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Two-Dimensional Protein Patterns of *Arabidopsis* Wild-Type and Auxin Insensitive Mutants, *axr1, axr2*, Reveal Interactions between Drought and Hormonal Responses

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In order to detect gene products involved in *Arabidopsis* drought adaptive strategy, 2D-PAGE protein patterns of two auxin-insensitive mutants, *axr1, axr2*, differentially affected in specific drought responses, were compared to the wild-type Columbia ecotype, in well-watered and drought-stressed conditions. Coupled to computer analysis of polypeptide amounts, 2D-electrophoresis revealed subtle changes in protein expression induced by progressive drought stress and/or mutations affecting the auxin response pathway.

The differential protein patterns of *axr1* and *axr2* were consistent with their contrasting drought responses. The specific leaf and root protein patterns of *axr1* showed that this mutation disrupts drought responses related to auxin regulation. In particular, the near absence of drought rhizogenesis in *axr1* was associated with a root protein pattern closer to the well-watered than to the water-stressed *axr2* and Columbia wild-type root protein patterns. Also, the largely different effects of *axr1* and *axr2* mutations suggest that they affect different pathways in auxin response. Several sets of polypeptides, whose regulation was affected by drought and/or mutation, were thus detected. These polypeptides could play a role both in the auxin and the drought response pathways. Their identification, through microsequencing, should be most informative.

Key words: *Arabidopsis thaliana* L. — Automatic quantification — Auxin-insensitive mutants — 2D-PAGE — Progressive drought stress — Protein expression.

Drought stress is a major factor limiting plant growth and development. The wide variety of responses, from the molecular to the morphological level, including adaptive strategies, is dependent upon the genetic potentials of species. Numerous drought- or dehydration-induced genes have been isolated by differential screening between drought-stressed and well-watered plants, particularly in the model species *Arabidopsis* (see for example Gosti et al. 1995, Mäntyla et al. 1995, Kiyosue et al. 1994, Yamaguchi-Shinozaki et al. 1992 and ref. there in). The predicted functions of the gene products, deduced from cDNA sequences, are supposed to protect cellular structures and to play a role in drought tolerance (for a review see Bray 1993, Bohnert et al. 1995). However the exact function of most of the drought-induced proteins and the signal transduction pathway still remain largely unknown (Giraudat et al. 1994).

An alternative strategy to get an insight into these mechanisms is the analysis of mutants differentially affected in specific drought responses as compared to the wild-type. *Arabidopsis* is well suited for such an approach. On the one hand, it displays characteristic drought responses, particularly the drought rhizogenesis: formation of roots that remain short, hairless and tuberized but are capable of rapid recovery, giving rise to a new absorbing root system upon rehydration (Vartanian et al. 1994, Couot-Gastelier and Vartanian 1995, Vartanian 1996a and ref. in). On the other hand, numerous monogenic mutants are available in *Arabidopsis* and especially hormonal mutants, whose behaviour was shown to be altered under progressive drought stress as compared to the wild-type (Vartanian et al. 1994, Vartanian 1996b). Differential, contrasting drought responses were thus observed, in particular within ABA-insensitive mutants (*abi1* vs *abi2*, Vartanian et al. 1994) as also within auxin-insensitive mutants (*axr1* vs *axr2*, Vartanian 1996b), indicating that ABA and auxin are involved in regulating drought adaptive processes. ABA-insensitive mutants have been extensively studied to analyse the role of endogenous ABA in the drought-induced regulation of gene expression (Giraudat et al. 1994). Interestingly, the opposite drought response of *abi1* and *abi2* was shown at morphogenetic level (near absence of drought rhizogenesis in *abi1* as compared to *abi2* and wild-type, Vartanian et al. 1994) as well as at the molecular level: Gosti et al. (1995) reported that the ABA-

Abbreviations: RH, relative atmospheric humidity, IEF, isoelectric focusing; ci, calibrated integrated intensities; ANOVA, analyses of variance; GST, glutathione S-transferase.

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dependent induction of two drought-regulated cDNA was differentially affected in *abi1* and *abi2*. Patel et al. (1994) also observed that exogenous ABA-regulation of several genes was impaired in *abi1* while the *abi2* mutation disrupted ABA-regulation of a smaller number of genes.

Unlike ABA-insensitive mutants, no data have been reported for differential drought responses of auxin-insensitive mutants at molecular level. Actually, although auxin controls numerous developmental processes, the molecular mechanisms of auxin action remain largely unknown (Hagen 1995). Thus the use of mutants affected in auxin response may contribute to a better understanding of these mechanisms (Abel and Theologis 1996).

