Available online at www.sciencedirect.com
SCIENCE DIRECT
Plant Science 169 (2005) 21–28
PLANT SCIENCE
www.elsevier.com/locate/plantsci

Comparison of ABP1 over-expressing *Arabidopsis* and under-expressing tobacco with an auxinic herbicide-resistant wild mustard (*Brassica kaber*) biotype

J. Mithila, J.C. Hall *

Canada Research Chair in Recombinant Antibody Technology, Department of Environmental Biology, University of Guelph, Guelph, Ont.,Canada N1G 2W1

Received 8 October 2004; received in revised form 2 February 2005; accepted 2 February 2005
Available online 31 March 2005

Abstract

An auxinic herbicide-resistant biotype of wild mustard was discovered in western Canada in a field sprayed once each year for ten years with combinations of 2,4-D, MCPA and dicamba. Earlier research suggests that the resistance may be due to altered binding of auxinic herbicides to an auxin-binding protein (ABP) in wild mustard. To further elucidate the role of ABP in inducing auxinic herbicide responses in wild mustard, a series of in vitro experiments were initiated. A protocol for generating cell suspensions of both auxinic herbicide-resistant (R) and -susceptible (S) wild mustard was developed from callus cultures. Auxinic herbicide-induced physiological responses (e.g. cell elongation or lateral root formation) of R and S biotypes were compared with ABP1 antisense tobacco (NAS1), as well as to ABP1 over-expressing *Arabidopsis* in cell suspension and seed culture experiments, respectively. Upon treatment with either NAA (synthetic auxin) or picloram (auxinic herbicide), there was an increased cell elongation in the S biotype and wild-type tobacco, whereas there was no cell elongation in cell suspensions of the R biotype and NAS1. A dose–response assay measuring picloram-induced lateral root formation in the in vitro seed cultures of wild mustard and *Arabidopsis* showed excessive lateral root formation in the S biotype (0.5–10 μM) and ABP1 over-expressing *Arabidopsis* (1 μM). These results correlate with the differences between ABP (a potential auxin receptor) levels in R and S wild mustard and this difference may be a possible mechanism of resistance to auxinic herbicides.

Keywords: *Arabidopsis*; Auxin-binding protein; Auxinic herbicides; Cell elongation; Lateral roots; Tobacco

1. Introduction

High doses of auxin and auxinic herbicides induce several similar effects including cell elongation, epinasty (downward curling of leaf petiole), hypertrophy (abnormal growth of internodal region), and excessive lateral root formation in sensitive species. However, the precise mechanism of action of both auxin and auxinic herbicides remain unknown 60 years after their discovery [1]. Knowledge about the mode of action of auxin can be related to the mechanism of auxinic herbicide action due to several structural and resulting physiological and biochemical similarities between these compounds [1].

Wild mustard (*Brassica kaber* syn: *Sinapis arvensis*) is a common weed in cereal crops and can be effectively controlled with auxinic herbicides such as dicamba, MCPA and 2,4-D. The discovery of a wild mustard biotype [2] that is resistant (R) to a combination of auxinic herbicides offers several avenues to characterize the physiological, biochemical and molecular basis of auxinic herbicide resistance in this species [3]. It has been reported that the resistance to auxinic herbicides (i.e., picloram, 2,4-D, dicamba and

Abbreviations: ABP, auxin-binding protein; BAP, benzyl amino-purine; 2,4-D, (2,4-dichlorophenoxy)acetic acid; IAA, indole acetic acid; KH_2PO_4, potassium di-hydrogen phosphate; MCPA, (4-chloro-2-methylphenoxy)acetic acid; MS, Murashige and Skoog; NAA, naphthalene acetic acid; NAS1, nicotiana antisense

* Corresponding author. Tel.: +1 519 824 4120x52740; fax: +1 519 837 0442.
E-mail address: jchall@uoguelph.ca (J.C. Hall).

0168-9452/$ – see front matter
doi:10.1016/j.plantsci.2005.02.010

MCPA) in R wild mustard is not due to changes in herbicide absorption, translocation, sequestration or metabolism [4]. However, analysis of ^{3}H-IAA binding to auxin-binding protein (ABP) indicates that there are both low- and high-affinity binding sites in auxinic herbicide-susceptible (S) wild mustard, whereas there is only a low-affinity binding site in the R biotype [5]. Furthermore, herbicide resistance was associated with the extent of auxinic herbicide binding to ABP(s) in R and S wild mustard [5,6]; consequently, the associated cascade of biochemical and physiological responses triggered by binding were different in the two biotypes.

ABP(s) have been well characterised in several systems, viz., corn, tobacco, radish and *Arabidopsis* [7]. Investigations uncovering the mode of action of auxins have also provided evidence for the role of ABP as a potential auxin receptor, responsible for several auxin-induced effects in plants [8,9]. The biochemical changes such as Ca^{2+} influx, plasma membrane ATPase activity and increased nucleic acid and protein synthesis are believed to be elicited by ABP which binds with auxin [10–12]. However, there is no conclusive evidence demonstrating the function of ABP(s) as auxin receptors and sites for triggering auxinic herbicide action.

To further elucidate the role of ABP(s) in auxinic herbicide resistance in wild mustard, this investigation was initiated using in vitro cell and seed culture systems based on the hypothesis that resistance may be due to differences in the interaction of auxinic herbicides with a primary target site (ABP) in wild mustard. Auxinic herbicide-R and -S biotypes collected from Gilbert plains and Minto Manitoba, Canada, respectively, were used in this research. The resistant biotype was identified in a field that was repeatedly treated with a combination of MCPA, dicamba and 2,4-D over a 10-year period. The specific objectives were (i) to use single-cell suspensions to evaluate the effects of auxin and auxinic herbicides on cell elongation of R and S wild mustard biotypes compared to ABP1 antisense and wild-type tobacco, and (ii) to assess the responses of an auxinic herbicide (picloram) on lateral root formation of R and S wild mustard as well as ABP1 over-expressing and wild-type *Arabidopsis*.

