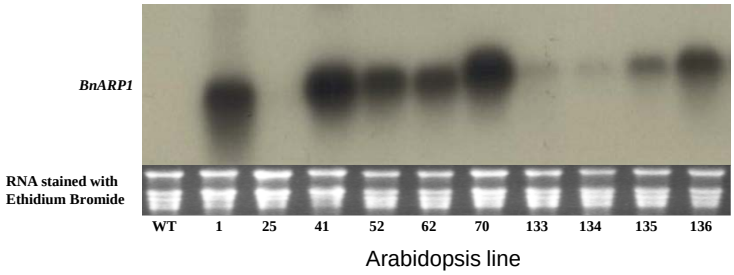


Figure 5. Representative Northern blot analysis of transgene expression level in seedling leaves of T_2 transgenic Arabidopsis *BnARP*1-*OE* lines. Leaves were taken from 14-day-old plants. Ethidium bromide stained rRNA shown as a gel loading control, and the probe was hybridized with membranes for overnight and membrane was exposed to film for 3 h.

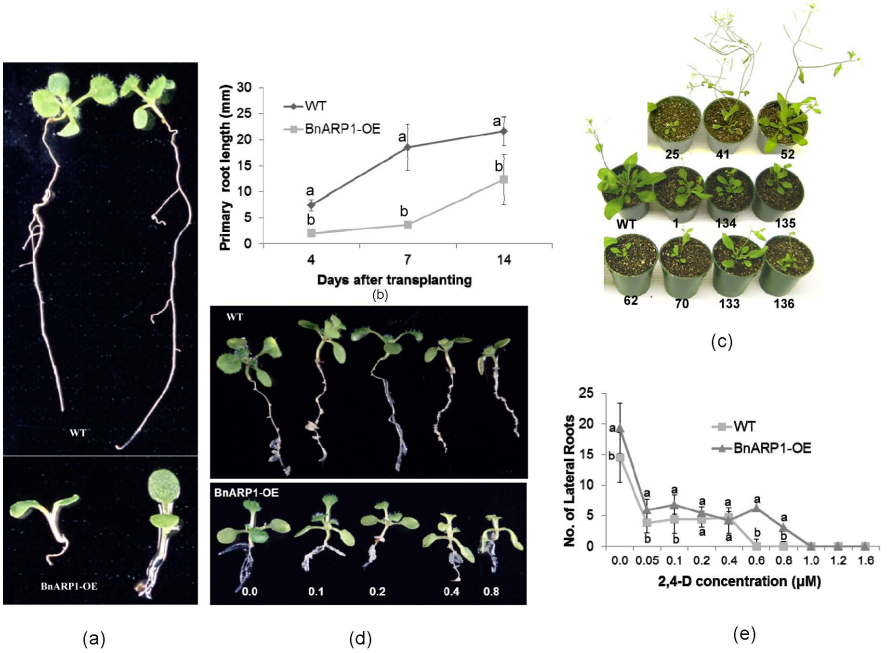


Figure 6. Comparison of representative phenotypes of Arabidopsis *BnARP*1*-OE* expression lines and wild type. (a) 10 days after germination on selection medium with kanamycin; (b) Primary root growth inhibition after transplanting into MS medium. Root length was measured after 4 d, 7 d, and 14 d of growth. Statistically significant were determined by a Student's t-test. Different letters represent significant differences of the means ± SD ($n = 10$); (c) Delayed phenotype of transplants at 5 weeks in soil; (d) Primary root growth response on 2, 4-D; (e) lateral root growth response to 2, 4-D for transgenic Arabidopsis line *BnARP*1-1 *OE*. Numbers of lateral roots were counted after 10 days growth on MS medium. Statistically significant were determined by a Student's t-test. Different letters represent significant differences of the means ± SD ($n = 10$).

3.6. *BnARP*1*-OE* Plants Reduced Hypocotyl Elongation

Lee *et al.* [32] reported that *BrARP*1 and *BrDRM*1-overexpression Arabidopsis plants showed reduced hypocotyl elongation, therefore, similar experiment was conducted to show whether *BnARP*1 has overlap function as that of *BrARP*1, which has 97.22% similarity in amino acids with *BnARP*1. Hypocotyl elongation was reduced in *BnARP*1*-OE* lines with and without the presence of 1 μM NAA when grown in the dark (**Figure 7**). These results imply that *BnARP*1 and *BrARP*1 have function overlap in hypocotyl elongation.

3.7. Auxin Affects Root Growth and Expression of the *BnARP*1 Transgene

Because auxin plays a role during primary root and lateral root development, and *BnARP*1*-OE* plants has short primary root and more lateral roots compared to WT (**Figure 6(a)**, **Figure 6(b) Figure 6(e)**), the relationship between root growth inhibition and auxin concentration was investigated in experiments using the synthetic auxin, 2, 4-D and a representative dwarf transgenic Arabidopsis line, *BnARP*1*-OE*-1, which had strong transgene expression. Application of a range of 2, 4-D (up to 1.6 μM) to this dwarfed line showed that primary root in

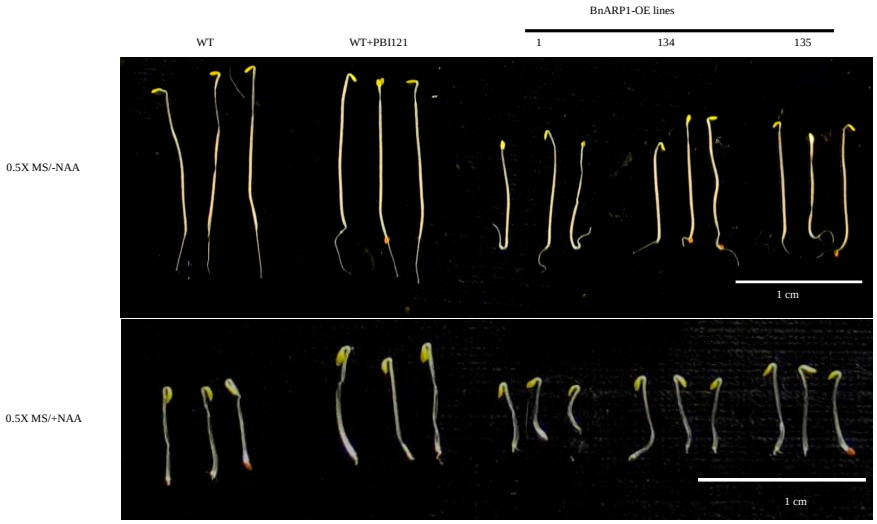


Figure 7. Inhibition of hypocotyl elongation in Arabidopsis *BnARP*1*-OE* lines. The *BnARP*1*-OE* lines showed reduced hypocotyl elongation with or without 1μM NAA compared to wild type being grown for 4 days in the dark at 22˚C.

hibition was not released, while primary root growth of WT roots was slightly depressed starting at 0.4 μM (**Figure 6(d)**). Lateral root growth was inhibited at a higher 2, 4-D concentration (1.0 uM) for *BnARP*1*-OE*-1 compared with 0.6 uM for WT seedlings, suggesting that the transgenic *BnARP*1*-OE*-1 plants are less sensitive to 2, 4-D than WT seedlings (**Figure 6(e)**).

The auxin-repressed transgene *RpARP* from black locust (*Robinia pseudoacacia*) is post-transcriptionally regulated (repressed) in response to exogenous auxin applied to transgenic expression plants [25]. Hence, Northern blot analysis was performed to see if the *BnARP*1 gene was also affected by exogenous auxin. Surprisingly, transcripts for *BnARP*1 accumulated strongly in *BnARP*1*-OE*-1 transgenic Arabidopsis lines after 2, 4-D application and increased even more by 2h and 6 h (**Figure 8**). A small part of this transcription could represent the Arabidopsis AT2G33830 gene, which has 92.59% amino acid identity with *BnARP*1 and could be detected weakly at 6 h of auxin treatment in the WT plant. However, the majority of this transcript induction would likely be due to transformation using the *BnARP*1 protein coding region without any UTRs and under the control of the 35*S* promoter.

3.8. BnARP1 Affects PIN1 and LOX2 Transcription

To determine the impact of *BnARP*1 on transcription of auxin genes, three independent Arabidopsis lines harboring high transgene expression levels (*BnARP*1*-OE*-1), medium levels (*BnARP*1*-OE*-52), and low levels (*BnARP*1*-OE*-134) were analyzed by Northern blotting with the following auxin signal transduction and transport genes (**Figure 5**). *BnARP*1 potentially could activate any one of the *GH*3 genes (eg. *DWARF IN LIGHT* 1, *DFL*1) to adenylate indole-3-acetic acid (IAA) and reduce free IAA level, shoot growth, and root growth [50], or repress *PIN*1 trans-membrane proteins in the auxin efflux carrier complex [51], or repress the *AUXIN-BINDING PROTEIN* 1 (*ABP*1) receptor

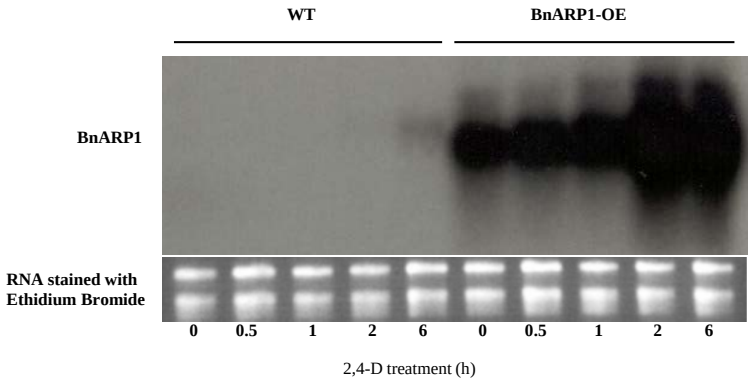


Figure 8. Representative Northern blot showing the effect of 2, 4-D application on *BnARP*1 expression in leaves of transgenic T_2 homozygous Arabidopsis line *BnARP*1-*OE*-1. RNAs isolated from leaves of 10-day-old seedlings up to 6 h after spraying with 20 μM 2, 4-D. Numbers below lanes indicate time (h) after treatment was initiated. Radiolabelled *BnARP*1 whole CDS was hybridized to membranes overnight and the membranes exposed to film for 3 h. Ribosomal RNAs stained with ethidium bromide (EB) indicates equal gel loading of sample RNAs.

controlling auxin-mediated plant cell expansion [52]. *BnARP*1 could also potentially affect auxin (Aux) response factors (*ARFs*; directly activating or repressing transcription of target genes), or the *Aux/IAA* proteins (regulated by auxin to repress *ARF* function), or the *AUXIN RESISTANCE* (signal transduction) gene *AXR*1 important for jasmonate-mediated responses [14] [20]. However, five of these six genes (*AtDFL*1, *AtABP*1, *AtARF*1, *AtAXR*1, and *AtIAA*1) showed no change in expression with the increased titre of *BnARP*1 in transgenic Arabidopsis (**Figure 9**). Only the *AtPIN*1 gene showed decreased expression in two transgenic Arabidopsis lines *BnARP*1-*OE*-52 and -134 with medium-to-low levels of the *BnARP*1 transgene (**Figure 9**). This indicates that *BnARP*1 may participate in auxin transportation.

