Differential Two-Dimensional Protein Patterns as Related to Tissue Specificity and Water Conditions in Brassica napus var oleifera Root System

ABSTRACT

Differential two-dimensional protein patterns as related to tissue specificity and water conditions were investigated within Brassica napus var oleifera root system. The different parts of the root system (tap root, lateral roots, and drought-induced short roots) were analyzed under various moisture regimes (regular watering at field capacity, progressive drought stress, and rewatering). Tissue specificity was evident from 25 differences in protein patterns (qualitative and quantitative) between well-watered lateral and tap roots. Twice as many polypeptides (53) were drought-affected and the response to the water stress was shown to be similar in both root types. In addition, more than half of the polypeptides detected as organ-specific were affected by drought. Based on the trend of variation observed under drought and rehydration, three categories of polypeptides could be defined that might be differently involved in drought susceptibility or tolerance. A highly differentiated protein pattern characterized the drought-induced short roots. This pattern appeared as far from the watered as from the water-stressed normal roots. In particular, 13 unique polypeptides were detected which could be relevant to their adaptive morphogenesis and/or their specific drought tolerance induction. Upon rehydration, their polypeptide pattern and their specific morphology returned to a normal well-watered lateral root type.

DIFFERENTIAL GENOMIC EXPRESSION

Differential genomic expression specific to the tissue, organ, or developmental stage has been studied in various species by two-dimensional electrophoresis of proteins (14, 15, 26). It is also extensively documented that environmental stress promotes important modifications in gene expression (16, 19), and tissue specificity of the stress response has been investigated under heat shock (2, 4) osmotic (3, 7), and salinity stress (12, 17, 18). Nevertheless, there seems to be no report of interaction between differential tissue-specific and differential drought stress-specific gene expression.

Drought rhizogenesis, an adaptive morphogenetic response to progressive drought stress, has been well described in Cruciferae and phylogenetically related families (1, 21–23). It involves de novo formation of short, tuberized and hair-deprived roots which, due to their high desiccation tolerance and ability to resume growth rapidly upon rehydration, represent a survival potential at the root level (24). In a short communication (25), we previously reported notable and reversible changes in two-dimensional protein patterns of Brassica napus var oleifera tap root under drought. Most polypeptides appearing under drought were also found in the drought-induced short roots.

It has been assumed that the main primary root, issued from the embryo radicle, was a different type of root from the laterals which arise from dedifferentiation and proliferation of pericyclic root cells (27). Some physiological studies have also shown a differential response of the tap root and laterals to environmental changes such as exogenous growth compounds (11) or water stress (21). For instance, in B. napus, twice as much proline and free amino-acids were observed in the tap root as in the laterals under drought stress (N Vartanian, unpublished data).

In order to reveal possible tissue-specific responses, we compared the two-dimensional protein patterns of all components of the root system under different moisture regimes. In the present paper, we show that tap root and laterals display specific protein patterns but respond similarly to drought stress; upon rehydration, the protein patterns of water-stressed roots are shifted towards their respective well-watered patterns. Moreover, the drought-induced short roots appear very different from both tap root and laterals, either well-watered or water-stressed. Rehydration which induces elongation of these organs makes their protein pattern very similar to that of the well-watered lateral roots.

MATERIALS AND METHODS

Plant Culture and Sampling Procedure. Rape plants (Brassica napus var oleifera, cv Darmor) were cultivated as in Vartanian et al. (25) under different moisture regimes. Young 48 h germinating seedlings were planted in a sandy soil initially watered at field capacity. Control plants were maintained at field capacity by regular waterings, while drought-stressed plants, due to the absence of any rewatering, were progressively subjected to increasing water deficit, as previously described (21, 22). Rewatering of water-stressed plants occurred after short tuberized root expression was achieved, i.e. in the stationary, survival phase of drought rhizogenesis (1) and when shoot water potential, measured with the Scholander pressure chamber method (20) had reached values lower than −3 MPa. Within 24 h, turgor recovery was already noticeable and after 3-d rehydration, the shoot water potential had not yet completely returned to the control value (−1.1 MPa versus −0.6 MPa).

Thirty-five d after sowing, 3 control, 25 water-stressed, and 6 rehydrated plants were harvested. The different root types, separately collected, were immediately frozen in liquid N₂, with the sampling pattern described in Table 1.

Two-Dimensional Electrophoresis of Proteins. Protein extraction and two-dimensional electrophoresis procedure were as described in Damerval et al. (5, 6) and in Vartanian et al. (25). The isoelectric focusing gel contained 75 and 25% of pharmalyte pH 5 to 8 and pH 5 to 6, respectively.