2. Materials and methods

Cell suspensions of R and S biotypes of wild mustard were developed from callus cultures. Cell suspensions of ABP1 antisense (NAS1) and wild-type tobacco as well as the seed of the two *Arabidopsis* ABP1 over-expressing lines (# 19, # 30) and a wild type (# 4) were kindly provided by Dr. A.M. Jones (department of biology, University of North Carolina at Chapel Hill, NC, USA). Over-expression of ABP1 in independent transformants (# 19, # 30) of *Arabidopsis* has been shown in Dr. Jones laboratory (personal communication).

2.1. Cell culture experiments

2.1.1. Development of cell suspensions of wild mustard

2.1.1.1. Callus induction from the R and S biotypes. About 50 seeds of both R and S wild mustard were surface sterilized with 80% alcohol for 2–3 min and treated for 10–12 min with 50% bleach containing a drop of Tween 20. The seeds were rinsed four to five times with sterile distilled water. The sterilized seeds were cultured aseptically on MS medium containing MS salts [13], MS vitamins and 3% sucrose. The medium was gelled with 8 g/L of agar. The pH of the medium was adjusted to 5.7 before autoclaving at 121 °C for 20 min.

Four to five seeds were cultured in each Petri dish (100 × 15 mm), which contained 20 ml of medium. Petri dishes were sealed with Parafilm and the cultures were incubated in the dark at room temperature. The seeds germinated after a week of initial culturing and 2-week-old seedlings (1 each) were transferred aseptically into Magenta boxes (300-ml plastic vessels, Magenta Corp., Chicago, IL, USA) containing 50 ml of the medium described above. The cultures were incubated in a growth room at 24 °C in light (16-h photoperiod; 50 μmol s^{-1} m^{-2}) provided by cool white fluorescent lamps (Philips Canada, Scarborough, ON). After 6–7 weeks of initial culturing, leaf discs of about 1 cm were excised aseptically using a scalpel from the seedlings of R and S wild mustard and cultured on various media combinations (described below) for callus induction.

In preliminary experiments, the callus induction medium contained MS salts, MS vitamins, and sucrose (3%), supplemented with either NAA (0.05–2 mg/L), BAP (0.5–2 mg/L), or combinations of the two chemicals. Although the leaf explants initiated callus on several media combinations, the callus was not friable and could not be used to make cell suspensions upon transfer to liquid medium. However, both R and S wild mustard produced friable callus, which was used to produce actively growing cell suspensions (see below), when grown on MS medium containing MS salts, sucrose (3%), myo-inositol (100 mg/L), and thiamine (1 mg/L), supplemented with NAA (0.1 mg/L) and BAP (1 mg/L). The pH of the medium was adjusted to 5.7 before autoclaving at 121 °C for 20 min. Every two weeks thereafter, the initial callus was sub-cultured on the fresh medium (same as the callus induction medium). Actively growing callus of both the R and S wild mustard was used for initiating cell suspensions.

2.1.1.2. Initiation of cell suspensions from callus cultures of R and S wild mustard. To develop single-cell suspensions of wild mustard, undifferentiated actively growing R and S wild mustard callus (pieces of 3–5 mm length) were transferred to 125-ml Erlenmeyer flasks containing 25 ml of liquid medium (same media composition as that of callus-induction medium except agar which was not added). These cultures were incubated in the dark on a rotary shaker at 150 rpm. One week following callus transfer, single cells

and cell aggregates were released into the medium. After 10 d, the cell suspensions (10 ml) from these cultures were sub-cultured into 50 ml of fresh liquid medium contained in sterile 125-ml Erlenmeyer flasks, and thereafter, the cell growth was recorded as follows.

Ten-day-old cell suspensions were transferred aseptically into sterile-disposable graduated centrifuge tubes (50 ml) and were centrifuged at 1000 rpm for 10 min. The volumes of the cells in the liquid were recorded. The content of each centrifuge tube was aseptically transferred to a new 125-ml sterile Erlenmeyer flasks and the cells were allowed to grow in the dark for seven additional days on a rotary shaker at 150 rpm. The cell suspensions were centrifuged again as described above. The new volume of the cells in each liquid culture was recorded. The active cell growth was assessed from the initial and final volumes. Subsequently, 1 ml of the actively growing cell suspension was transferred to 50 ml of fresh liquid medium in a new 125-ml sterile Erlenmeyer flask every two weeks for continuous growth of the cells.

2.1.2. Maintenance of cell suspensions of tobacco

Cell suspensions of ABP1 antisense (NAS1) and wild-type (BY-2) tobacco were maintained by regular sub-culturing as follows: one millilitre of actively growing cell suspension of each tobacco cell line was transferred separately to a 250-ml sterile Erlenmeyer flask containing 50 ml of the liquid medium. The medium consisted of MS salts, sucrose (3%), thiamine (1 mg/L), myo-inositol (100 mg/L), KH_2 PO_4 (255 mg/L), supplemented with 2,4-D (0.2 mg/L). The medium (pH 5.0) was autoclaved at 121 °C for 20 min. Cultures were incubated in the dark on a rotary shaker (150 rpm).