Jasmonate-inducible allene oxide synthase (*AOS*) and lipoxygenase 2 (*LOX*2) genes are involved in JA biosynthesis and are elevated by MeJA treatment [53]. One study showed that *LOX*2 and *AOS* induction by IAA was suppressed in the axr1-24 mutant line, indicating a link between the level of jasmonate synthesis and auxin signaling [20]. Surprisingly, *LOX*2 expression was reduced in all three *BnARP*1-*OE* lines (1, 52, and 134) compared with WT (**Figure 9**). These data suggest that *BnARP*1 could be involved in regulating the level of jasmonate biosynthesis.

3.9. Expression of *BnARP*1 Improves Arabidopsis Resistance to *S. sclerotiorum*

In addition to transcript enhancement after flea beetle feeding, drought, and cold, *S. sclerotiorum* infection also caused very strong increases in *BnARP*1 in *B. napus* seedlings (**Figure 2**). Therefore, bioassays were conducted to determine whether transgenic Arabidopsis expressing this *Brassica* gene could improve tolerance to *S. sclerotiorum*. Two *BnARP*1-*OE* transgenic lines (No. 134 with weak

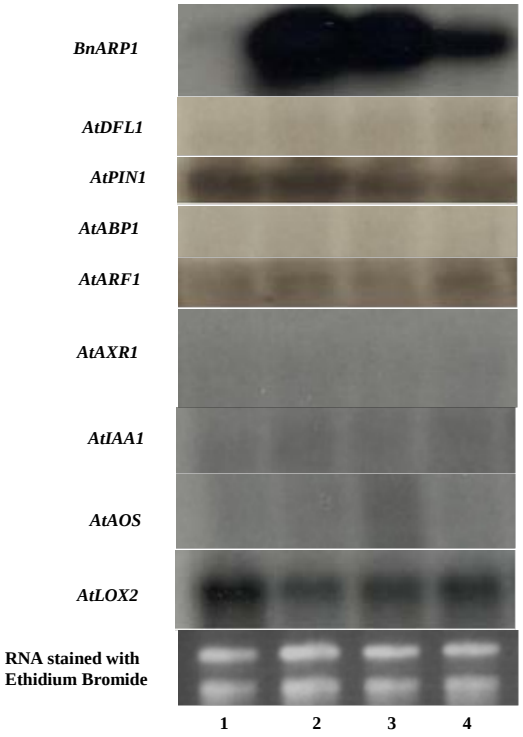


Figure 9. Representative Northern blot showing expression pattern of *BnARP*1 and eight other auxin-responsive genes in three transgenic T_2 homozygous *BnARP*1-*OE* lines. 1) Wild type, 2) *BnARP*1-*OE*-1 (high transgene expression), 3) *BnARP*1-*OE*-52 (moderate transgene expression), 4) *BnARP*1-*OE*-134 (weak transgene expression). Ribosomal RNA bands stained with ethidium bromide are shown as RNA loading controls in original gel.

trans-gene expression and No. 135 with moderate transgene expression) showed greater seedling survival after *S. sclerotiorum* infection compared with wild type or empty vector transformed plants (Figure10). The strongest resistance was provided by the *BnARP*1-*OE*-135 line (mean of 70% seedling survival). Only *BnARP*1-*OE*-1 (with high *BnARP*1 transgene expression) showed no significant difference in response to *S. sclerotiorum*. Variation for seedling survival was also higher in individual transgenic lines than in control lines (Figure 10).

4. Discussion

4.1. Tissue/Stress Specificity and Diversity of the *BnARP* Gene Family

A survey of expressed genes in a *Brassica napus* flea beetle damaged leaf EST library revealed an abundance of transcripts for *auxin repressed proteins*. An expanded search for these genes within our *Brassica* EST libraries revealed gene family consisting of at least 25 *ARP* genes (ESTs) that each are differentially expressed in different tissues, developmental stages or in response to different stresses. *BnARP* gene family is comprised of five sub-groups of genes. The data indicate much larger gene family than previously reported in *B. napus* or in other *Brassica* species and support the broad conservation known across higher plants for *ARP* [25]. Individual sequences may be diversified to fit local condi-

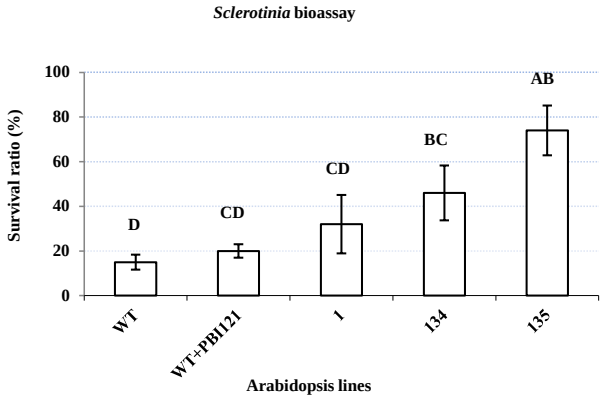


Figure 10. Seedling survival after *S. sclerotiorum* infection in Arabidopsis lines expressing *BnARP*1. *S. sclerotiorum* inocula were sprayed onto 21-day old plants and leaves assessed after 7 days. WT: wild type, WT + PBI121: transgenic wild type plants with empty PBI121 vector. *BnARP*1-*OE*-1, *BnARP*1-*OE*-134 and *BnARP*1-*OE*-135 are three representative Arabidopsis transgenic lines expressing the *BnARP*1 gene. Error bars indicate SE ($n = 4$). ANOVA and a post-hoc t-test were conducted, such that the different letter indicates significant difference of the means at $p < 0.05$.

tions in individual tissues or under specific stress conditions. However, more closely related *ARPs* may also have some over-lapping roles or functional redundancy, since our data show that representation of individual *BnARP* genes is not completely unique to each tissue or stress and both *BnARP*1 (in this study) and *BrARP*1overexprssion lines arrest hypocotyl elongation [32].

ARPs are known to be dormancy-associated proteins or induced by abiotic/biotic stress [22] [23] [28] [30]. Their induced expression may cause a temporary arrest of plant growth under unfavorable conditions to re-allocate resources into combating stress, as proposed by Lee *et al.* [32]. *BnARP*1 gene is strongly induced by flea beetle and *S. sclerotiorum* damage on *B. napus* leaves and moderately induced by drought and freezing temperatures. The closest *BnARP*2 orthologue (*BrARP*1) is also induced by drought and cold and the expression levels of these two orthologues are similar in mature leaves (high), in cotyledon and young leaves (low), and roots (reduced) in transgenic Arabidopsis expression lines [32]. A previously reported auxin-repressed gene (GenBank, GU189578) is also induced by drought and high salinity, and appears to be *BnARP*1, although the scope of its function was only tested in a limited way [31].

4.2. BnARP1 May Link Auxin Signaling and JA Synthesis

A lack of apical dominance, a block in primary root extension, and expansion of lateral roots in our Arabidopsis lines confirmed that transgenic expression of *BnARP*1 in Arabidopsis can cause an "auxin-depletion" phenotype. Reduction of *AtPIN*1 transcript levels in two out of three transgenic Arabidopsis *BnARP*1-*OE* test lines and reduction in *LOX* transcripts in all three transgenic *BnARP*1-*OE* test lines also suggest that the *BnARP*1 protein is an auxin-responsive negative regulator that can facilitate a slow-down in plant development by reducing transcription of an auxin transport protein and affecting a JA signalling gene. IAA

induction of *LOX*2 and *AOS* was suppressed in the axr1-24 mutant supporting this link between jasmonate and auxin signaling [20]. Since *BnARP*1-*OE* lines have similar phenotype with axr1-24 mutant lines, and *LOX*2 gene was suppressed in both types of Arabidopsis lines, it indicates that the *BnARP*1 gene has opposite function to *AtAXR*1 gene. Since axr1 lines is more susceptible to the fungus *P. irregulare* in Arabidopsis demonstrated by Tiryaki and Staswick [20], *BnARP*1-*OE* lines are also expected to be more susceptible to the fungus *S. sclerotiorum*. But our study demonstrated an opposite results in term of resistance to fungus. The reasons for this could be the differentiation of the fungus and *BnARP*1 is at the different location as *ARX*1 to link jasmonate and auxin signalling pathways. Unfortunately, our experiment on the impact of exogenous auxin on *BnARP*1 was limited by the lack of UTRs and other regulatory elements on the *BnARP*1 binary vector introduced into Arabidopsis. In support of this finding, the *RpARP* gene from the black locust tree was also not repressed when exogenous auxin was applied to over-expression transgenic plants developed with an *RpARP* transgene devoid of UTRs [25]. Oddly, the *BnARP*1 expression signal was actually increased as early as 2h after treatment of the transgenic Arabidopsis with exogenous auxin. This could be due in part to hybridization of the *BnARP*1 probe with the highly similar Arabidopsis *ARP*1 (**Figure S3**). However, the applied auxin must also have an enhancing effect on the 35*S* promoter in these transgenic plants. A TGTCTA element did exist on 35*S* promoter, which is only one nucleotide different from the auxin response element (AuxRE) TGTCTC [54]. This element and many other DNA cis elements exiting on 35*S* (data not shown) may explain the induction of *BnARP*1 in overexpression lines by IAA application. The limited test sample for this preliminary experiment must now be expanded to determine whether this phenomenon would occur on a broader set of transgenic lines.