Scoring Procedure. At least four gels, corresponding to two
independent protein extracts, were examined for every sample. The more basic portion of the gels was not taken into account because of poor resolution. Presence/absence and relative intensity variations were determined by two independent observers. Dissimilarity indices between the different root types and moisture regimes were computed as the ratio of variant spots to the total number of reproducible spots. Principal coordinate analysis (10) was performed on the dissimilarity matrix to visualize the relationships between the types of roots under different water conditions.

RESULTS

Four hundred and twenty-four reproducible spots were retained in the analysis of the eight two-dimensional protein patterns obtained for the different root types of plants grown under the various moisture regimes described (Table I). Three hundred and twenty-three spots were found invariant and 101 spots (23.8%) were varying according to the type of root —49—, the water conditions —38—, or both —14— (Fig. 1, A and B).

**Comparison of Two-Dimensional Protein Patterns.** The W-Rt and W-RI displayed 373 identical spots and 25 different ones (6.3%): 8 spots were present or absent according to the organ, and 17 spots had different intensities (Fig. 2, A1 and B1).

The S-Rt and S-RI displayed a few more spots than the well-watered roots (Fig. 2, A2 and B2). In water-stressed tap root as compared to the control, 362 spots were found invariant, 13 spots appeared and 12 increased in intensity, while 2 spots disappeared and 18 decreased in intensity (25). In the case of laterals, 366 spots remained unchanged, 14 spots appeared and 7 increased in intensity while 2 spots disappeared and 19 decreased in intensity. The number of drought-affected spots was of the same magnitude (45 versus 42) in the two root types. Since most of these spots coincided between the two sorts of roots, in all only 52 different polypeptides appeared to be drought-affected (see Table II for details). In no case did a particular polypeptide increase in abundance in one root type and decrease in another under drought stress. Fourteen of the 25 spots found different between well-watered tap root and laterals were affected by drought; for 10 of them no difference could any longer be noticed between the 2 water-stressed root types. Among the 373 spots previously found in the same intensity in W-Rt and W-RI, 22 were drought-affected to the same extent in both root types and 3 other spots were modified either in tap root or in laterals. The protein pattern of the S-Rs appeared very different from both hydrated and water-stressed normal root patterns (Fig. 3A).

This is shown by the number of specific spots—13—and intensity differences—23—between S-Rs and both tap root and laterals. Moreover 3 spots, unaffected by drought in the normal root system, were never seen in S-Rs. As far as the drought-

**Table I. Eight Samples of Roots Harvested from Plants Grown under Different Water Conditions**

<table>
<thead>
<tr>
<th>Root</th>
<th>Watered (W)</th>
<th>Water-Stressed (S)</th>
<th>Rehydrated (SW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap</td>
<td>W-Rt</td>
<td>S-Rt</td>
<td>SW-Rt</td>
</tr>
<tr>
<td>Lateral</td>
<td>W-RI</td>
<td>S-Rl</td>
<td>SW-RI</td>
</tr>
<tr>
<td>Short</td>
<td>S-Rs</td>
<td></td>
<td>SW-Rs</td>
</tr>
</tbody>
</table>

affected spots are concerned, the 11 spots increasing in intensity were present. Interestingly among the 13 appearing spots, just one was lacking: only 1 of the 3 disappearing and only 10 of the 21 decreasing spots were detected, with an intensity inferior or similar to the water-stressed organs.

After 3-d rehydration and although the plant water potential had not yet completely returned to the control value (−1.1 MPa versus −0.6 MPa), 29 of the 45 spots affected in SW-Rt and 28 of the 42 spots affected in SW-RI were already identical to the controls. The spots still unmodified were mostly spots increasing (10 in SW-Rt and 5 in SW-RI) or appearing (5 in SW-Rt) under drought. Four spots, previously unaffected by drought, showed a peculiar behavior upon rehydration: one disappeared and another one increased in intensity in SW-Rt; 2 others increased in intensity in SW-RI (Fig. 2, A3 and B3).

In short tuberized roots, rehydration promotes a shift of the pattern into a 'lateral type' one (SW-Rs, Fig. 3B), which is demonstrated by the small number of differences between the rehydrated short roots and both control and rehydrated laterals (9 and 15, respectively).