The *axr1* mutant (due to a single recessive mutation located on chromosome 1) is characterized by phenotypic alterations which are consistent with a decrease in auxin sensitivity in all plant tissues: reduction in plant height due to a decrease in cell number, in apical dominance and fertility, defects in root gravitropism and vascular bundle differentiation in stems (Lincoln et al. 1990). These pleiotropic effects suggested that the *AXR1* gene encodes an essential function associated with auxin action. In fact, this gene, isolated and characterized by Leyser et al. (1993), encodes a protein with significant sequence similarity to the ubiquitin-activating enzyme E1. However, the AXR1 protein is diverged from the E1 enzyme and, in particular, lacks a cysteine residue known to be essential for E1 activity. AXR1 may define a new class of enzymes in the ubiquitin pathway, recycling an ABP-AUX1 protein complex (Millner 1995) or it may have a novel function in cellular regulation which is unrelated to ubiquitin conjugation (Hobbie and Estelle 1994). Actually, Timpte et al. (1995) have recently shown that *axr1* plants display a pronounced deficiency in the rapid auxin-induced accumulation of the *SAUR-AC1* mRNA in all *Arabidopsis* tissues. These results and others (Abel et al. 1994) indicated that the *axr1* gene is essential for early auxin-mediated responses.

The *axr2* mutant (resulting from a single dominant mutation located on chromosome 3) is characterized by a dwarf modified phenotype with dark green wrinkled leaves (Wilson et al. 1990) and pleiotropic defects in shoot and root gravitropism, in stomata distribution and a dramatic reduction in cell length (Timpte et al. 1992). Although the *axr2* mutation confers additionnal insensitivity to ethylene and ABA, the studies of Wilson et al. (1990) and Timpte et al. (1992) suggested that the extreme dwarf phenotype and the altered gravitropic behaviour of *axr2* mutant plants result primarily from defects in auxin action. In addition the *axr2* mutation was also shown, like *axr1*, to reduce expression of the *Arabidopsis SAUR-AC1* gene (Gil et al. 1994). Altogether, these results suggested that the *axr2* mutation disrupts auxin action at an early step in the signal transduction pathway (Hobbie and Estelle 1994).

The differential drought behaviour of *axr1* and *axr2* as compared to the wild-type, during the gradual soil moisture decline, concerned the transpiration rate (slowered in *axr2*), the rosette leaf survival duration (twice higher in *axr2*: 8 weeks vs 4 in wild-type and 4.5 in *axr1*) and was particularly noticeable at the root level: the drought rhizogenesis index (DRI, number of short roots per mg of root biomass) was 0.5 in *axr1* vs 21 in *axr2* and 9 in Columbia wild-type (Vartanian et al. 1994, Vartanian 1996b).

Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) is a powerful technique for analysing genome expression at the mature protein level. In *Brassica napus* drought-adapted leaves, this technique made it possible to reveal the induction of a 22 kDa protein showing homologies with the Künitz protease inhibitor family (Reviron et al. 1992, Downing et al. 1992). 2D-PAGE, associated with computer analysis of polypeptide amounts, allowed the characterization of three *Arabidopsis* developmental mutants by a set of proteins showing a differential expression when compared with the wild-type plant (Santoni et al. 1994). Such an approach, which had not yet been undertaken to analyse *Arabidopsis* drought responses, can bring complementary information to the cDNA differential screening performed in *Arabidopsis* wild-types subjected to progressive drought stress (Gosti et al. 1995).

In the present study, 2D-PAGE, coupled to automatic quantification of 2-D gels, was used to analyse the modifications in protein expression induced by the *axr1* and *axr2* mutations as compared to the wild-type Columbia, under well-watered and drought-stressed conditions. The differential *axr1, axr2* protein patterns allowed to identify several sets of polypeptides, whose regulation was shown to be affected by drought and/or mutations. These polypeptides may be involved both in the auxin and drought response pathways.

Materials and Methods

Plant culture and experimental conditions—The *Arabidopsis thaliana* (L.) Heynh lines used in this study were the Columbia wild-type and the derived (EMS mutagenesis) auxin resistant mutants: *axr1-3* (Estelle and Somerville 1987) and *axr2* (Wilson et al. 1990). Genetic analyses had shown that no other mutation was segregating in these mutants (Lincoln et al. 1990, Wilson et al. 1990). Thus the Columbia wild-type and the mutants are isogenic lines, differing at only one gene locus, i.e. *axr1* or *axr2*. Seeds of the mutants were kindly provided by Dr. M. Estelle.

The experimental protocol used for progressive drought has been described previously (Vartanian et al. 1994 and ref. there in). Briefly, plants were grown in a microphytotron at 22°C under a photon flux density of about 200 μmol photon $m^{-2} s^{-1}$ PAR during a 8 h photoperiod (9 am–5 pm). Young seedlings were planted in a sandy soil watered to field capacity (5.6 % dry weight humidity at a matrix potential of − 0.01 MPa). The soil surface was protected from evaporation by a sheet of parafilm. Plants were first allowed to grow in well-watered conditions: about 75% Relative Atmospheric Humidity (RH) and soil moisture maintained at field capacity.