4.3. Expression of *BnARP*1 Improves Tolerance to *S. sclerotiorum* Infection

Although *BnARP*1 was nearly unique among their gene family members in their strong response to flea beetle feeding, transgenic Arabidopsis plants expressing *BnARP*1 did not improve resistance to flea beetle feeding, drought, or cold, although this gene was induced by these conditions in *B. napus*. In contrast, expression of *BnARP*1 transgenes in Arabidopsis, strongly improved survival after *S. sclerotiorum* infection. To *BnARP*1, its strong induction in stressed *B. napus* leaves, its effect on growth and failure to improve flea beetle, drought, and cold tolerance, coupled with improved survival after *S. sclerotiorum* infection in transgenic overexpression Arabidopsis, suggests both an indirect role in slowing plant growth to cope with stress and a direct role in *S. sclerotiorum* resistance.

4.4. Summary of This Study

In summary, ESTs for an *Auxin Repressed Protein* 1 (*BnARP*1) were highly represented (expressed) in a *Brassica napus* subtractive library developed after

leaf damage by the crucifer flea beetle (*Phyllotreta cruciferae*). Expression of this gene was under different developmental control in *B. napus*, and it was co-induced in *B. napus* by flea beetle feeding, *S. sclerotiorum* infection, drought, and cold. A total of 25 *BnARP* genes represented in different *B. napus* stress and development EST libraries indicated larger, diversified families than known earlier. Dwarf phenotypes, primary root growth inhibition, lateral root enhancement, reduced sensitivity to 2, 4-D, and reduced *PIN*1 and *LOX* expression in transgenic Arabidopsis expression lines suggest that *BnARP*1 is an auxin repressor that may prevent auxin transport and supports an interaction between auxin and jasmonate-signaling pathways. The increased survival after *S. sclerotiorum* infection in transgenic Arabidopsis suggests that *BnARP*1 may have a direct role in *S. sclerotiorum* resistance through regulating JA pathway. Therefore, this study also points to a practical use for these *BnARP* genes and a need for testing their ability to protect *Brassica* oilseed and vegetable from disease in crop zones with limited growing seasons.

Acknowledgements

Limin Wu was the recipient of an NSERC Visiting Fellowship to a Canadian Government Laboratory. This study was supported by a grant to M. Gruber and D. Hegedus from the Genomics Research & Development Initiative (GRDI) of Agriculture and Agri-Food Canada.

References

[1] Grillo, G., Stotz, H.U., Pittendrigh, B.R., Kroymann, J., Weniger, K., Fritsche, J., Bauke, A. and Mitchell-Olds, T. (2000) Induced Plant Defense Responses against Chewing Insects. Ethylene Signaling Reduces Resistance of Arabidopsis against Egyptian Cotton Worm but Not Diamondback Moth. *Plant Physiology*, **124**, 1007-1017. https://doi.org/10.1104/pp.124.3.1007

[2] Kessler, A. and Baldwin, I.T. (2002) Plant Responses to Insect Herbivory: the Emerging Molecular Analysis. *Annual Review of Plant Biology*, **53**, 299-328. https://doi.org/10.1146/annurev.arplant.53.100301.135207

[3] Walling, L.L. (2000) The Myriad Plant Responses to Herbivores. *Journal of Plant Growth Regulation*, **19**, 195-216.

[4] Moran, P.J. and Thompson, G.A. (2001) Molecular Responses to Aphid Feeding in Arabidopsis in Relation to Plant Defense Pathways. *Plant Physiology*, **125**, 1074-1085. https://doi.org/10.1104/pp.125.2.1074

[5] Xu, Y., Chang, P.-F.L., Liu, D., Narasimhan, M.L., Raghothama, K.G., Hasegawa, P.M. and Bressan, R.A. (1994) Plant Defense Genes Are Synergistically Induced by Ethylene and Methyl Jasmonate. *Plant Cell*, **6**, 1077-1085. https://doi.org/10.1105/tpc.6.8.1077

[6] O'Donnell, P.J., Calvert, C., Atzorn, R., Wasternack, C., Leyser, H.M.O. and Bowles, D.J. (1996) Ethylene as a Signal Mediating the Wound Response of Tomato Plants. *Science*, **274**, 1914-1917. https://doi.org/10.1126/science.274.5294.1914

[7] Penninckx, I.A., Thomma, B.P., Buchala, A., Métraux, J-P. and Broekaert, W.F. (1998) Concomitant Activation of Jasmonate and Ethylene Response Pathways Is Required for Induction of a Plant Defensin Gene in Arabidopsis. *Plant Cell*, **10**, 2103-2113. https://doi.org/10.1105/tpc.10.12.2103

[8] Kahl, J., Siemens, D.H., Aerts, R.J., Gäbler, R., Kühnemann, F., Preston, C.A. and Baldwin, I.T. (2000) Herbivore-Induced Ethylene Suppresses a Direct Defense but Not a Putative Indirect Defense against an Adapted Herbivore. *Planta*, **210**, 336-342. https://doi.org/10.1007/PL00008142

[9] Guo, H. and Ecker, J.R. (2004) The Ethylene Signaling Pathway: New Insights. *Current Opinion in Plant Biology*, **7**, 40-49. https://doi.org/10.1016/j.pbi.2003.11.011

[10] Johnson, P.R. and Ecker, J.R. (1998) The Ethylene Gas Signal Transduction Pathway: A Molecular Perspective. *Annual Review of Genetics*, **32**, 227-254. https://doi.org/10.1146/annurev.genet.32.1.227

[11] Shinozaki, K., Yamaguchi-Shinozaki, K. and Seki, M. (2003) Regulatory Network of Gene Expression in the Drought and Cold Stress Responses. *Current Opinion in Plant Biology*, **6**, 410-417. https://doi.org/10.1016/S1369-5266(03)00092-X

[12] Anderson, J.P., Badruzsaufari, E., Schenk, P.M., Manners, J.M., Desmond, O.J., Ehlert, C., Maclean, D.J., Ebert, P.R. and Kazan, K. (2004) Antagonistic Interaction between Abscisic Acid and Jasmonate-Ethylene Signaling Pathways Modulates Defense Gene Expression and Disease Resistance in Arabidopsis. *Plant Cell*, **16**, 3460-3479. https://doi.org/10.1105/tpc.104.025833

[13] Davies, P.J. (1995) The Plant Hormones: Their Nature, Occurrence, and Functions. In: Davies, P.J., Ed., *Plant Hormones: Physiology, Biochemistry, and Molecular Biology*, Kluwer Academic Publishers, Dordrecht, The Netherlands, 1-5. https://doi.org/10.1007/978-94-011-0473-9_1

[14] Leyser, O. (2001) Auxin Signalling: The Beginning, the Middle and the End. *Current Opinion in Plant Biology*, **4**, 382-386. https://doi.org/10.1016/S1369-5266(00)00189-8

[15] Zhao, Y. (2010) Auxin Biosynthesis and Its Role in Plant Development. *Annual Review of Plant Biology*, **61**, 49-64. https://doi.org/10.1146/annurev-arplant-042809-112308

[16] Abel, S., Oeller, P.W. and Theologis, A. (1994) Early Auxin-Induced Genes Encode Short-Lived Nuclear Proteins. *Proceedings of the National Academy of Sciences of the United States of America*, **91**, 326-330. https://doi.org/10.1073/pnas.91.1.326

[17] Overwoode, P.I., Ikushima, Y., Alonso, J.M., Chare, A., Chan, C., Ecker, J.R., Hughest, B., Liu, A., Onodera, C., Quach, H., Smith, A., Yu, G. and Theologis, A. (2005) Functional Genomic Analysis of the AUXIN/INDOLE-3-ACETIC ACID Gene Family Members in *Arabidopsis thaliana*. *Plant Cell*, **17**, 3282-3300. https://doi.org/10.1105/tpc.105.036723

[18] Ulmasov, T., Hagen, G. and Guilfoyle, T.J. (1999) Activation and Repression of Transcription by Auxin-Response Factors. *Proceedings of the National Academy of Sciences of the United States of America*, **96**, 5844-5849. https://doi.org/10.1073/pnas.96.10.5844

[19] Sieberer, T., Seifert, G.J., Hauser, M.T., Grisafi, P., Fink, G.R. and Luschnig, C. (2000) Post-Transcriptional Control of the Arabidopsis Auxin Efflux Carrier EIR1 requires AXR1. *Current Biology*, **10**, 1595-1598. https://doi.org/10.1016/S0960-9822(00)00861-7

[20] Tiryaki, I. and Staswick, P.E. (2002) An Arabidopsis Mutant Defective in Jasmonate Response Is Allelic to the Auxin-Signaling Mutant axr1. *Plant Physiology*, **130**, 887-894. https://doi.org/10.1104/pp.005272

[21] Reddy, A.S.N. and Poovaiah, B.W. (1990) Molecular Cloning and Sequencing of a cDNA for an Auxin-Repressed Messenger-RNA-Correlation between Fruit Growth and Repression of the Auxin Regulated Gene. *Plant Molecular Biology*, **14**, 127-136. https://doi.org/10.1007/BF00018554

[22] Stafstrom, J.P., Ripley, B.D., Devitt, M.L. and Drake, B. (1998) Dormancy-Associated Gene Expression in Pea Axillary Buds. *Planta*, **205**, 547-552. https://doi.org/10.1007/s004250050354

[23] Kebrom, T.H., Burson, B.L., and Finlayson, S.A. (2006) Phytochrome B Represses *Toesinte Branched*1 Expression and Induces Sorghum Axillary Bud Outgrowth in Response to Light Signals. *Plant Physiology*, **140**, 1109-1117. https://doi.org/10.1104/pp.105.074856

[24] Steiner, C., Bauer, J., Amrhein, N. and Bucher, M. (2003) Two Novel Genes Are Differentially Expressed during Early Germination of the Male Gametophyte of *Nicotiana tabacum*. *Biochimica et Biophysica Acta*, **1625**, 123-133. https://doi.org/10.1016/S0167-4781(02)00598-5

[25] Park, S. and Han, K.-H. (2003) An Auxin-Repressed Gene (RpARP) from Black Locust (*Robinia pseudoacacia*) Is Post-Transcriptionally Regulated and Negatively Associated with Shoot Elongation. *Tree Physiology*, **23**, 815-823. https://doi.org/10.1093/treephys/23.12.815

[26] Kim, H.B., Lee, H., Oh, C.J., Lee, N.H. and An, C.S. (2007) Expression of Eu-NOD-ARP1 Encoding Auxin-Repressed Protein Homolog Is Upregulated by Auxin and Localized to the Fixation Zone in Root Nodules of *Elaeagnus umbellata*. *Molecules and Cells*, **23**, 115-121.