2.1.3. Cell suspensions and treatment with auxin and auxinic herbicides

One week after sub-culturing, the actively growing cell suspensions of R and S wild mustard, as well as ABP antisense and wild-type tobacco-suspension cultures were treated with either NAA or picloram. Cell suspensions were grown on auxin-free cell suspension medium (as described above for the R and S wild mustard as well as NAS1 and wild-type tobacco) for 3 d before treatment. Twenty-four well tissue-culture plates (Corning Inc.; NY, USA) were used for these experiments. A cell suspension (1 ml) of each genotype (R and S wild mustard, as well as NAS1 and wild-type tobacco) were aseptically added to each well and NAA or picloram at 0, 0.1, 0.5, 1, 5, 10 or 100 μM was added. Each treatment was replicated twice. The plates were sealed with two layers of Parafilm and incubated in the dark on a rotary shaker at 150 rpm.

Four and eight days after treatment, samples from tobacco and wild mustard cell cultures were observed under a light microscope (Axiostar; Zeiss Inc., Germany). The images of the cells were taken through a digital camera (Sony, 3.3 mega pixels) attached to the microscope and the cell length (μm) of all the treated cells including controls was recorded using a software package (Axiovision; Zeiss Inc., Germany). In each replication, about 25 observations were recorded; all experiments were repeated twice.

2.2. Seed culture experiments

Seeds of R and S wild mustard (from the same source as mentioned before) as well as ABP over-expressing and wild-type (Columbia) *Arabidopsis* were cultured aseptically on MS medium. About 120–130 (∼200 mg) seeds of R and S wild mustard were surface and sterilized for 2–3 min with 80% alcohol and then treated for 10–20 min with 50% bleach containing a drop of Tween 20. Seeds were rinsed 4–5 times with sterile distilled water. For *Arabidopsis*, ∼3 mg of ABP over-expressing lines (# 19, # 30) and wild-type (# 4) seed were surface-sterilized for 1–2 min with 80% alcohol prior to treatment with 30% bleach for 10–15 min. The seeds were rinsed four to five times with sterile distilled water.

The sterilized seeds of R and S wild mustard were cultured aseptically on MS medium containing MS salts, vitamins, and 3% sucrose supplemented with NAA or picloram at 0, 0.1, 0.5, 1, 5 or 10 μM (added to the medium after autoclaving). The medium was gelled with 8 g/L of agar and the pH of the medium was adjusted to 5.7 before autoclaving at 121 °C for 20 min. Four to five seeds were cultured in each Petri dish (100 × 15 mm) containing 20 ml medium. Petri dishes were sealed with Parafilm and the cultures were incubated in the dark at room temperature. *Arabidopsis* seed was also cultured and treated similarly except that the medium containing 10 μM estradiol as an inducer (for trasgene expression). Furthermore, the *Arabidopsis* cultures were maintained in the dark at 4 °C for 3 d (to enhance seed germination) before being incubated at room temperature. Each treatment was replicated 3–4 times for wild mustard and *Arabidopsis*, respectively.

All the seed cultures were assessed for lateral root formation. Fresh weight of roots (mg) was recorded 3 and 5 weeks after culturing wild mustard and *Arabidopsis*, respectively.

2.3. Statistical analyses

All experiments (cell culture and seed culture) consisted of a completely randomized design and all experiments were repeated at least twice. The data from each experiment were subjected to an analysis of variance (ANOVA; $P \leq 0.01$) and means were separated using LSD ($P \leq 0.01$).

3. Results and discussion

Mutants with resistance to herbicides are excellent tools to uncover and/or understand pathways responsible for the physiological and biochemical effects induced by these compounds. Several auxin-resistant mutants have been

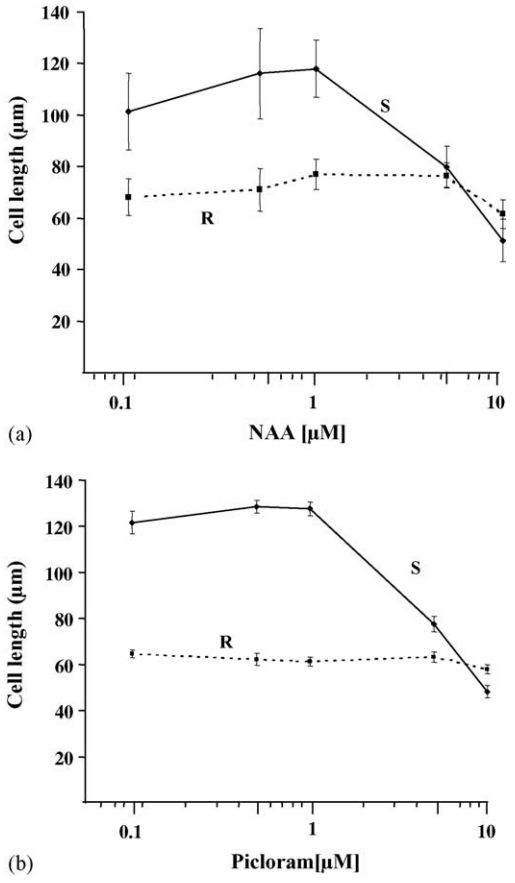

Fig. 1. Effect of 0, 0.1, 0.5, 1, 5, and 10 µM NAA (a) and picloram (b) on cell elongation of susceptible ('-' line;◆) and resistant ('- -'line;■) wild mustard biotypes. Bars represent SEM. Data on the *x*-axis are in logarithmic scale. Analysis of variance indicated a significant ($P \leq 0.01$) dose × biotype interaction.