When the rosettes were 2 cm wide, plants were exposed to a drier atmosphere (50% RH) and the progressive drought stress was initiated by withholding water. Soil moisture in control plants was maintained at field capacity by daily watering. From the onset of the progressive drought stress, water loss was monitored by weighing each pot every day at the same hour. These values were then used to calculate the remaining soil moisture. Water loss occurred through plant transpiration only, since evaporation was prevented by the parafilm sheet covering the soil surface. Also, the increase in plant biomass was negligible compared with the water loss. Plants were harvested when the soil moisture content had reached 0.8% dry weight humidity. This critical soil moisture corresponds to the fall in the transpiration rate to a basal level (Vartanian et al. 1994), and was also previously used as a harvest index for a differential screening of drought-regulated transcripts (Gosti et al. 1995). For each genotype, control and stressed plants were harvested at the same time. Rosette leaves and root systems were separately frozen in liquid nitrogen.

The mutant *axr2*, which grows more slowly than the wild-type (62 vs. 34 days at the onset of drought initiation), was older than the other genotypes upon harvest. However, no significant age effect on the protein patterns was observed (data not shown).

2-D electrophoresis—Proteins were extracted according to Damerval et al. (1986) with 30 μl and 50 μl of solubilization solution used to resuspend 1 mg of pellet for roots and leaves respectively. Isoelectric focusing (IEF) was performed using an ampholyte mixture of Pharmalyte pH 5-8 and Pharmalyte pH 5-6 (3 : 1), according to Leonardi et al. (1987), except that the run was 35,000 Vh, with about 50 μg proteins per gel as determined according to Scopes (1974). The second-dimension sodium dodecyl sulfate (SDS) electrophoresis and the silver staining were performed according to Damerval et al. (1987). One protein extract was obtained from a mix of at least three plants (root systems or rosette leaves). For each organ of each genotype in a given water condition, at least three replicates were carried out with independent extracts. Coelectrophoresis of root and leaf extracts (1 : 1) were performed for the wild-type in drought condition to identify proteins that are common to both organs. The apparent molecular mass of polypeptides was determined using the Low Molecular Weight Pharmacia calibration kit. The pH gradient displaying a linear evolution from 5 to 7, the pI could be precisely determined for each polypeptide.

Analysis of 2-D gels—A low selectivity screening of spots affected by mutation and/or drought stress was made visually. 2D gels of well-watered genotypes were first compared. For each genotype, gels of well-watered plants were then compared to gels of drought-stressed plants. The selected spots were then quantified on each gel. Image acquisition was performed using an Eikonix 7899 scanner with a spatial resolution of 100 microns per pixel, an optical density range from 0 to 1.2 and 256 grey levels. Each gel was scanned in a 2,048*2,048 pixel format, sampled to have 1,024*1,024 pixel image that can be analysed with the 2-D analyser Bioimage, version 6.0 (Bioimage Corp., Ann Arbor. Mich., U.S.A.). A detection threshold, corresponding to the mean background intensity, was defined to visualize the spots. Spots were detected as the gel surface whose intensity was over the threshold. The spot intensity was then estimated by the integration of the gel intensity over the threshold on the whole spot surface.

Scaling procedure to compensate for between-gel differences due to global factors (e.g. silver staining variability, Burstin et al. 1993) is necessary to compare the integrated spot intensities between genotypes. A calibration set of 20 spots, scattered all over the gel surface, which did not seem to vary according to visual analysis, was defined. For each spot of each gel the calibrated integrated intensity was then calculated as:

$$ci_{jg} = ri_{jg} \cdot m/m_g$$

ci_{jg}: calibrated intensity of spot j in gel g
ri_{jg}: raw intensity of spot j in gel g
m: mean intensity of spots of the calibration set for all the gels
m_g: mean intensity of spots of the calibration set in the gel g

Statistical analyses—Statistical analyses were carried out on calibrated integrated intensities (ci) using the SAS software package (version 6.03). Two-way analyses of variance (ANOVA) with interaction were performed for each spot, with genotype and water condition as factors. To take into account both quantitative and qualitative (presence/absence) variations, unrooted trees were constructed. For each spot in one organ, up to 3 classes of relative intensity were defined, based on the significant differences between the various genotype*conditions (one genotype in one condition) revealed by the ANOVA. The absence of a spot was encoded 0. A distance matrix between all the genotype*condition was calculated. The distance value was the ratio of the number of spots with different intensities between 2 genotype*conditions to the total number of spots. Based on this matrix, unrooted trees were constructed with the Fitch and Margoliash (1967) method and drawtree programs of the Phylip package (Felsenstein 1989), using the "Bisance" service (Dessen et al. 1990). The UPGMA method resulted in the same grouping (data not shown).