[27] Shimizu, M., Suzuki, K., Miyazawa, Y., Fujii, N. and Takahashi, H. (2006) Differential Accumulation of the mRNA of the Auxin-Repressed Gene CsGRP1 and the Auxin-Induced Peg Formation during Gravimorphogenesis of Cucumber Seedlings. *Planta*, **225**, 13-22. https://doi.org/10.1007/s00425-006-0324-y

[28] Tatematsu, K., Ward, S., Leyser, O., Kamiya, Y. and Nambara, E. (2005) Identification of cis-Elements That Regulate Gene Expression during Initiation of Axillary Bud Outgrowth in Arabidopsis. *Plant Physiology*, **138**, 757-766. https://doi.org/10.1104/pp.104.057984

[29] Hwang, E.W., Kim, K.A., Park, S.C., Jeong, M.J., Byun, M.O. and Kwon, H.B. (2005) Expression Profiles of Hot Pepper (*Capsicum annum*) Genes under Cold Stress Condition. *Journal of Biosciences*, **30**, 657-667. https://doi.org/10.1007/BF02703566

[30] Mantri, N.L., Ford, R., Coram, T.E. and Pang, E.C.K. (2007) Transcriptional Profiling of Chickpea Genes Differentially Regulated in Response to High-Salinity, Cold and Drought. *BMC Genomics*, **8**, 303. https://doi.org/10.1186/1471-2164-8-303

[31] Chen, L., Ren, F., Zhong, H., Jiang, W. and Li, X. (2010) Identification and Expression Analysis of Genes in Response to High-Salinity and Drought Stresses in *Brassica napus*. *Acta Biochim Biophys Sin*, **42**, 154-164. https://doi.org/10.1093/abbs/gmp113

[32] Lee, J., Han, C.-T. and Hur, Y. (2013) Molecular Characterization of the *Brassica rapa* Auxin-Repressed, Superfamily Genes, BrARP1 and BrDRM1. *Molecular Biology Reports*, **40**, 197-209. https://doi.org/10.1007/s11033-012-2050-9

[33] Gruber, G., Wu, L., Links, M., Gjetvaj, B., Durkin, J., Lydiate, D. and Hegedus, D. (2012) Analysis of Expressed Sequence Tags in *Brassica napus* Cotyledons Damaged by Crucifer Flea Beetle Feeding. *Genome*, **55**, 118-133. https://doi.org/10.1139/g11-083

[34] Moreno-Hagelsieb, G. and Latimer, K. (2008) Choosing BLAST Options for Better Detection of Orthologs as Reciprocal Best Hits. *Bioinformatics*, **24**, 319-324. https://doi.org/10.1093/bioinformatics/btm585

[35] Clough, S.J. and Bent, A.F. (1998) Floral Dip: A Simplified Method for Agrobacterium-Mediated Transformation of *Arabidopsis thaliana*. *The Plant Journal*, **16**, 735-

743. https://doi.org/10.1046/j.1365-313x.1998.00343.x

[36] Klimyuk, V.I., Carroll, B.J., Thomas, C.M. and Jones, J.D.G. (1993) Alkali Treatment for Rapid Preparation of Plant Material for Reliable PCR Analysis. *The Plant Journal*, **3**, 493-494. https://doi.org/10.1111/j.1365-313X.1993.tb00169.x

[37] Murray, M.G. and Thompson, W.F. (1980) Rapid Isolation of High Molecular Weight Plant DNA. *Nucleic Acids Research*, **8**, 4321-4325. https://doi.org/10.1093/nar/8.19.4321

[38] Southern, E.M. (1975) Detection of Specific Sequences among DNA Fragments Separated by Gel Electrophoresis. *Journal of Molecular Biology*, **98**, 503-517. https://doi.org/10.1016/S0022-2836(75)80083-0

[39] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual. II Edition, Cold Spring Harbour Laboratory. Press, Cold Spring Harbour, New York.

[40] Fukaki, H., Tameda, S., Masuda, H. and Tasaka, M. (2002) Lateral Root Formation Is Blocked by a Gain-of-Function Mutation in the SOLITARY-ROOT/IAA14 Gene of Arabidopsis. *The Plant Journal*, **29**, 153-168. https://doi.org/10.1046/j.0960-7412.2001.01201.x

[41] Okushima, Y., Fukaki, H., Onoda, M., Theologis, A. and Tasaka, M. (2007) ARF7 and ARF19 Regulate Lateral Root Formation via Direct Activation of LBD/ASL Genes in Arabidopsis. *Plant Cell*, **19**, 118-130. https://doi.org/10.1105/tpc.106.047761

[42] Palaniswamy, P., Lamb, R.J. and McVetty, P.B.E. (1992) Screening for Antixenosis Resistance to Flea Beetles, *Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae) in Rapeseed and Related Ccrucifers. *The Canadian Entomologist*, **124**, 895-906. https://doi.org/10.4039/Ent124895-5

[43] Hallett, R.H., Ray, H., Holowachuk, J., Soroka, J.J. and Gruber, M.Y. (2005) Bioassay for Assessing Resistance of *Arabidopsis thaliana* L. (Heynh.) to the Adult Crucifer Flea Beetle, *Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae). *Canadian Journal of Plant Science*, **85**, 225-235. https://doi.org/10.4141/P03-122

[44] Vogel, J.T., Zarka, D.G., Buskirk, H.A.V., Fowler, S.G. and Thomashow, M.F. (2005) Roles of the CBF2 and ZAT12 Transcription Factors in Configuring the Low Temperature Transcriptome of Arabidopsis. *The Plant Journal*, **41**, 195-211. https://doi.org/10.1111/j.1365-313X.2004.02288.x

[45] Pedras, M.S.C. and Ahiahonu, P.W.K. (2004) Phytotoxin Production and Phytoalexin Elicitation by the Phytopathogenic Fungus *Sclerotinia sclerotiorum*. *Journal of Chemical Ecology*, **30**, 2163-2179. https://doi.org/10.1023/B:JOEC.0000048781.72203.6c

[46] SAS Institute, Inc (2001) The SAS System. Version 9.0., SAS Institute, Inc., Cary, North Carolina.

[47] Bailey, L.C., Searls, D.B. and Overton, G.C. (1998) Analysis of EST-Driven Gene Annotation in Human Genomic Sequence. *Genome Research*, **8**, 362-376. https://doi.org/10.1101/gr.8.4.362

[48] Lamb, R.J. (1984) Effects of Flea Beetles, *Phyllotreta* spp. (Chrysomelidae: Coleoptera), on the Survival, Growth, Seed Yield and Quality of Canola, Rape and Yellow Mustard. *The Canadian Entomologist*, **116**, 269-280. https://doi.org/10.4039/Ent116269-2

[49] Parkin, I., Gulden, S.M., Sharpe, A.G., Lukens, L., Trick, M., Osborn, T.C. and Lydiate, D.J. (2005) Segmental Structure of the *Brassica napus* Genome Based on Comparative Analysis with *Arabidopsis thaliana*. *Genetics*, **171**, 765-781. https://doi.org/10.1534/genetics.105.042093

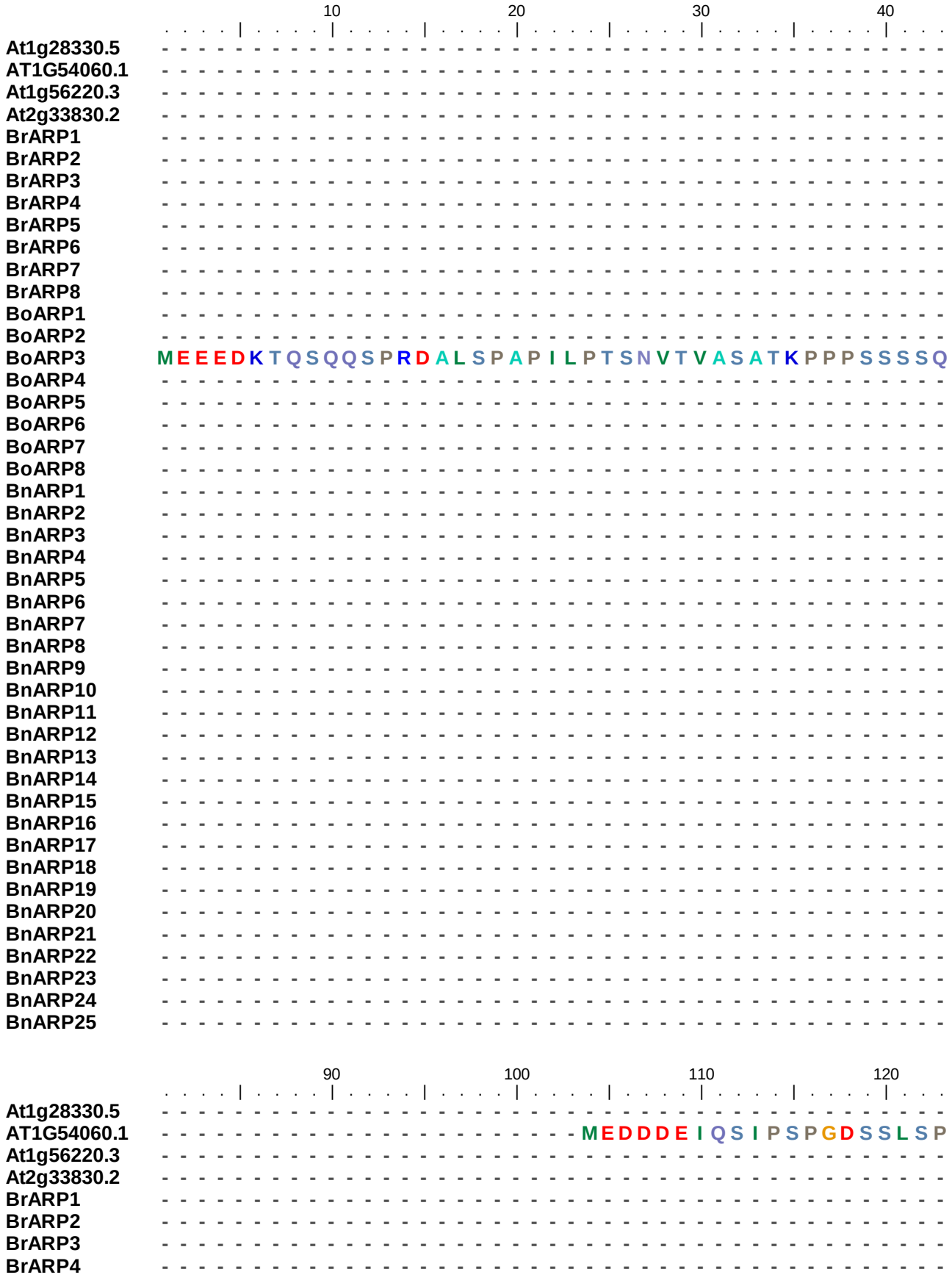
[50] Nakazawa, M., Yabe, N., Ichikawa, T., Yamamoto, Y.Y., Yoshizumi, T., Hasunuma, K. and Matsui, M. (2001) DFL1, an Auxin-Responsive GH3 Gene Homologue, Negatively Regulates Shoot Cell Elongation and Lateral Root Formation, and Positively Regulates the Light Response of Hypocotyl Length. *The Plant Journal*, **25**, 213-221. https://doi.org/10.1111/j.1365-313X.2001.00957.x