**Relationships between Protein Patterns as Revealed by Principal Coordinate Analysis.** Dissimilarity indices between the eight two-dimensional protein patterns are shown on Table III. The two first planes (1-2 and 1-3) of the principal coordinate analysis account for about 90 and 75% of the variance, respectively (Fig. 4, A and B). In the first plane (1-2, Fig. 4A), the first axis discriminates the drought-induced short roots from the normal root system, while along the second axis, the water-stressed normal roots appear separated from their rehydrated and well-watered counterparts. Most striking is the shift, upon rewatering, of the rehydrated pattern of the drought-induced short roots in the well-watered and rehydrated normal lateral root patterns (Fig. 4, A and B). In the second plane (1-3, Fig. 4B), the third axis discriminates the two components (tap root and laterals) of the normal root system. Furthermore, for each root type, the relative positions of the three moisture regimes appear similar.

**DISCUSSION**

The analysis of denatured proteins of *Brassica napus* root system by two-dimensional electrophoresis allowed us to follow the tissue-specific gene expression under different moisture regimes. Twenty-five differences have been detected within the normal watered roots by comparing the main axis to the laterals. The nature and function of the gene products concerned are unknown, but might be related to different structural and/or physiological characteristics: embryological origin, pattern of differentiation, physiological age, number of meristems and growing apices in the laterals (27).

More than half of the spots which differ between the main axis and the laterals appear also affected by drought, suggesting a differential, physiological rather than structural, role of these gene products in the two root types. Drought promotes the same trend of variation for 35 spots in both tap root and laterals whereas differential effects concern only 10 and 7 spots, in tap root and laterals, respectively. Hence the relatively large number of spots affected by drought, together with the similar shift of most of them in both root types, lead to a strongly differentiated protein pattern of the water-stressed organs, which appear closer to each other than to their respective control on the first plane of the principal coordinate analysis (Fig. 4A).

In response to other environmental stresses, roots often appeared to be a major site of change of gene expression. For example, the ability to synthesize heat shock proteins was higher in maize roots than in leaves, and within the root itself, elongating portions exhibited a stronger heat shock response than mature

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1 Abbreviations: W-Rt, W-RI, well-watered tap root and laterals, respectively; S-Rt, S-RI, water-stressed tap root and laterals, respectively; S-Rs, drought-induced roots; SW-Rt, SW-RI, SW-Rs, rehydrated tap root, laterals and drought-induced roots, respectively.
Drought Behavior of Spots Tap Root Tap Root and Lateral Roots Lateral Roots Total No.

Appear 3 10 4* 17
Increase 4(1)* 7 0 11
Disappear 1 1 1 3
Decrease 2 16 3 21

* Indicates spots lacking in W-Rt and appearing in S-Rl, which were already present in W-Rt. This spot appears in S-Rl but increases in intensity in S-Rt as compared to W-Rt; it is counted with the spots appearing in S-Rl.

FIG. 1. Maps of the reproducible spots retained in the two-dimensional protein patterns of B. napus roots. A. Spots varying according to the type of root. The triangles represent the tap root spots, the squares the lateral root spots, and the diamonds the drought-induced short root spots; the black symbols are for presence/absence variants and the white symbols for intensity variants. The three symbols in each class of variant can be combined; for example (▲), spot present in both laterals and tap root but absent from short tuberized roots; (△), spot more intense in tap root than in both laterals and short tuberized roots; (○), spot more intense in laterals and short tuberized roots than in tap root; (●), spots varying in rehydrated normal roots. (The black points are for spots affected by drought, see B). B. Spots: affected by drought in the normal root system. The symbols representing the three types of roots are as in A. The black symbols are for appearing spots, the white symbols for disappearing spots, the striped tall symbols (▲) for spots increasing in intensity and the striped small symbols (●) for spots decreasing in intensity. The stars point to spots for which the short tuberized roots are different from the normal roots. Capital A beside the star means that the spot is lacking in the short tuberized roots. Capital V is for spots found variant between the watered tap root and laterals. (The black points are for spots not affected by drought, see A). Mol wt are indicated on the left in kilodaltons.

In roots and shoots of barley seedlings, distinct proteins and mRNAs, unique to each tissue, were induced by salinity stress (17, 18), whereas, in wheat, only root tissues exhibited changes in gene expression, in both salt-tolerant and salt-sensitive genotypes (12).

