

Communication

Drought-Induced Changes in Protein Patterns of *Brassica napus* var. *oleifera* Roots

Received for publication March 13, 1987 and in revised form April 22, 1987

NICOLE VARTANIAN*, CATHERINE DAMERVAL, AND DOMINIQUE DE VIENNE¹
Institut de Physiologie Végétale, Centre National de la Recherche Scientifique, 91190 Gif-sur-Yvette, France (N.V.), and Laboratoire de Génétique des Systèmes Végétaux, Centre National de la Recherche Scientifique, Institut National de la Recherche Agronomique, Université de Paris-Sud, La Ferme du Moulon, 91190 Gif-sur-Yvette, France (C.D., D.V.)

ABSTRACT

Drought-induced changes in two-dimensional silver stained protein patterns of *Brassica napus* L. var. *oleifera* M. root system were detected both at quantitative and qualitative levels. Particularly, 13 new polypeptides of low molecular weight were evidenced in the drought-stressed tap root, 12 of which were also present in the short tuberized roots, a specific drought-induced root type. The reversibility of these modifications, observed after 3 days rehydration, suggests that they might be involved in drought tolerance.

In spite of intensive research in the field of water stress and plant physiology, few drought-induced changes in protein patterns have been mentioned to date (17, 21). Heat shock proteins, detected in various species of higher plants under experimental conditions (15) were recently observed in field grown plants subjected to high temperature stress (4, 16). Although under field conditions heat shock and water stress often occur together, it has not yet been possible to separate both effects, and only heat shock proteins and mRNA induced by the heat shock have been reported until now, either in etiolated seedlings or in mature leaves (4, 16).

When higher plants have to cope with limited water supply, an essential role in adaptation is assigned to the root system. Hence if drought-induced proteins are of frequent occurrence, they should rather be found within the root tissues. Drought rhizogenesis (27), a specific, adaptive, morphogenetic response to progressive drought, appeared as a very convenient model for such an investigation. In Cruciferae and other dicotyledonous, phylogenetically related families (28), a new root system was evidenced, under progressively increasing water deficit. From a threshold water potential (20), the roots produced remain short, hair deprived and may take a tuberized shape associated with cortical cell hypertrophy and starch accumulation. The drought-induced roots are highly desiccation tolerant and keep alive and turgid, despite whole plant wilting through water stress-induced senescence (1). Furthermore, upon rehydration, the short roots appear able to resume growth rapidly and give rise to a new absorbing root system, which helps plant recovery and represents

a potentiality for drought survival in natural environment (29). Thus, such a system allows not only to examine the effects of water deficit on the protein pattern of normal roots, but also to determine to what extent the new drought-adapted short roots display a peculiar, specific pattern.

The two-dimensional electrophoresis of denatured proteins (10, 18), which allows to detect simultaneously several hundreds of gene products and to follow the modifications of gene expression in different organs (25, 30) and under various conditions, was the valuable tool to such an approach.

In this paper we show that reversible changes in protein patterns of the tap root of *Brassica napus* var. *oleifera* are induced by drought and that most of the drought specific proteins are also found in the short tuberized roots.

MATERIALS AND METHODS

Plant Culture. Seeds of rape plants (*Brassica napus* L. var. *oleifera* Metz. cv Darmor) were germinated in Petri dishes on moistened filter paper. When radicles were 5 mm long, after 48 h in darkness, young seedlings were transferred to 15 cm high plastic pots filled with 1.5 kg of sandy soil watered at field capacity (5.6% dry weight humidity at a soil matrix potential of -0.1 MPa). The soil surface was covered with a sheet of Parafilm to avoid evaporation. The plants were grown in controlled conditions (microphytotron) at a photon flux density of approximately $200 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ PAR, under a 16 h photoperiod, with a constant temperature of 22°C and 50% relative atmospheric humidity.

Ten d after cotyledon emergence, plants were subjected to different water treatments. In one set, control plants were maintained at field capacity by regular waterings, every 2 d at the same time (10:00 AM). The shoot water potential, determined with the Scholander pressure chamber method (22) ranged from -0.4 to -0.6 MPa throughout the whole growth period. Another set of plants was subjected to progressively increasing water deficit: as no water was added, plants dehydrated slowing during growth and could develop the morphogenetic adaptive response at the root level, as previously described (27). After short tuberized roots expression was achieved, *i.e.* in the stationary, survival phase of drought rhizogenesis (20, 27), and when shoot water potential reached -3.5 MPa (on d 30), a few plants were rehydrated by rewatering the soil at field capacity. Turgor recovery occurred within 24 h and plants were harvested after 3 d rehydration. The shoot water potential (-1 MPa) had not yet fully returned to the value of control plants.

Plant Harvesting and Sampling Procedure. Thirty-three d after

¹ Present address; Department of Genetics, University of California, Davis, CA 95616.

planting, 25 water-stressed, 3 control and 6 rehydrated plants were harvested. The detopped root systems were carefully dry cleaned from sand and rapidly washed in cold water. The tap roots of W-Rt,² S-Rt, and SW-Rt plants, and S-Rs excised from the tap roots of water-stressed plants, were immediately frozen in liquid nitrogen. Wound effects should not be noticeable with this methodology (23).