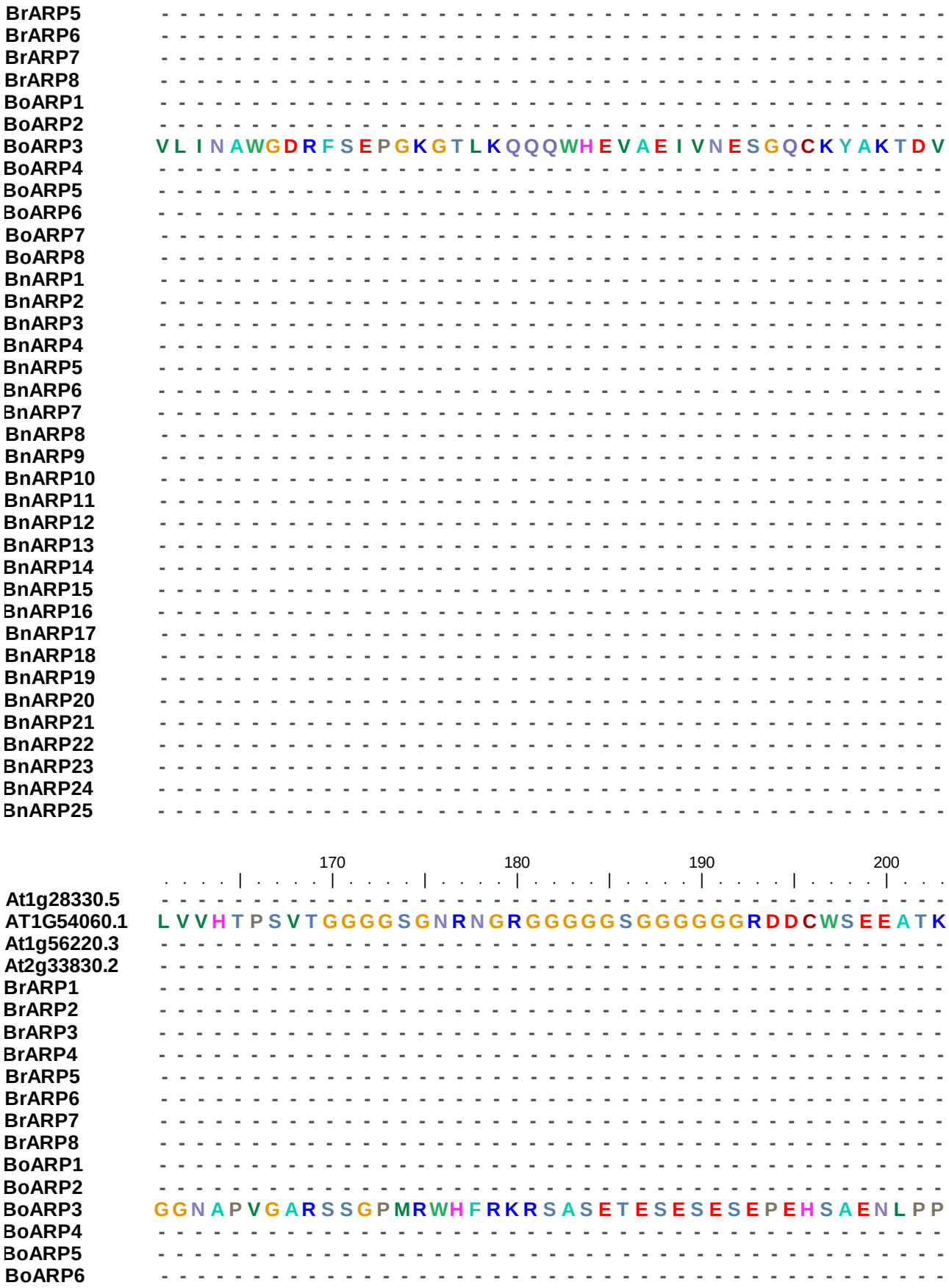
[51] Gälweiler, L., Guan, C., Müller, A., Wisman, E., Mendgen, K., Yephremov, A. and Palme, K. (1998) Regulation of Polar Auxin Transport by AtPIN1 in Arabidopsis Vascular Tissue. *Science*, **282**, 2226-2230. https://doi.org/10.1126/science.282.5397.2226

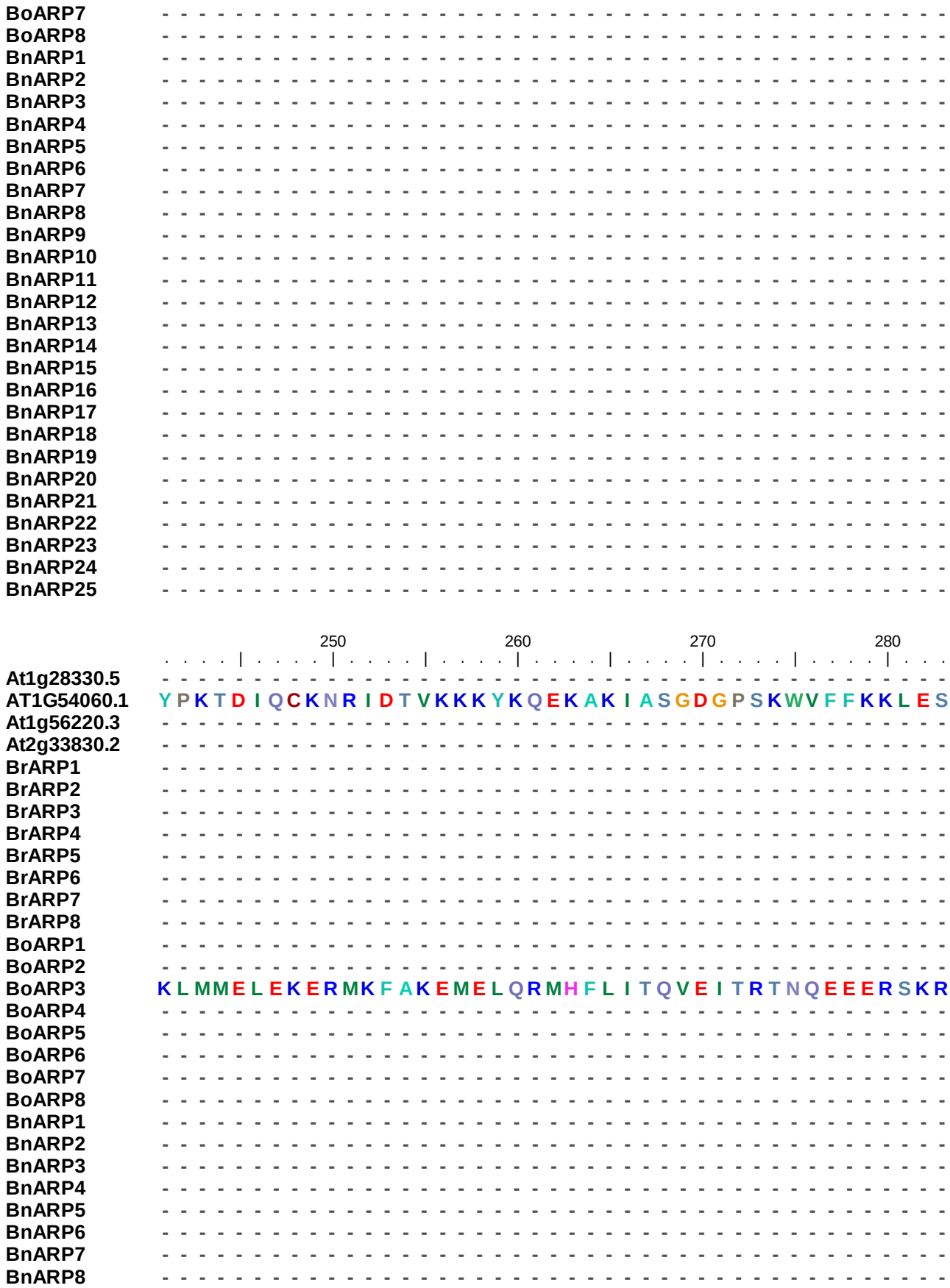
[52] Jones, A.M., Im, K.H., Savka, M.A., Wu, M.J., DeWitt, N.G., Shillito, R. and Binns, A.N. (1998) Auxin-Dependent Cell Expansion Mediated by Overexpressed Auxin-Binding Protein 1. *Science*, **282**, 1114-1117. https://doi.org/10.1126/science.282.5391.1114