Most of the drought-induced changes observed in B. napus were shown to be reversible after 3-d rehydration. The trend of response to drought in one or both normal root types (Table II), together with the subsequent recovery to control intensity in the whole root system enable most polypeptides to be classified into three sets, the physiological function of which might be different. First, the 24 spots that decrease or disappear in water-stressed organs are mostly characterized by a recovery within 3-d to the control intensity. They probably correspond to gene products whose turnover was shifted towards degradation under drought, as mentioned in other responses to environmental stress (16, 19).
A contrasted behavior was found for the 11 spots that increased in intensity in one or both tissues under drought, since upon rehydration 3 of them only show a partial or complete decline to control intensity. The remaining set of 13 new spots seems to be relatively homogeneous since 10 of them were shared by the three types of roots under drought stress and all of them disappear in at least one of the three rehydrated organs, at variance with the increasing spots; this could reveal a different role of these drought-induced, unique polypeptides. In this connection, a different recovery behavior was observed in salt-adapted cultured tobacco cells (8). Though enhanced protein synthesis returned progressively to control level within a few days after cells were transferred to control medium, in contrast, the level of a 26 kD new protein became undetectable only after 2 or 3 passages through control medium. The reversibility of the modifications induced by salinity in the pattern of protein synthesis of barley roots, upon transfer to control medium, suggested that expression of genes for these proteins might be involved in the salt-tolerance ability (13).

The drought-induced short roots display particular morphological, biochemical and physiological characteristics (1, 9, 22, 23). The specificity of this organ is actually shown at the polypeptide level as can be seen on the two planes of the principal coordinate analysis (Fig. 4). Attention should be drawn to the fact that normal water-stressed roots and drought-induced short roots represent quite different systems with reference to their drought response and behavior: growth and differentiation had already occurred in tap root and laterals while water was still available, whereas the short new roots, initiated from a threshold water deficit, developed during drought stress under a different hormonal and metabolic internal environment (1, 9). Hence, drought tolerance in the normal water-stressed roots will result from changes occurring in already differentiated and functional tissues, whereas drought adaptation in the short roots is induced through early morphogenetic and physiological adjustment, from the onset of their emergence. Thirteen of the 24 polypeptides decreasing or disappearing in the normal root system are lacking in the short tuberized roots, suggesting that these gene products are not involved in any adaptive structural or functional modification. On the contrary all the spots increasing in intensity in...
Table III. Dissimilarity Index Matrix

<table>
<thead>
<tr>
<th>Root</th>
<th>W-Rt</th>
<th>S-Rt</th>
<th>SW-Rt</th>
<th>W-RI</th>
<th>S-RI</th>
<th>SW-RI</th>
<th>S-Rs</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-Rt</td>
<td>0.108</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SW-Rt</td>
<td>0.047</td>
<td>0.078</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W-RI</td>
<td>0.059</td>
<td>0.137</td>
<td>0.075</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-RI</td>
<td>0.122</td>
<td>0.057</td>
<td>0.099</td>
<td>0.101</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SW-Rl</td>
<td>0.071</td>
<td>0.113</td>
<td>0.051</td>
<td>0.040</td>
<td>0.073</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-Rs</td>
<td>0.207</td>
<td>0.186</td>
<td>0.202</td>
<td>0.186</td>
<td>0.162</td>
<td>0.188</td>
<td></td>
</tr>
<tr>
<td>SW-Rs</td>
<td>0.052</td>
<td>0.118</td>
<td>0.059</td>
<td>0.024</td>
<td>0.082</td>
<td>0.035</td>
<td>0.186</td>
</tr>
</tbody>
</table>

Fig. 3. Two-dimensional gels of the drought-induced short roots. A, Under drought stress; B, upon rehydration. IEF and SDS dimensions are as in Figure 1. The scale on the left of Figure 3A indicates mol wt standards.

Fig. 4. 1-2 and 1-3 planes of the principal coordinate analysis performed on the dissimilarity index matrix. A, 1-2, this plane accounts for about 90% of the variance; B, 1-3, this plane accounts for about 75% of the variance.

Upon rehydration, the short tuberized roots begin elongation and give rise to a new absorbing root system in lateral position on the tap root. This indeed corresponds to what is observed at the polypeptide level: the 13 tissue-specific spots have disappeared and the rehydrated pattern strongly resembles that of the lateral roots.

Thus, the specific model of drought rhizogenesis has proved to be very informative for the study of differential genomic

the normal water-stressed roots are present in the short tuberized roots, together with 12 of the 13 drought appearing polypeptides. Because the short roots are known to be an adaptive response of the plant, this suggests an actual role of these gene products in drought tolerance, rather than an indirect response to an environmental change. The 13 gene products specific to the short tuberized roots might be related to their differential morphogenesis and/or peculiar functioning during drought.
expression according to the tissue and water condition. Further studies should determine possible relations between tissue-specific changes observed at the protein level and drought tolerance.

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LITERATURE CITED