Results

About six hundred reproducible spots were observed on leaf or on root 2D gels, in a pI range from 5 to 7 and M_r range from 20 to 100 kDa (Fig. 1). Thirty polypeptides were affected by drought and/or at least one mutation specifically in leaves, 15 in roots and 8 others in both organs. Among these modifications, statistical analyses discriminated 3 overlapping classes of polypeptides resulting from genotype effect, condition effect or interactions of both effects (Table 1).

Alterations of the wild-type drought protein pattern by the mutations axr1 and axr2 (table 2, A and B)-Twenty two polypeptides were affected by drought in Columbia wild-type, in at least one organ. Most of them were appearing or increasing in intensity, only 3 polypeptides were decreased by drought (C6 and L17 in leaves, C19 in roots). Six were affected in both organs (Table 2A), 11 in leaves only, and 5 in roots only (Table 2B). Among these polypeptides affected by drought in Columbia wild-type, 7 were shown to be altered by one or the other mutation in well-watered conditions (Table 2A, B). The effect of drought was identical in the wild-type and in both mutants for 7 (C5, C1, C2, C3, C4, L12, L17) of the 17 polypeptides affected in leaves and 2 only (C5, C19) of the 11 polypeptides affected in roots.

The behaviour of 1 polypeptide in leaves (L58) and 4 in roots (C1, C2, C3, C17), affected by drought in the wild-type, was specifically altered by the mutation *axr1*. The mutation *axr2* affected specifically 2 polypeptides in leaves (C7, C9) and 1 in roots (C6, Fig. 2). Five other polypeptides (C8, L28, L46, L53 in leaves and R11 in roots) were