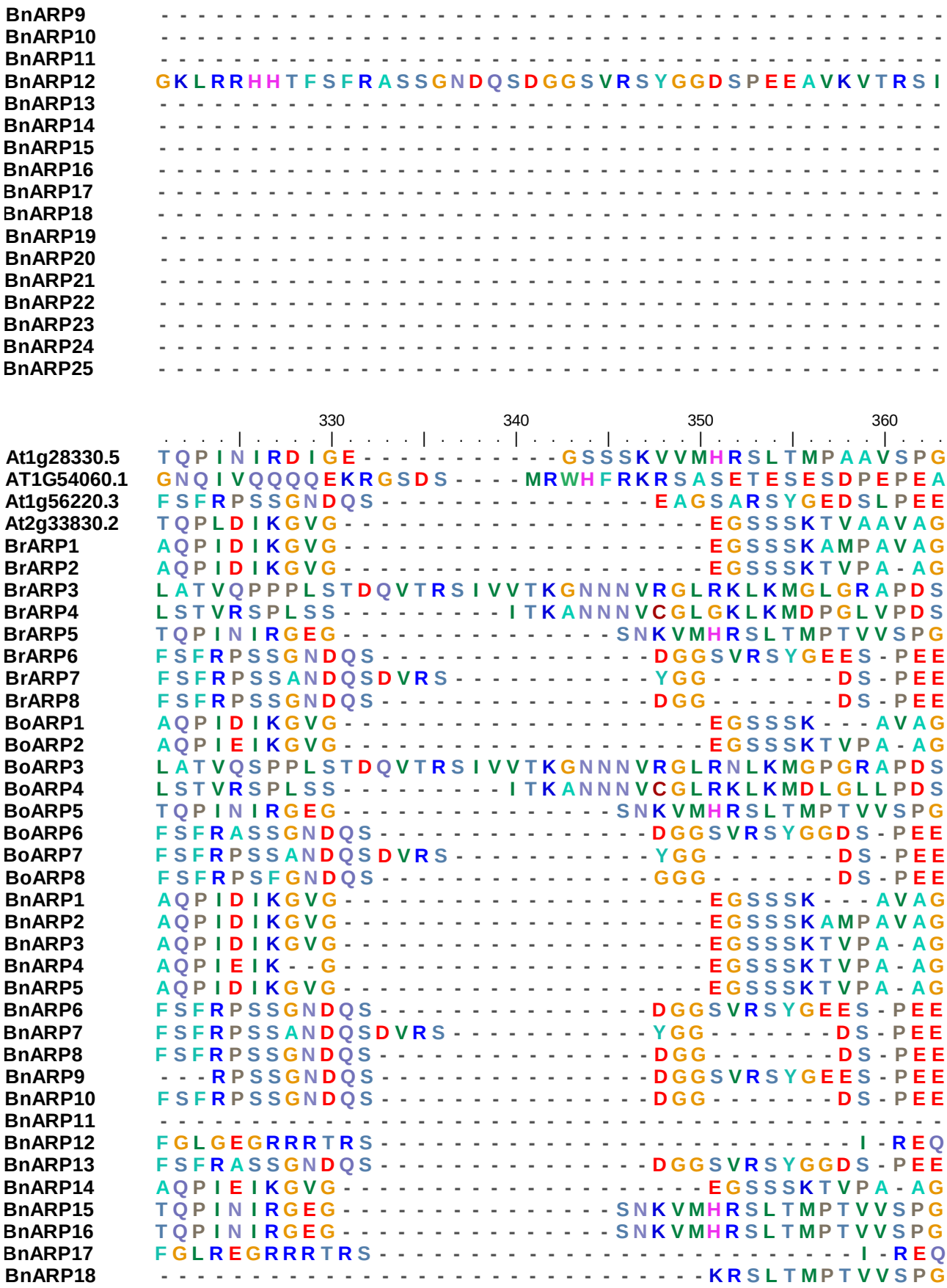
[53] Creelman, R.A. and Mullet, J.E. (1997) Biosynthesis and Action of Jasmonates in Plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, **48**, 355-381. https://doi.org/10.1146/annurev.arplant.48.1.355

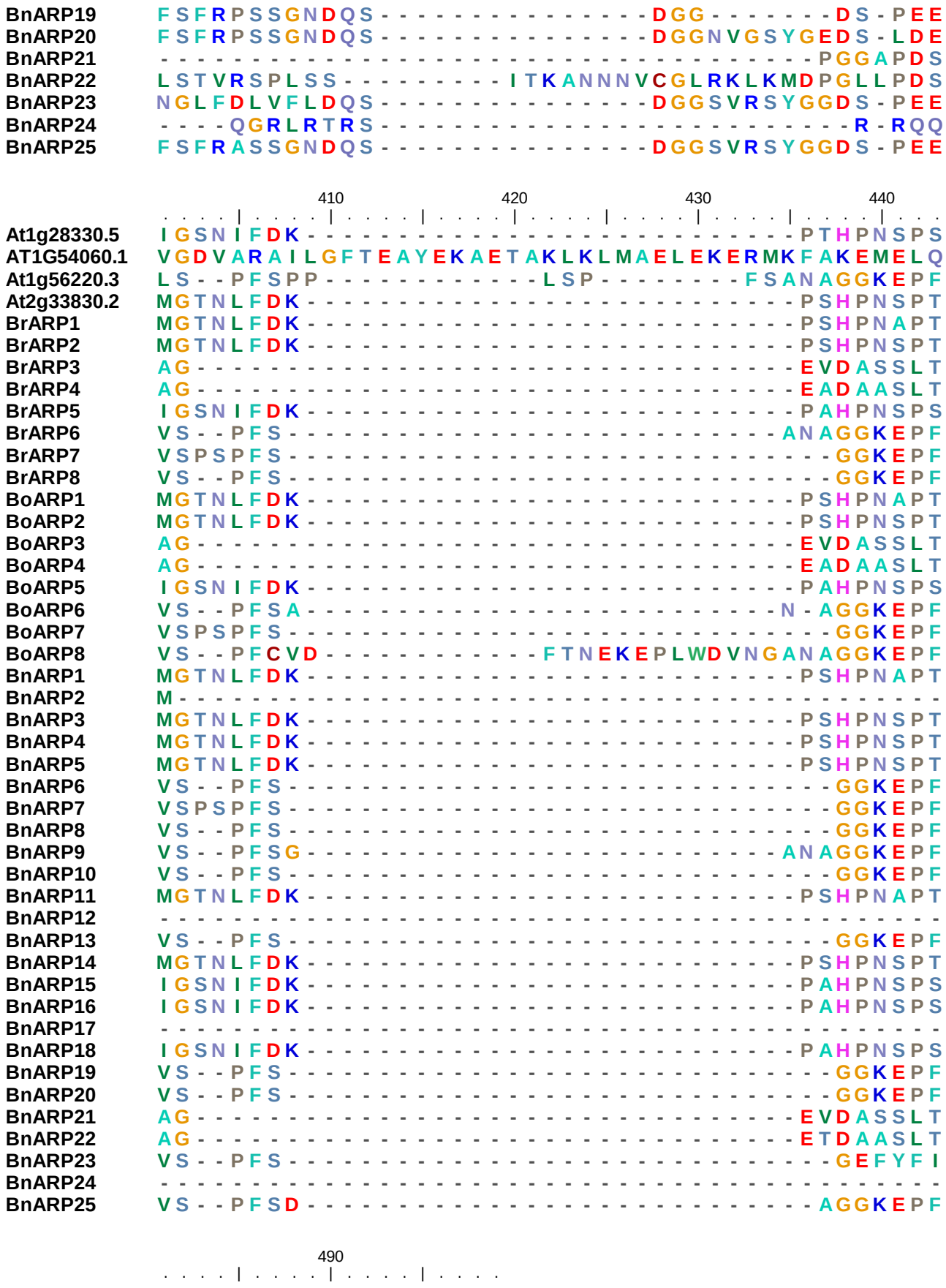
[54] Tiwari, S.B., Hagen, G. and Guilfoyle, T. (2003) The Roles of Auxin Response Factor Domains in Auxin-Responsive Transcription. *Plant Cell*, **15**, 533-543. https://doi.org/10.1105/tpc.008417


10
20
30
40
At1g28330.5
AT1G54060.1
At1g56220.3
At2g33830.2
BrARP1
BrARP2
BrARP3
BrARP4
BrARP5
BrARP6
BrARP7
BrARP8
BoARP1
BoARP2
BoARP3
BoARP4
BoARP5
BoARP6
BoARP7
BoARP8
BnARP1
BnARP2
BnARP3
BnARP4
BnARP5
BnARP6
BnARP7
BnARP8
BnARP9
BnARP10
BnARP11
BnARP12
BnARP13
BnARP14
BnARP15
BnARP16
BnARP17
BnARP18
BnARP19
BnARP20
BnARP21
BnARP22
BnARP23
BnARP24
BnARP25
MEEEDKTQSQQSPRDALSPAPILPTSNVTVASATKPPPSSSSQ
90
100
110
120
At1g28330.5
AT1G54060.1
At1g56220.3
At2g33830.2
BrARP1
BrARP2
BrARP3
BrARP4
MEDDDEIQSIPSPGDSSLSP


BrARP5
BrARP6
BrARP7
BrARP8
BoARP1
BoARP2
BoARP3 V L I N A W G D R F S E P G K G T L K Q Q Q W H E V A E I V N E S G Q C K Y A K T D V
BoARP4
BoARP5
BoARP6
BoARP7
BoARP8
BnARP1
BnARP2
BnARP3
BnARP4
BnARP5
BnARP6
BnARP7
BnARP8
BnARP9
BnARP10
BnARP11
BnARP12
BnARP13
BnARP14
BnARP15
BnARP16
BnARP17
BnARP18
BnARP19
BnARP20
BnARP21
BnARP22
BnARP23
BnARP24
BnARP25
170
180
190
200
At1g28330.5
AT1G54060.1 L V V H T P S V T G G G G S G N R N G R G G G G G S G G G G G G R D D C W S E E A T K
At1g56220.3
At2g33830.2
BrARP1
BrARP2
BrARP3
BrARP4
BrARP5
BrARP6
BrARP7
BrARP8
BoARP1
BoARP2
BoARP3 G G N A P V G A R S S G P M R W H F R K R S A S E T E S E S E S E P E H S A E N L P P
BoARP4
BoARP5
BoARP6