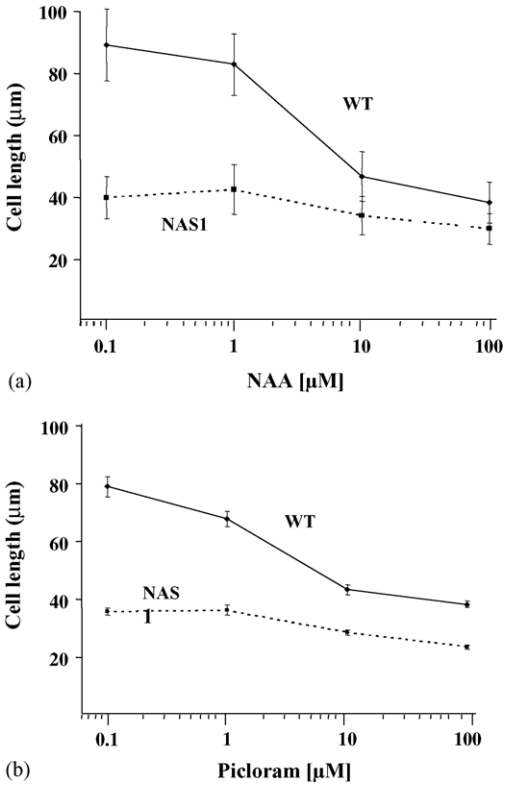

Fig. 2. Effect of 0, 0.1, 1, 10 and 100 µM NAA (a) and picloram (b) on cell elongation of wild-type ('-' line;◆) and ABP1 antisense ('- -'line;■) lines of tobacco. Bars represent SEM. Data on the *x*-axis is in logarithmic scale. Analysis of variance indicated a significant ($P \leq 0.01$) dose × line interaction.

developed in *Arabidopsis* through induced mutations [14–17] and have played pivotal roles in elucidating potential mechanisms of auxin's action. Similarly, herbicide-resistant biotypes also play a primary role in illustrating the mechanism of action of these compounds [3–5]. Auxinic herbicide-resistant *Brassica kaber* thus offers an excellent system to understand the mechanism of action of these agriculturally important compounds. In this study, auxinic herbicide R and S wild mustard were used to show auxinic herbicide elicited in vitro physiological responses in cell and seed cultures and compare their responses with those of ABP1 over- and under-expressing *Arabidopsis* and tobacco lines, respectively.

The most important and well-established auxin and auxinic herbicide-induced effects in plants include cell elongation, cell division, and lateral root formation [18–21]. Both auxin and auxinic herbicide-induced cell elongation and lateral root formation were the focus of this investigation. To test the hypothesis that auxinic herbicide resistance in R wild mustard is due to ABP concentration [5], cell elongation in R and ABP1 antisense tobacco (NAS1) were compared following the treatment with NAA or Picloram. (ABP1 was undetectable in NAS1 when probed with several ABP1 specific antibodies [22]). Conversely, lateral root formation in the S biotype of wild mustard, which has a higher concentration of ABP than R [5] was compared to ABP1 over-expressing *Arabidopsis* following treatment with picloram. Therefore, ABP1 antisense tobacco and ABP1 over-expressing *Arabidopsis* may be considered as surrogates of R and S wild mustard, respectively.

This is the first report of the establishment of callus cultures of wild mustard. Furthermore, a protocol for producing single-cell suspensions of R and S wild mustard was developed. MS medium containing MS salts, sucrose (3%), myo-inositol (100 mg/L), and thiamine (1 mg/L), supplemented with NAA (0.1 mg/L) and BAP (1 mg/L) was found to be optimal for initiating the growth of callus as well as single-cell suspensions of both R and S wild mustard. Dose–response assays were also developed to assess picloram-induced lateral root formation in seed cultures of wild mustard and *Arabidopsis*.

3.1. Effect of NAA and picloram on cell elongation of wild mustard and tobacco

Single-cell suspensions of wild mustard and tobacco responded similarly to both picloram and NAA, 4 and 8 days after treatment, respectively. There was a dose-dependent increase in cell elongation in the S biotype of wild mustard