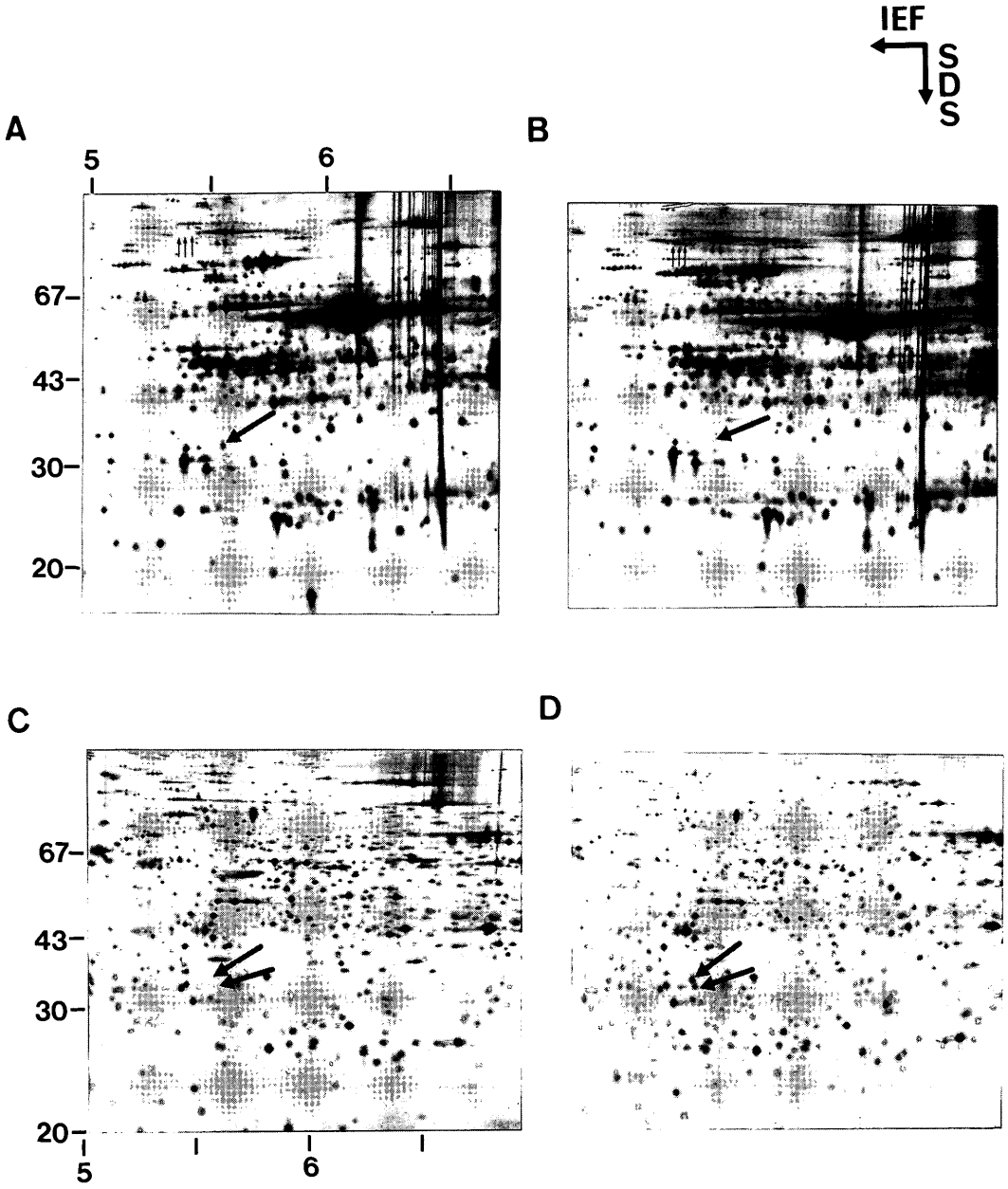

Fig. 1 Wild-type Columbia 2D-protein patterns. (A) Leaves, well-watered. (B) Leaves, drought-stressed. The large arrow points to polypeptide C6 whose amount decreased under drought. The thin arrows point to 3 drought-induced polypeptides C1, C2 and C3 (pI: 5.41; 5.43; 5.45 respectively). (C) Roots, well-watered. (D) Roots, drought-stressed. The arrows point to C6 and C7 whose amount increased under drought.

Table 1 Results of ANOVA ($p<0.05$): number of spots displaying a genotype, condition or genotype*condition interaction effects

Organ	Genotype effect	Condition effect	Interaction
Both	1	6	5
Leaves	14	30	28
Roots	3	16	15

differentially regulated by drought in the 3 genotypes. Three polypeptides in leaves (C6, C12, L4) and 3 in roots (C7 (Fig. 2), C18, R28) were altered identically by both mutations as compared to the wild-type.

Specific axr1, axr2 drought-induced changes (Table 3) —Twenty three polypeptides were drought-affected in one or both mutants while not in the wild-type. In leaves, 3 polypeptides (L44, L16, L36) displayed identical changes in expression in the two mutants. Six others (C14, L29, L41, L49, L54, L55) were specifically affected by the *axr1* mutation and only one (C13) by the *axr2* mutation. Four other

Table 2 Polypeptides whose expression is affected by drought in wild type and their behaviour in the mutants under well-watered or drought-stressed conditions

A

Spot	M_r	pI	Leaves						Roots					
			Well-watered			Drought-stressed			Well-watered			Drought-stressed		
			Col	*axr1*	*axr2*	Col	*axr1*	*axr2*	Col	*axr1*	*axr2*	Col	*axr1*	*axr2*
C1[a]	92	5.41	0	0	0	1	1	1	0	0	0	1	0	1
C2	92	5.43	0	0	0	1	1	1	0	0	0	1	0	1
C3	92	5.45	0	0	0	1	1	1	0	0	0	1	0	1
C7	32	5.58	0	0	1	1	1	1	0	0	0	1	2	2
C6	34	5.56	2	2	2	1	0	0	1	1	1	2	2	1
C5	77	5.53	1	1	1	2	2	2	0	0	0	1	1	1

B

Organ	Spot	M_r	pI	Well-watered			Drought-stressed		
				Col	*axr1*	*axr2*	Col	*axr1*	*axr2*
Leaves	L58[b]	24	5.72	0	1	0	3	2	3
	C9	74	5.46	0	0	1	1	1	1
	C8	26	6.01	0	1	2	1	2	2
	L28	37	5.51	0	2	1	3	3	3
	L46	55	5.84	0	1	2	1	1	3
	L53	29	6.14	0	0	2	1	0	2
	C12	36.6	6.15	1	1	1	2	1	1
	L4	22	5.16	1	2	2	3	3	3
	C4	92	5.47	0	0	0	1	1	1
	L12	93	5.42	0	0	0	1	1	1
	L17	92	5.6	2	2	2	1	1	1
Roots	C17	47	5.9	1	1	1	3	2	3
	R11[b]	44	5.48	2	2	2	3	1	2
	C18	58	5.85	0	0	0	1	0	0
	R28	22	5.88	0	0	0	1	0	0
	C19	52	6.10	2	2	2	1	1	1

A: in both organs. B: in leaves or roots.

[a] The spots "C" are present in both organs (but not systematically affected in both organs).

[b] The spots "L" are present in leaves only, the spots "R" are present in roots only.

Col: Columbia wild type. M_r: molecular mass (kDa). pI: isoelectric point. 0=absent. 1, 2, 3=classes of increasing intensity. These class values are relative to each spot in one organ (1 in roots can be different from 1 in leaves for example).

An alteration is considered as specific when a differential regulation of spot intensity under drought is observed as compared to the wild-type, irrespective of the final spot intensity level.

polypeptides (C10, C11, L3, L64) were differentially affected by both mutations. In roots, the expression of one polypeptide (C20) was similarly altered in both mutants. Three polypeptides (C21, R16, C8) were specifically affected by the *axr1* mutation and 5 (C22, C23, R10, R38, C4) by the *axr2* mutation.