BoARP7
BoARP8
BnARP1
BnARP2
BnARP3
BnARP4
BnARP5
BnARP6
BnARP7
BnARP8
BnARP9
BnARP10
BnARP11
BnARP12
BnARP13
BnARP14
BnARP15
BnARP16
BnARP17
BnARP18
BnARP19
BnARP20
BnARP21
BnARP22
BnARP23
BnARP24
BnARP25
250
260
270
280
At1g28330.5
AT1G54060.1 Y P K T D I Q C K N R I D T V K K K Y K Q E K A K I A S G D G P S K W V F F K K L E S
At1g56220.3
At2g33830.2
BrARP1
BrARP2
BrARP3
BrARP4
BrARP5
BrARP6
BrARP7
BrARP8
BoARP1
BoARP2
BoARP3 K L M M E L E K E R M K F A K E M E L Q R M H F L I T Q V E I T R T N Q E E E R S K R
BoARP4
BoARP5
BoARP6
BoARP7
BoARP8
BnARP1
BnARP2
BnARP3
BnARP4
BnARP5
BnARP6
BnARP7
BnARP8


BnARP9
BnARP10
BnARP11
BnARP12
BnARP13
BnARP14
BnARP15
BnARP16
BnARP17
BnARP18
BnARP19
BnARP20
BnARP21
BnARP22
BnARP23
BnARP24
BnARP25
330
340
350
360
At1g28330.5
AT1G54060.1
At1g56220.3
At2g33830.2
BrARP1
BrARP2
BrARP3
BrARP4
BrARP5
BrARP6
BrARP7
BrARP8
BoARP1
BoARP2
BoARP3
BoARP4
BoARP5
BoARP6
BoARP7
BoARP8
BnARP1
BnARP2
BnARP3
BnARP4
BnARP5
BnARP6
BnARP7
BnARP8
BnARP9
BnARP10
BnARP11
BnARP12
BnARP13
BnARP14
BnARP15
BnARP16
BnARP17
BnARP18


BnARP19
BnARP20
BnARP21
BnARP22
BnARP23
BnARP24
BnARP25
410
420
430
440
At1g28330.5
AT1G54060.1
At1g56220.3
At2g33830.2
BrARP1
BrARP2
BrARP3
BrARP4
BrARP5
BrARP6
BrARP7
BrARP8
BoARP1
BoARP2
BoARP3
BoARP4
BoARP5
BoARP6
BoARP7
BoARP8
BnARP1
BnARP2
BnARP3
BnARP4
BnARP5
BnARP6
BnARP7
BnARP8
BnARP9
BnARP10
BnARP11
BnARP12
BnARP13
BnARP14
BnARP15
BnARP16
BnARP17
BnARP18
BnARP19
BnARP20
BnARP21
BnARP22
BnARP23
BnARP24
BnARP25
490

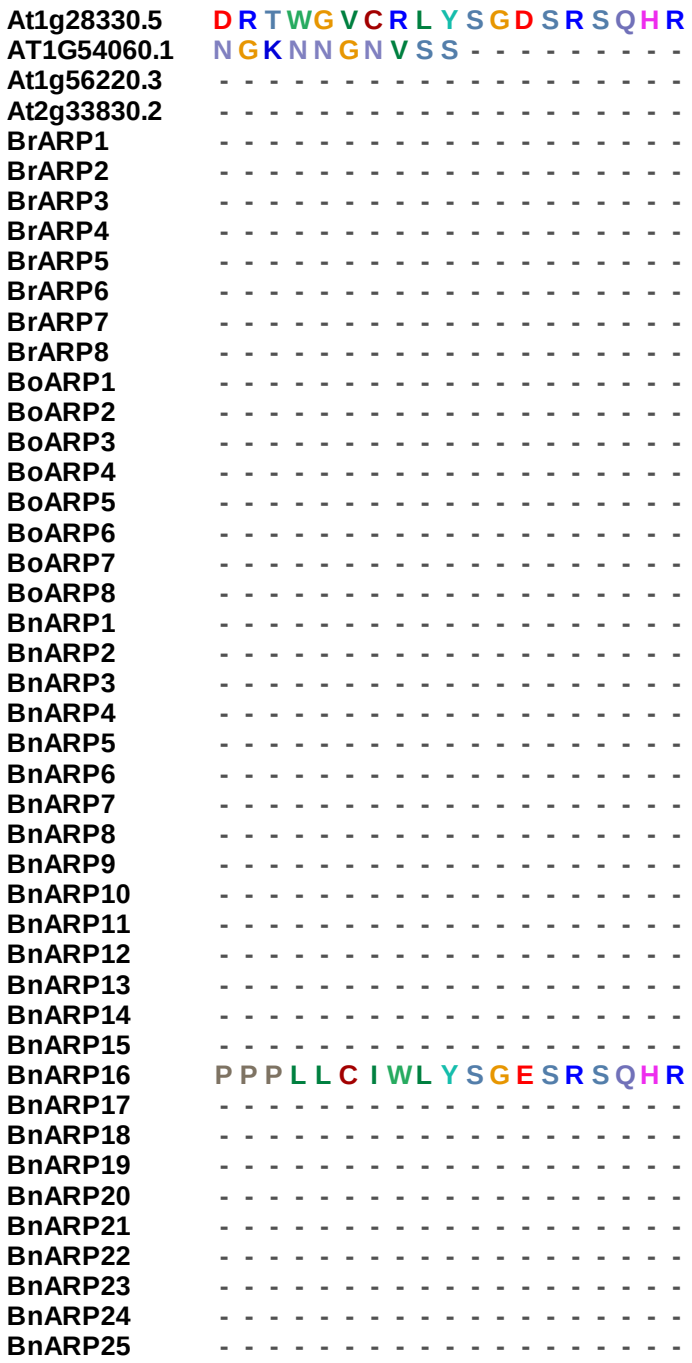


Figure S1 Sequence alignment of auxin-repressed proteins from *Arabidopsis*, *B. rapa*, *B. oleracea* and *B. napus*. The sequence alignment was generated using the ClustalW Multiple alignment method in BioEdit (http://www.mbio.ncsu.edu/bioedit/bioedit.html).

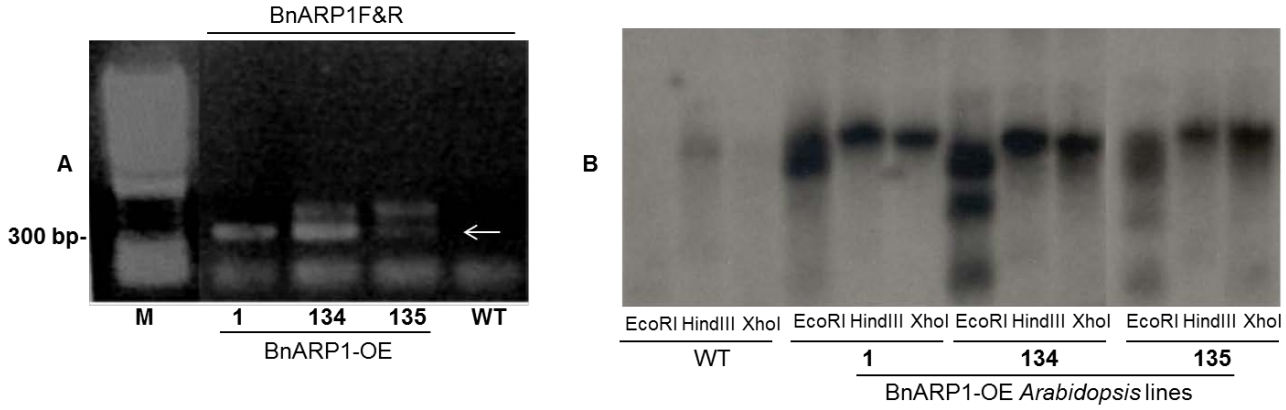


Figure S2. Molecular confirmation of the transgenes in *Arabidopsis* plants. A) Agarose gel showing PCR amplification of transgenes in 3 representative transgenic plants with *BnARP*1 primers. M: 1 kb plus DNA marker; 1: *BnARP*1*-OE*-1; 134: *BnARP*1*-OE*-134; 135: *BnARP*1*-OE*-135; WT: wild type. The 318 bp bands amplified by *BnARP*1 primers are *BnARP*1 CDSs, but the larger band (~500 bp) amplified by *BnARP*1 primers are part of AT2G33830 genes (see **Figure S3**). B) Representative Southern analysis of the *BnARP*1 transgene on restriction enzyme-digested gDNA for wild type and three *BnARP*1*-OE* expression lines. Since no restriction cut sites for all three enzymes on *BnARP*1 CDS probe, it should be only one band on each line, but more than one bands appeared on EcoRI lanes. This may because the probe was hybridized with AT2G33830 genes, which shows very high sequence similarity (see **Figure S3**).

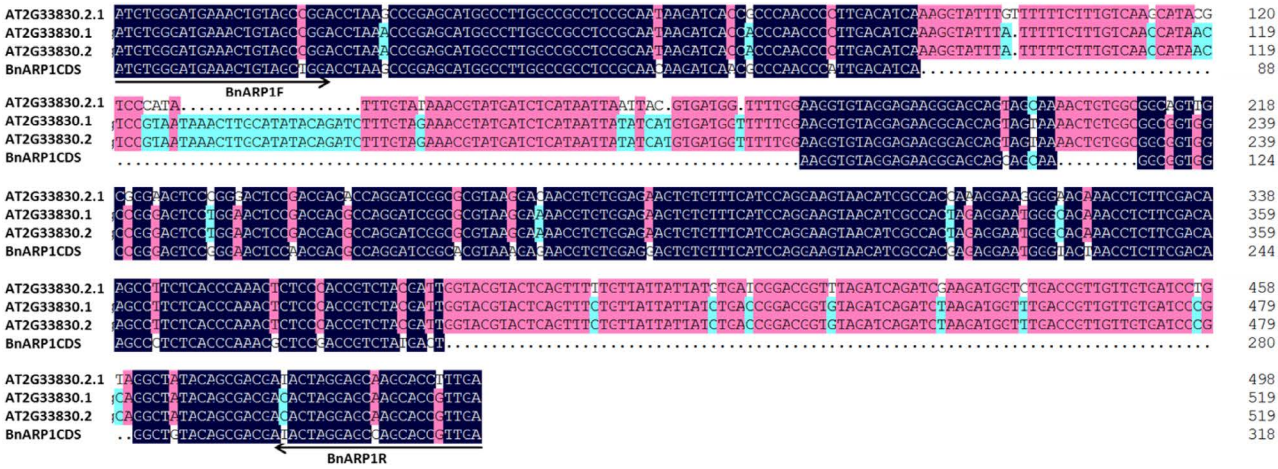


Figure S3. Alignment of nucleotide sequences of *BnARP*1 CDS and AT2G33830 DNAs.