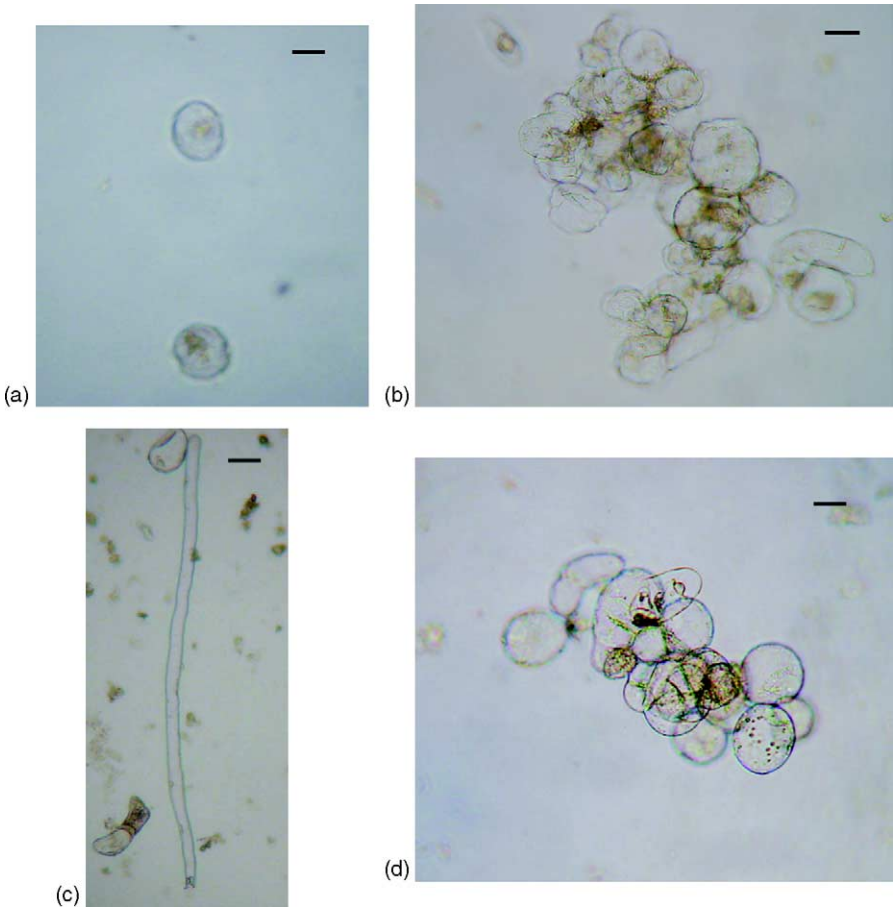

Fig. 3. Single-cell suspensions of wild mustard: a and b show the untreated cells of susceptible (S) and resistant (R) biotypes, respectively; c and d show the effect of picloram (1 μM) on the S and R cells, respectively (scale bar: 10 μm).

and the wild-type tobacco in response to both NAA and picloram treatment (see Figs. 1–4). In contrast, there was little or no cell elongation in suspensions of R wild mustard and NAS1 tobacco treated with the same doses of NAA and picloram (see Figs. 1–4). The effect of NAA on cell elongation of both NAS1 and wild-type tobacco observed in this study was in accordance with the results reported by Chen et al. [22] using the same tobacco lines.

3.2. Effect of picloram on lateral root formation of wild mustard and Arabidopsis

Seed culture experiments indicate that addition of picloram to the medium induced excessive lateral root formation with a concomitant increase in fresh-root biomass in the S biotype of wild mustard, whereas the R biotype formed normal roots at all doses of picloram (Fig. 5). In the case of *Arabidopsis*, ABP over-expressing lines # 19 and # 30 were ∼5 times more sensitive (1 μM) to picloram than the wild type (5 μM; Figs. 6 and 7a–f).

Seed culture experiments were also conducted using NAA as the source of auxin. While significant differences (similar to picloram) were found between lateral root formation in R and S wild mustard, there was no significant effect of NAA on ABP1 over-expressing and wild-type *Arabidopsis* (data not shown). It has been reported [23] that the dose-dependent physiological and biochemical effects produced by auxins and auxinic herbicides depend largely on the chemical structure of these compounds (the structure–activity relationship, i.e., potency depends on structure), their persistence (i.e., half-life of molecule) in the plant, cellular concentration, tissue type, and the stage of organ development. Therefore, differences in root biomass in response to NAA and picloram may be attributed to differences in their potency (picloram > NAA) and/or persistence (picloram > NAA). Seed culture experiments were not conducted with wild-type and ABP1 antisense tobacco lines because the transgenic lines were developed as cell suspensions (Dr. Jones laboratory) and the cells would not differentiate to produce plants, and subsequently the seed (data not shown).

The results of these experiments suggest that cell elongation was similar in ABP1 antisense tobacco and R wild mustard when either NAA or picloram was used (Figs. 1a and b; 2a and b; 3a–d; 4a–d). Likewise, lateral root growth of ABP1 over-expressing *Arabidopsis* (lines # 19, # 30) and S wild mustard was similar following treatment with picloram (Figs. 5, 6 and 7a–f), suggesting that differences in

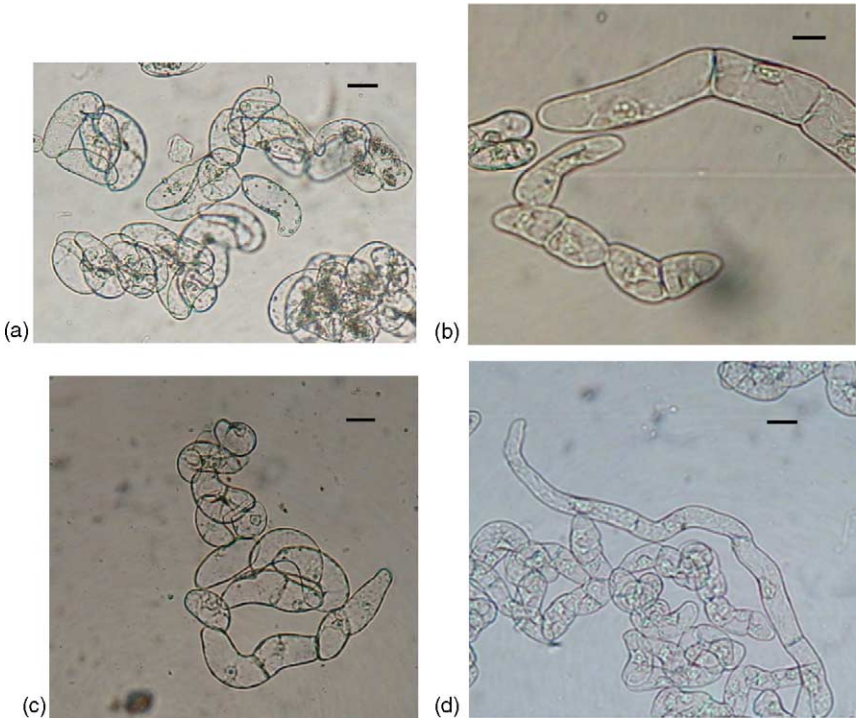

Fig. 4. Single-cell suspensions of tobacco: a and b show the untreated suspension of NAS1 and wild type, respectively; c and d show the effect of picloram (1 μM) on NAS1 and wild-type cells, respectively. (Scale bar: 10 μm).

the concentration of ABP in R (low) and S (higher) wild mustard may influence their sensitivity to auxinic herbicides.