Changes induced by the mutations in the well-watered wild-type protein pattern—The expression of ten polypeptides (7 in leaves, 3 in roots), not affected by drought in the wild-type, was altered by one or the other mutation under well-watered conditions (Table 4A, B). Eight of them were also modified by drought in the corresponding mutant (Table 4A). Among the 2 polypeptides affected by mutation only (Table 4B), L47 was differentially altered while C16 (Fig. 2) was identically affected by both mutations.

Global trends in protein expression changes in rosette leaves and root systems—All—qualitative (presence/absence) and quantitative—variable spots (38 in leaves, 23 in roots) were used to build the distance matrix. Such analy-

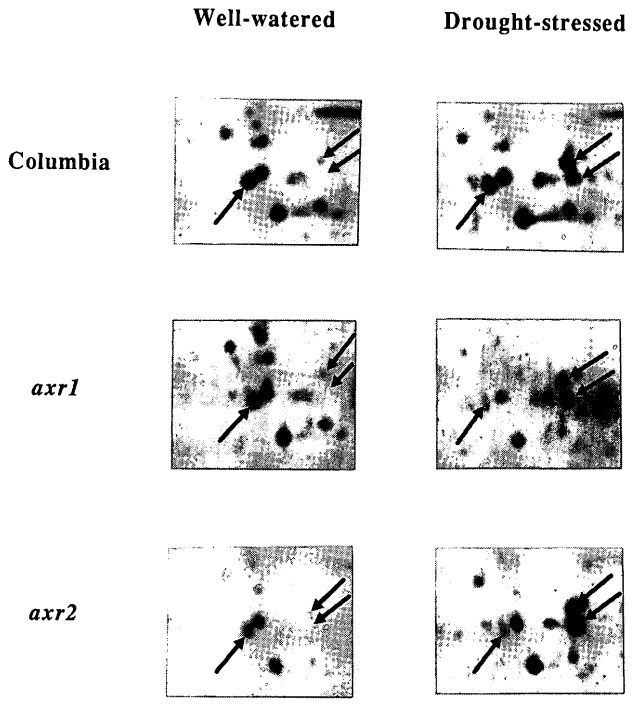

Fig. 2 Portion of root 2D protein patterns showing the behaviour of 3 polypeptides in Columbia wild-type, *axr1* and *axr2* under well-watered and drought conditions. The left arrow points to polypeptide C16 affected only by mutation (decreased intensity in *axr1* and *axr2*, Table 4B). The right arrows point to polypeptides C6 (upper) and C7 (lower) differentially affected by drought in wild-type and mutants (Table 2A).

sis, that takes into account the variation of numerous polypeptides simultaneously, allows to visualize changes in the pattern of gene expression induced by mutation and/or water condition. Actually, a clear discrimination between well-watered and drought conditions could immediately be observed for both organs (Fig. 3A, B). In leaves (Fig. 3A), well-watered genotypes were tightly grouped together, Columbia and the *axr1* mutant being slightly closer to each other than to *axr2*. Drought-stressed genotypes were differently associated: both mutants were distant from the wild-type and *axr1* was the most divergent. In roots (Fig. 3B), the three well-watered genotypes also appeared very close to each other. In contrast with the well-watered leaf pattern, the *axr1* mutation resulted in a higher global effect than the *axr2* mutation, as compared to the wild-type. The same trend was observed under drought: Columbia and *axr2* being closer to each other than to *axr1*. In addition, drought-stressed *axr1* was nearer to the well-watered genotypes than to the other drought-stressed genotypes.

A principal component analysis, performed with the spots displaying only quantitative variations, revealed the same trends (data not shown) as unrooted trees.

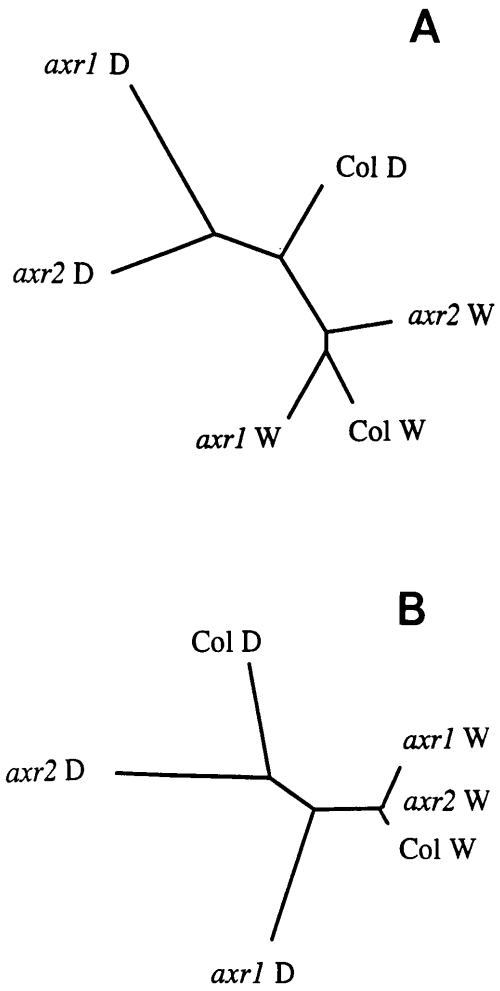

Fig. 3 Unrooted trees from protein pattern data. Col, Columbia wild-type; W, well-watered condition. D, drought-stressed condition. (A) Leaves. (B) Roots.