Supplementary Table 1. Primers for *BnARP*1 coding sequences and Northern blotting.

Specific primers	ATG index	Amplicon size (bp)	Sequence
		Coding sequence amplification	
*BnARP*1*F*	NA	334 (318)	5'-gctctagaATGTGGGATGAAACTGTAGCTGGA-3'
*BnARP*1*R*			5'-ttgagctcTCAACGGTGCTGGCTCCTAGTAT -3'
		Probe amplification	
*AtDFL*1*F*	AT5G52510	926	5'-GAGGTTTCTGATGAGAGC-3'
*AtDFL*1*R*			5'-TGTGACATTGTTCCAGTC-3'
*AtPIN*1*F*	AT1G73590	912	5'-ATGATTACGGCGGCGGACT-3'
*AtPIN*1*R*			5'-AGCAGCCGTCGGTTTAGCA-3'
*AtABP*1*F*	AT4G02980	597	5'-ATGATCGTACTTTCTGTTGG-3'
*AtABP*1*R*			5'-TTAAAGCTCGTCTTTTTGTG-3'
*AtARF*1*F*	AT1G59750	938	5'-CAATGAAAGGTAATCGTGG-3'
*AtARF*1*R*			5'-CTGATCGAACGGAACAAT-3'
*AtAXR*1*F*	AT1G0510	452	5'-AATTGTGGCCCTACTGGTTCC-3'
*AtAXR*1*R*			5'-TTATTCAGGCGGAGGTCGTC-3'
*AtIAA*1*F*	AT4G14560	504	5'-ATGGAAGTCACCAATGGGCTTAACC-3'
*AtIAA*1*R*			5'-TAAGGCAGTAGGAGCTTCGGATCC-3'
AtAOSF	AT5G42650	1157	5'-ATGGCTTCTATTTCAACCCC-3'
AtAOSR			5'-CTAAAAGCTAGCTTTCCTTAACG-3'
*AtLOX*2*F*	AT3G45140	339	5'-TATTGTAGAGAGTCCTTGTCG-3'
*AtLOX*2*R*			5'-CTTGGCGCTAATAAGCTC -3'

NA: not applicable. Brackets ( ) show smaller size of BnARP1 amplicons used as probes for Northern blotting. Larger amplicons were generated by adding XbaI and SstI restriction sites (for cloning purposes).

Supplementary Table 2. The orthologues of *BnARPs* in *B. rapa*, *B. olerecea* and *A. thaliana.*

B. napus Genome		*B. rapa* Genome		*B. oleracea* Genome		*A. thaliana* Genome
BnARPs	Best two maches	*BrARPs*	*B. rapa* Chiifu protein matches	*BoARPs*	*B. oleracea* Capitata protein matches	Best matches
*BnARP*1	BnaC03g18820D		NF	*BoARP*1	Bol036995	AT2G33830.2
	BnaA03g15610D	*BrARP*1	Bra022955	*BoARP*1	Bol036995	AT2G33830.2
*BnARP*2	BnaA03g15610D	*BrARP*1	Bra022955	*BoARP*1	Bol036995	AT2G33830.2
	BnaC03g18820D		NF	*BoARP*1	Bol036995	AT2G33830.2
*BnARP*3	BnaA05g09830D	*BrARP*2	Bra005469	*BoARP*2	Bol027300	AT2G33830.2
	chrUn_random		NF		NF	NF
*BnARP*4	chrUn_random		NF		NF	NF
	BnaA05g09830D	*BrARP*2	Bra005469	*BoARP*2	Bol027300	AT2G33830.2
*BnARP*5	BnaA05g09830D	*BrARP*2	Bra005469	*BoARP*2	Bol027300	AT2G33830.2
	chrUn_random		NF		NF	NF
*BnARP*6	BnaA03g57280D	*BrARP*6	Bra000867	*BoARP*6	Bol030650	AT1G56220.3
	BnaC03g30710D	*BrARP*6	Bra000867	*BoARP*6	Bol030650	AT4G02740.1

Continued

BnARP7	BnaC02g27240D	*BrARP7*	Bra018532	*BoARP7*	Bol016535	AT1G56220.3
	BnaA02g20750D	*BrARP7*	Bra018532	*BoARP7*	Bol016535	NF
BnARP8	BnaA09g01040D	*BrARP8*	Bra036244	*BoARP8*	Bol006083	NF
	BnaC09g00190D		NF		NF	AT1G56220.3
BnARP9	BnaA03g57280D	*BrARP6*	Bra000867	*BoARP6*	Bol030650	AT1G56220.3
	BnaC03g30720D	*BrARP6*	Bra000867	*BoARP6*	Bol030650	AT1G56220.3
BnARP10	BnaA09g01040D	*BrARP8*	Bra036244	*BoARP8*	Bol006083	NF
	BnaC09g00190D		NF		NF	AT1G56220.3
BnARP11	BnaC03g18820D		NF	*BoARP1*	Bol036995	AT2G33830.2
	BnaA03g15610D	*BrARP1*	Bra022955	*BoARP1*	Bol036995	AT2G33830.2
BnARP12	BnaC03g30720D	*BrARP6*	Bra000867	*BoARP6*	Bol030650	AT1G56220.3
	BnaA03g57280D	*BrARP6*	Bra000867	*BoARP6*	Bol030650	AT1G56220.3
BnARP13	BnaC02g27240D	*BrARP7*	Bra018532	*BoARP7*	Bol016535	AT1G56220.3
	BnaA02g20750D	*BrARP7*	Bra018532	*BoARP7*	Bol016535	NF
BnARP14	chrUn_random		NF		NF	NF
	BnaA05g09830D	*BrARP2*	Bra005469	*BoARP2*	Bol027300	AT2G33830.2
BnARP15	BnaA09g27350D	*BrARP5*	Bra032894	*BoARP5*	Bol015732	AT1G28330.5
	BnaC05g21780D	*BrARP5*	Bra032894	*BoARP5*	Bol015732	AT1G28330.5
BnARP16	BnaA09g27350D	*BrARP5*	Bra032894	*BoARP5*	Bol015732	AT1G28330.5
	BnaC05g21780D	*BrARP5*	Bra032894	*BoARP5*	Bol015732	AT1G28330.5
BnARP17	BnaC03g30720D	*BrARP6*	Bra000867	*BoARP6*	Bol030650	AT1G56220.3
	BnaA03g57280D	*BrARP6*	Bra000867	*BoARP6*	Bol030650	AT1G56220.3
BnARP18	BnaC05g21780D	*BrARP5*	Bra032894	*BoARP5*	Bol015732	AT1G28330.5
	BnaA09g27350D	*BrARP5*	Bra032894	*BoARP5*	Bol015732	AT1G28330.5
BnARP19	BnaA09g01040D	*BrARP8*	Bra036244	*BoARP8*	Bol006083	NF
	BnaC09g00190D		NF		NF	AT1G56220.3
BnARP20	BnaC09g00190D		NF		NF	AT1G56220.3
	BnaC03g70780D		NF		NF	AT1G56220.3
BnARP21	BnaC06g06860D	*BrARP3*	Bra037961	*BoARP3*	Bol039069	AT1G54060.1
	BnaA06g01350D	*BrARP3*	Bra037961		NF	AT1G54070.1
BnARP22	BnaC06g10490D	*BrARP4*	Bra014369	*BoARP4*	Bol013372	NF
	BnaA05g14020D	*BrARP4*	Bra014369	*BoARP4*	Bol013372	NF
BnARP23	BnaC03g30720D	*BrARP6*	Bra000867	*BoARP6*	Bol030650	AT1G56220.3
	BnaA03g57280D	*BrARP6*	Bra000867	*BoARP6*	Bol030650	AT1G56220.3
BnARP24	BnaC03g70780D		NF		NF	AT1G56220.3
	BnaC09g00190D		NF		NF	AT1G56220.3
BnARP25	BnaC03g30720D	*BrARP6*	Bra000867	*BoARP6*	Bol030650	AT1G56220.3
	BnaA03g57280D	*BrARP6*	Bra000867	*BoARP6*	Bol030650	AT1G56220.3

Note: Original ESTs (represent *BnARPs*) were used to BLAST on the Genoscope website at http://www.genoscope.cns.fr/blat-server/cgi-bin/colza/webBlat. NF: Not Found.

Supplementary Table 3. Diverse growth phenotypes for transgenic Arabidopsis expressing *BnARP*1

*BnARP*1 -*OE*	T_1 phenotypes for independent transgenic events	*Km*^{r}, *PCR*$^+$ T_1 *plants*	T_2 phenotypes for selected T_1 families
T_1 Very dwarfed seedlings	Dwarf adult, late harvest time	3	3; WT-like growth, flowered, green leaf (2 yellow seed)
	WT-like adult, late harvest time	2	2; WT-like growth, flowered, green leaf
	WT-like adult, WT-like harvest time	13	2; WT-like growth, more siliques than WT, purple leaf
T_1 Dwarfed seedlings	Dwarf adult, late harvest time	1	1; WT-like growth, flowered, green leaf
	WT-like adult, WT-like harvest time	30	
T_1 WT-like seedlings	Dwarf adult, WT-like harvest time	1	1; WT-like growth, more siliques than WT, purple leaf
	WT-like adult, WT-like harvest time	49	1; WT-like growth, flowered, green leaf
Total Confirmed T_1 transformants		99	