Auxin-induced cell elongation has been well characterised in several systems; this effect has been related to the H^+ efflux leading to acidification of cell walls, i.e. the apoplast matrix [18]. The resulting high pH activates enzymes, which act on cell wall extensibility [24]. Similarly, auxinic herbicide-induced cell elongation has also been reported due to activation of plasma membrane ATPase, resulting in H^+ extrusion through the plasma membrane into the cell wall [21,25]. Furthermore in wild mustard, a light scattering study indicated that the signal amplitudes from picloram-treated S protoplasts resulted in the increased H^+ efflux, whereas the protoplasts from R biotype were unaffected [6]. Therefore, picloram and NAA-induced cell elongation in both S wild mustard and wild-type tobacco is probably due to increased H^+ efflux into the cell wall.

Several proteins have been shown to bind auxins and auxinic herbicides and the likelihood of these ABPs being auxin receptors with physiological and biochemical activity has been extensively discussed [9,26]. Among a number of ABPs discovered, only ABP1 has been reported to have a high-affinity binding site coupled with good specificity for

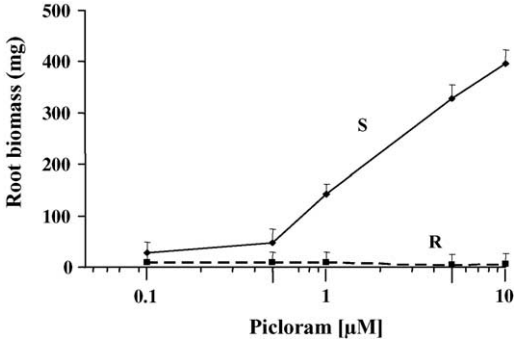

Fig. 5. Effect of picloram on root biomass of susceptible ('-' line;◆) and resistant ('- -'line;■) wild mustard biotypes. Bars represent SEM. Data on the *x*-axis is in logarithmic scale. SEM. bars are not visible where they are smaller than the symbol. Analysis of variance indicate a significant ($P \leq 0.01$) dose × biotype interaction.

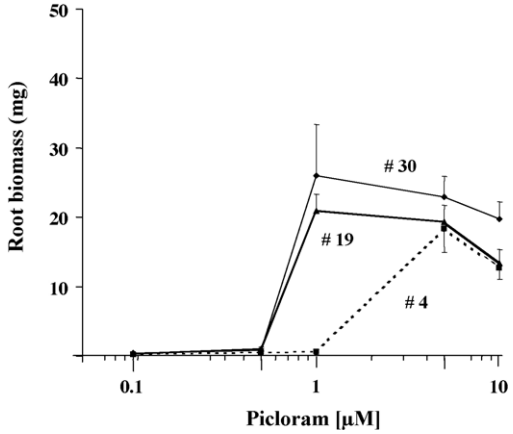

Fig. 6. Effect of picloram on root biomass of *Arabidopsis* wild-type ('- -'line;■) ABP1 over-expressing [('-' line;▲) and ('-' line;◆)] lines. Bars represent SEM. Data on the *x*-axis are in logarithmic scale. SEM bars are not visible where they are smaller than the symbol. Analysis of variance indicated a significant ($P \leq 0.01$) dose × line interaction.