Discussion

Two-dimensional electrophoresis of denatured proteins, coupled to automatic quantification of polypeptide spot intensity, allowed to reveal subtle changes at the level of the translated genome induced by progressive drought stress and/or monogenic mutations affecting the auxin signal transduction pathway.

The effects of the *axr1* and *axr2* mutations on the wild-type protein patterns appeared distinct whatever the water condition: not only identical polypeptides were differentially altered, but also, different polypeptides were affected. These results suggest that no mutation is fully epistatic on the other, and that the two mutations affect different pathways in auxin response.

The fact that, in well-watered conditions, 14 and 13 polypeptides were affected by the *axr1* and *axr2* mutations respectively is consistent with the known pleiotropic morphological and physiological effects of these mutations at

Table 3 Polypeptides whose expression is drought-affected in either mutant and not in wild type in leaves or roots: behaviour according to the genotype and the environmental condition

Organ	Spot	M_r	pI	Well-watered			Drought-stressed		
				Col	*axr1*	*axr2*	Col	*axr1*	*axr2*
Leaves	L44	68	5.93	0	0	0	0	1	1
	L16	93	5.48	0	0	0	0	1	1
	L36	20	5.44	0	0	0	0	1	1
	C14	26.5	5.8	1	1	1	1	2	1
	L29	39	5.57	1	1	1	1	2	1
	L41	72	5.91	1	1	1	1	2	1
	L49	38	5.85	1	1	1	1	2	1
	L54	28	5.78	1	1	1	1	2	1
	L55	27	5.88	1	1	1	1	2	1
	C13	30	5.91	1	1	1	1	1	2
	C10	70	5.51	1	1	1	1	2	0
	C11	71	5.85	1	1	1	1	3	2
	L3	33	5.05	1	1	1	1	3	2
	L64	53	6.26	0	0	0	0	2	1
Roots	C20	42	5.60	2	2	2	2	1	1
	C21	43	5.55	2	2	2	2	1	2
	R16	50	5.7	0	0	0	0	1	0
	C8	26	6.01	1	1	1	1	2	1
	C22	47	5.55	2	2	2	2	2	1
	C23	42	5.43	1	1	1	1	1	2
	R10	45	5.36	2	2	2	2	2	1
	R38	46	6.3	2	2	2	2	2	1
	C4	92	5.47	0	0	0	0	0	1

Legend as in Table 2.

the whole plant level (Lincoln et al. 1990, Wilson et al. 1990). Identification of these proteins may contribute to the understanding of the molecular mechanisms of auxin action in plant growth and development.

The auxin insensitive *axr1* and *axr2* mutants were initially chosen for specific differential drought responses as compared to the wild-type Columbia (Vartanian et al. 1994, Vartanian 1996b). Actually, one or the other mutation was shown to modify also pleiotropically gene expression in response to drought. Interestingly, more than two thirds of the polypeptides whose amount was altered by drought in the wild-type were not regulated in the same way in the drought-stressed mutants (Table 2A, B). Moreover, 31 polypeptides insensitive to drought in the wild-type became responsive in one or the other mutant (Table 3, 4A). Such changes in gene expression suggest interactions between the molecular mechanisms involved in auxin and drought responses. To date, only few examples of putative interactions have been reported. The *Arabidopsis AWI 34* gene expression is induced by an exogenous 2,4 D treatment and by environmental stresses such as drought, salt and cold (Yang et al. 1995). Another example is the characterization, in *Vigna radiata*, of an auxin-regulated gene, *ARG2*, showing sequence homology to a gene for an atypical cotton LEA5-A protein (Yamamoto et al. 1992, Yamamoto 1994). It was suggested that this gene might respond to changes in water potential in plant cells during auxin-induced elongation. Interestingly, the *Arabidopsis* soluble epoxide hydrolase (*AtsEH*) gene expression is strongly induced by auxin and slightly by dehydration (Kiyosue et al. 1994). The physiological functions of this gene are still unknown. A glutathione S-transferase (GST) activity was reported for the *Arabidopsis thaliana At103-1a* gene product. Moreover, the *At103-1a* gene was induced in roots not only by auxin but also by a high ABA concentration (100 μM) (van der Kop et al. 1996). Also, Kiyosue et al. (1993) had characterized two cDNAs, induced by dehydration in *Arabidopsis*, encoding for putative GST. Thus, altogether these results suggested (Kiyosue et al. 1994) that ATsEH and GST could play a role in detoxification, protecting cells against oxidizing compounds (for example due to lipid peroxidation) produced by auxin (Droog et al. 1993) and/or water stress (Olsson 1995). However, although there are currently many reports for a role of exogenous