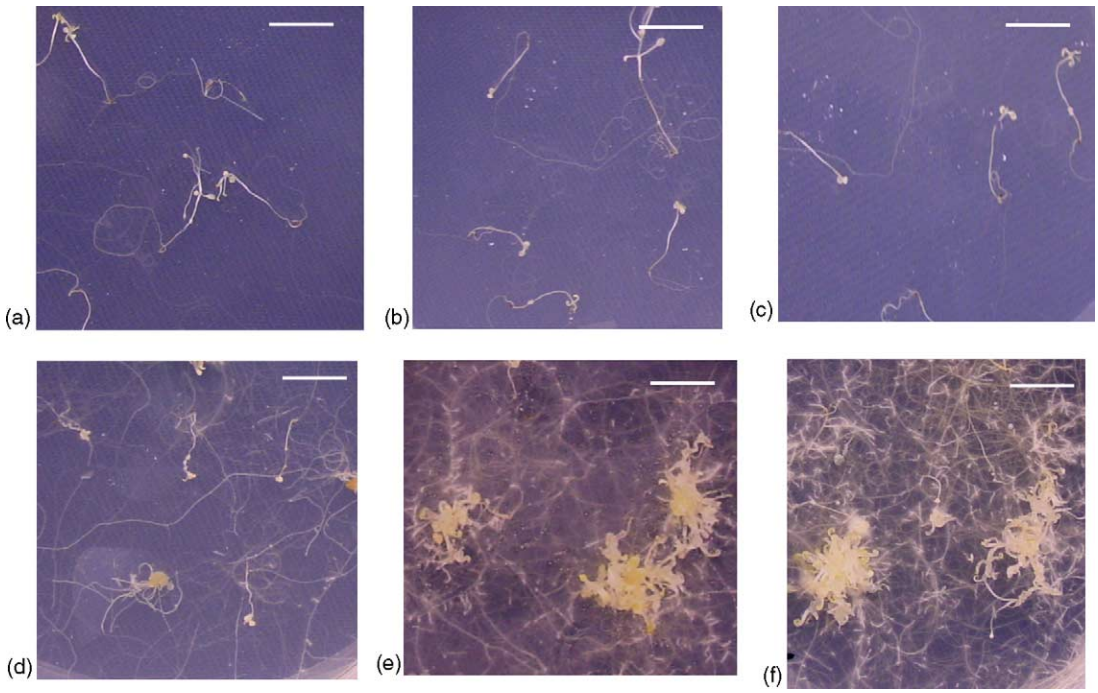

Fig. 7. *Arabidopsis* seed cultures. a, b and c represent untreated cultures of wild type (# 4) and ABP over-expressors (# 19 and # 30), respectively; d, e and f illustrate the effect of picloram (1 μM) on seed explants of # 4, # 19 and # 30, respectively (scale bar: 10 mm).

active auxin [27]. Although the other ABPs have sites for binding to hydrophobic ligands, these sites show low specificity and no regulatory function, and therefore they are not considered to be functional receptor proteins [27]. In addition, the best understood candidate for auxin-induced cell elongation in plants is ABP1 [9,22,28]. The role of ABP1 in activating proton pump ATPase through hyperpolarization [12], flux through potassium channels [29] and voltage dependent anion channels [30] has also been shown in plants. Recently for example, over-expression of ABP1 in transgenic tobacco plants has been demonstrated to have significant influence on K^+ fluxes in intact guard cells [31]. Furthermore, embryogenesis was arrested in *Arabidopsis* ABP1 homozygous knockout plants. Addition of a transgenic functional copy of ABP1 rescued this embryonic-lethal phenotype [22]. Chen et al. [22] also suggest that ABP1 is required early in plant development and the ABP1 knockout embryos cease to develop after the globular stage because of improper orientation of the cell wall resulting in failure of cell elongation. Therefore, the responses of ABP1 antisense tobacco and R wild mustard cultures (i.e. no response to auxin-induced cell elongation) observed in this research may be related to decreased affinity or quantities of ABP, which may result in reduced sensitivity of both types of plant cell cultures to NAA and picloram.

Initiation and stimulation of lateral and adventitious roots have also been touted as an auxin-induced response. Auxin stimulates cells to divide in the pericycle region resulting in formation of lateral and adventitious roots. In *Arabidopsis*, *alf1* (aberrant lateral root formation) mutants exhibit excessive proliferation of lateral roots and this effect was associated with increased endogenous auxin levels [20]. Also in several auxinic herbicide-susceptible species, production of excessive adventitious roots has been observed after treatment with these herbicides.

In conclusion, comparison of the response of both ABP1 antisense tobacco cells and ABP1 over-expressing *Arabidopsis* plants with R and S wild mustard following treatment with NAA or picloram, suggests that differences in ABP concentrations in R (lower ABP concentration) and S (higher ABP concentration) wild mustard may explain their respective sensitivity to the auxinic herbicides. However, the conclusive link between ABP concentration and sensitivity to auxinic herbicides in wild mustard has not yet been demonstrated. Differential expression of ABP from R and S wild mustard in ABP null mutants of *Arabidopsis* may help us discern whether the level of ABP expression determines the sensitivity of the respective wild mustard biotype to auxin and auxinic herbicides.

Acknowledgement

This research was supported by grants to JCH from the Natural Science and Engineering Research Council of Canada (NSERC) and Ontario Ministry of Agriculture and Food (OMAF). The post graduate scholarship to MJ from NSERC is gratefully acknowledged. We also thank Dr. A. M. Jones, department of biology, University of North Carolina at Chapel Hill, NC, USA for providing the transgenic lines of tobacco and *Arabidopsis* and valuable information on his research with these lines.

References

[1] T.M. Sterling, J.C. Hall, Mechanism of action of natural auxins and auxinic herbicides, in: R.M. Roe, J.D. Burton, R.J. Kuhr (Eds.), Herbicide Activity: Toxicology, Biochemistry and Molecular Biology, IOS Press, Amsterdam, 1997, pp. 111–141.

[2] I.M. Heap, I.N. Morrison, Resistance to auxin-type herbicides in wild mustard (*Sinapis arvensis* L) populations in western Canada, Annu. Meeting of Weed Sci. Soc. Am. 32 (1992) 164, Abstract #32.

[3] H.G. Zheng, J.C. Hall, Understanding auxinic herbicide resistance in *Sinapis arvensis* L.: physiological, biochemical and molecular genetic approaches, Weed Sci. 49 (2001) 276–281.

[4] M.G. Penuik, M.L. Romano, J.C. Hall, Physiological investigations into the resistance of a wild mustard (*Sinapis arvensis* L) biotype to auxinic herbicides, Weed Res. 33 (1993) 431–440.

[5] S.R. Webb, J.C. Hall, Auxinic herbicide-resistant and susceptible wild mustard (*Sinapis arvensis* L.) biotypes: effect of auxinic herbicides on seedling growth and auxin-binding activity, Pestic Biochem. Physiol. 52 (1995) 137–148.

[6] S. Deshpande, J.C. Hall, Auxinic herbicide resistance may be modulated at an auxin-binding site in wild mustard (*Sinapis arvensis* L): A light scattering study, Pestic Biochem. Physiol. 66 (2000) 41–48.

[7] A.M. Jones, Auxin-binding proteins, Annu. Rev. Plant Physiol. 45 (1994) 393–420.