Table 4 Polypeptides whose expression is affected by the mutations under well-watered conditions and not by drought in the wild-type: behaviour according to the genotype and the environmental condition

A

Organ	Spot	M_r	pI	Well-watered			Drought-stressed		
				Col	*axr1*	*axr2*	Col	*axr1*	*axr2*
Leaves	L22	73	5.62	1	2	1	1	1	1
	L38	20	5.56	1	2	1	1	1	1
	L26	38	5.29	2	1	1	2	2	1
	L24	44	5.36	2	1	1	2	3	1
	L6	93	5.4	0	0	1	0	0	2
	L59	21	5.86	0	1	1	0	1	2
Roots	C15	50	5.65	2	3	2	2	0	1
	R35	26	5.8	2	1	2	2	2	2

B

Organ	Spot	M_r	pI	Well-watered			Drought-stressed		
				Col	*axr1*	*axr2*	Col	*axr1*	*axr2*
Leaves	L47	46	5.75	3	2	1	3	2	1
Roots	C16	34	5.4	2	1	1	2	1	1

A: polypeptides whose expression is affected by mutation, in *axr1* or *axr2*, under well-watered and drought stress conditions. B: polypeptides affected only by mutation under well-watered condition in leaves or roots.
Legend as in Table 2.

auxin on stomata regulation, in particular counteracting ABA or CO_2 closing effects, changes in endogenous auxin content in response to water stress remain to be elucidated before assuming that interactions between ABA and auxin may be important in the control of plant water balance (Mansfield and McAinsh 1995).

In fact, the specific drought *axr1* root and leaf protein patterns (Fig. 3) clearly showed that the *axr1* mutation disrupts drought responses related to auxin regulation. In particular, the drought-stressed *axr1* root protein pattern appeared more similar to the well-watered than to the drought-stressed root patterns, as revealed by global analyses as well as by individual spot behaviour (Table 2, 3, 4). The fact that the *axr1* root protein pattern shared much less common variations with the wild-type than *axr2* may be specifically related to the intensity of the drought rhizogenesis: dramatically reduced in *axr1* while highly enhanced in *axr2* as compared to the wild-type (Vartanian et al. 1994, Vartanian 1996b). Actually, a 2D-PAGE study of different root types (tap roots, lateral roots and drought-induced short roots) in another *Brassicaceae* species (*Brassica napus*) had revealed a very specific protein pattern of the drought-induced roots as compared to other root types (Vartanian et al. 1987, Damerval et al. 1988). Thus, the distinct root protein pattern of *axr1* may be related to the lack of this adaptive process, as a result of auxin insensitivity (Vartanian et al. 1994).

Although morphological features of the *axr2* rosette leaves (wrinkled, dark green) were very different from Columbia wild-type and *axr1* in normal well-watered conditions (Wilson et al. 1990, Lincoln et al. 1990), the *axr2* leaf protein pattern was but slightly diverged from *axr1* and Columbia wild-type. It should be kept in mind that the present analyses take into account only differences in protein patterns in the pH range 5–7 and M_r range 20–100 kDa. Furthermore, it is not excluded that the specific changes observed in individual polypeptide behaviour, as mentioned above, are implied in leaf morphology of well-watered *axr2*. In contrast, the extreme drought tolerance of the *axr2* rosette leaves as compared to the wild-type (Vartanian 1996b) was associated with a global protein pattern relatively distant from *axr1* and Columbia wild-type. The *axr2* mutation affected specifically the wild-type drought protein patterns for 13 polypeptides in leaves and 8 in roots (Table 2, 3, 4). In addition, since *axr2* was also shown to be resistant to ABA, some of these polypeptides might reflect interactions between ABA and auxin regulatory pathways.

Based upon their differential drought behaviour in *axr1, axr2* mutants and Columbia wild-type, several classes of polypeptides were thus identified. Both sets of polypeptides—the ones responsive to drought in the wild-type and differentially affected in either or both mutants, and the ones insensitive to drought in the wild-type but responsive in either mutant—provide new tools to understand the ge-

netic and molecular basis of auxin action, particularly interactions, still largely unknown, between drought and auxin responses. In addition, the differential effect, at the level of the translated genome, of the *axr1* and *axr2* mutations allow further identification of proteins that may help to characterize their different alterations in the auxin signal transduction cascade. The characterization of these polypeptides through microsequencing, as proposed by Bauw et al. (1992), currently in progress in the laboratory, should be most informative.

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