[8] R.M. Napier, M.A. Venis, Monoclonal antibodies detect an auxin-induced conformational change in the maize auxin-binding protein, Planta 182 (1990) 313–318.

[9] A.M. Jones, K-H. Im, M.A. Savka, M.J. Wu, N.G. DeWitt, R. Shillito, A.N. Binns, Auxin-dependent cell expansion mediated by over-expressed auxin-binding protein1, Science 282 (1998) 1114–1117.

[10] G. Ephritikhine, H. Barbier-Brygoo, J.F. Muller, J. Guern, Auxin effect on the transmembrane potential difference of wild-type and mutant tobacco protoplasts exhibiting a differential sensitivity to auxin, Plant Physiol. 84 (1987) 801–804.

[11] H. Barbier-Brygoo, G. Ephritikhine, C. Maurel, J. Guern, Perception of auxin signal at the plasma membrane of tobacco mesophyll protoplasts, Biochem. Soc. Trans. 20 (1992) 59–63.

[12] A. Ruck, K. Palme, M.A. Venis, R.M. Napier, R.H. Felle, Patch-clamp analysis establishes a role for an auxin-binding protein in the auxin stimulation of plasma membrane current in *Zea mays* protoplasts, Plant J. 4 (1993) 41–46.

[13] T. Murashige, F. Skoog, A revised medium for rapid growth and bioassays with tobacco tissue culture, Physiol. Plant 15 (1962) 473–497.

[14] C. Lincoln, M. Estelle, The axr1 mutation of *Arabidopsis* is expressed in both roots and shoots, J. Iowa Acad. Sci. 98 (1991) 68–71.

[15] A. Cernac, C. Lincoln, D. Lammer, M. Estelle, The SAR1 gene of *Arabidopsis* acts downstream of the AXR1 gene in auxin response, Development 124 (1997) 1583–1591.

[16] L. Hobbie, M. Estelle, The axr4 auxin-resistant mutants of *Arabidopsis thaliana* define a gene important for root gravitropism and lateral root initiation, Plant J. 7 (1995) 211–220.

[17] L. Hobbie, M. McGovern, L.R. Hurwitz, A. Pierro, N.Y. Liu, A. Bandyopadhyay, M. Estelle, The *axr6* mutants of *Arabidopsis thaliana* define a gene involved in auxin response and early development, Development 127 (2000) 23–32.

[18] M.L. Evans, The action of auxin on plant cell elongation, CRC Crit. Rev. Plant Sci. 2 (1985) 317–365.

[19] Y. Takahashi, H. Kudora, T. Tanaka, Y. Machida, I. Takebe, T. Nagata, Isolation of auxin-regulated *par* gene cDNA expressed during the transition from G0 to S phase in tobacco mesophyll protoplasts, Proc. Natl. Acad. Sci. USA 86 (1989) 9279–9283.

[20] J.L. Celenza, P.L. Grisafi, G.R. Fink, A pathway for lateral root formation in *Arabidopsis thaliana*, Genes Dev. 9 (1995) 2131–2142.

[21] M.D. Devine, S.O. Duke, C. Fedtke, Herbicides with auxin activity, in: Physiology of Herbicide Action, P.T.R. Pretice Hall, Englewood Cliffs, NJ, USA, 1993, pp. 295–309.

[22] J.G. Chen, H. Ullah, J.C. Young, M.R. Sussman, A.M. Jones, ABP1 is required for organized cell elongation and division in *Arabidopsis* embryogenesis, Genes Dev. 15 (2001) 902–911.

[23] D. Coupland, Herbicide Resistance, in: S.B. Plants, A.M. Powel, Holtum (Eds.), Resistance to the Auxin Analog Herbicides, CRC Press, Florida, 1994, pp. 172–173.

[24] D.L. Rayle, R.E. Cleland, Evidence that auxin-induced growth of soybean hypocotyls involves proton extrusion, Plant Physiol. 66 (1980) 433–437.

[25] C.L. Foy, Picloram and related compounds, in: P.C. Kearney, D.D. Kaufman (Eds.), Herbicides: Chemistry, Degradation, and Mode of Action, Second ed., Marcel Dekker Inc, New York, 1975, Vol 2.

[26] M.A. Venis, R.M. Napier, Auxin receptors and auxin-binding proteins, Crit. Rev. Plant Sci. 14 (1995) 27–47.

[27] R.M. Napier, Models of auxin binding, J. Plant Growth Regul. 20 (2001) 244–254.

[28] J.G. Chen, S. Shimomura, F. Sitbon, G. Sandberg, A.M. Jones, The role of auxin-binding protein 1 in the expansion of tobacco leaf cells, Plant J. 28 (2001) 607–617.

[29] G. Thiel, M.R. Blatt, M.D. Fricker, I.R. White, P. Millner, Modulation of K^+ channels in vicia stomatal guard cells by peptide homologs to the auxin-binding protein C terminus, Proc. Natl. Acad. Sci. USA 90 (1993) 11493–11497.

[30] S. Zimmermann, S. Thomine, J. Guern, H. Barbier-Brygoo, An anion current at the plasma membrane of tobacco protoplasts show ATP-dependent voltage regulation and is modulated by auxin, Plant J. 6 (1994) 707–716.

[31] J.M. Bauly, I.M. Sealy, H. Macdonald, J. Brearley, S. Droge, S. Hillmer, D.G. Robinson, M.A. Venis, M.R. Blatt, C.M. Lazarus, R.M. Napier, Overexpression of auxin-binding protein enhances the sensitivity of guard cells to auxin, Plant Physiol. 124 (2000) 1229–1238.