Protein Extraction and Two-Dimensional Electrophoresis. Extraction, denaturation and resolubilization of proteins were performed as described in Zivy (31) and Damerval *et al.* (6). This method avoids protease activity during protein extraction. Thirty μ l of resolubilization solution per mg of protein pellet were used, and 30 μ l of extract were loaded on isoelectrofocusing rod gels. The two-dimensional electrophoresis procedure was modified from O'Farrell (18) according to Damerval *et al.* (7) except that isoelectrofocusing gels were 1 mm in diameter and second dimension gels were 1 mm thick. The silver staining of proteins was a simplification (7) of the Heukeshoven and Dernick's (14) procedure applied on sets of 19 gels bound to Gelbond PAG (12).

Two independent extracts per root type were made, and at least two gels per extract were visually scored. Faint spots whose reproducibility is difficult to ascertain were discarded (about 20%). The most basic part of the gels was not analyzed because of poor resolution. Qualitative and quantitative variations were determined by two independent observers. Molecular mass of the polypeptides were estimated using standard proteins (Pharmacia calibration kit): phosphorylase b (94 kD), bovine serum albumin (64 kD), ovalbumin (43 kD), carbonic anhydrase (30 kD) and soybean trypsin inhibitor (20.1 kD).

RESULTS AND DISCUSSION

Four hundred and twenty-two reproducible spots were observable in the four types of *Brassica napus* root protein patterns studied: 394 in the W-Rt, 405 in the S-Rt, 397 in the SW-Rt, and 407 in the S-Rs. Three hundred and twenty-seven spots appeared invariable whatever the organ and the water conditions.

Drought effects on the protein pattern of the tap root were revealed through 45 differences between W-Rt and S-Rt. Intensity differences occurred for 30 spots: 18 polypeptides being less abundant and 12 more abundant in S-Rt than in W-Rt. Two spots disappeared in S-Rt. Moreover, 13 polypeptides which were not detected in W-Rt, appeared in S-Rt (Fig. 1). The apparent molecular mass of these polypeptides was rather low, ranging from 20 to 30 kD for most of them (9), between 30 and 43 kD for 3 of them and between 43 and 64 kD for another one.

The inducing effect of drought was further assessed by the behavior of the 45 spots after 3 d rehydration, in SW-Rt. Although the shoot water potential of rehydrated plants had not yet quite recovered the value of control watered plants, 9 out of the 13 newly appeared polypeptides were no longer detectable in SW-Rt, 2 declined and only 2 remained unmodified. Among the 18 spots of decreased intensity in S-Rt, 16 had recovered the same intensity as in W-Rt. The two spots that had disappeared under drought were again present. Among the 12 spots of higher intensity in S-Rt, only one was exactly identical in SW-Rt and W-Rt, and another one had an intermediate intensity between S-Rt and W-Rt; the 10 others were still identical to S-Rt. The return to the W-Rt protein pattern type appears slower in the set of spots increasing in intensity under drought than in the 3 other sets. A similar pattern in intensity recovery upon release of water stress was reported by Bewley *et al.* (3).

Under stress conditions (heat shock, osmotic stress, anoxia), modifications of protein synthesis are well-known, and particu-

larly, whereas preexisting protein synthesis is repressed, initiation of synthesis of specific polypeptides, often characterized by a low mol wt, has been widely reported (17, 21). Protein degradation can also take place under water stress-induced senescence (9). Thus, the 13 appearing polypeptides might correspond to an increased synthesis of preexisting gene products, to *de novo* synthesis, and/or to proteolysis of some proteins, for instance those having disappeared or decreased in S-Rt as compared to W-Rt. The two first hypotheses could be cleared up with labeling of *in vivo* synthesized proteins and analysis of *in vitro* translation products from mRNA isolated from watered and water-stressed roots.

The third hypothesis would imply that some of the changes observed in the tap root might be associated with water stress-induced senescence, as already shown for senescence-induced modifications in protein patterns (8). However, such process seems very unlikely with regards to the presence and behavior of most of the drought-affected polypeptides in the drought-induced and adapted short roots. As a matter of fact, 12 out of the 13 newly appearing spots were found in S-Rs: 4 just identical to S-Rt, 3 more intense and 5 less intense. All of the 12 spots increasing in intensity in S-Rt were present in S-Rs, but about half of the disappearing and decreasing (11/21) spots were lacking. Thus, 33 of the 45 drought-affected spots were observable in S-Rs, and for 21 of them with the same abundance or the same trend of variation as in the tap root. Some other important differences between S-Rs and S-Rt were evident: in addition to 30 intensity differences, 3 spots were specific of the tap root, while 15 were unique to the short tuberized roots. Such a result agrees with the specific morphological, biochemical, and physiological features of this organ (1, 11, 20, 27), which attest from its neof ormation as a response to progressive drought-stress. Whereas the normal root system is subjected to water stress-induced senescence (1), cell proliferation, cell differentiation (27) and high level of synthesis activity (11) take place in the drought-induced roots until low values of water potential (about -2 MPa) are attained. Moreover their solute osmoticum content (K^+ , proline, total soluble amino acids), increases as compared to the other parts of the root system (N Vartanian, F Lahrer, unpublished data). Solute accumulation would reduce water-stress effects and limit senescence-induced protein degradation through osmoregulation (9). Hence, all these findings tend to rule out a proteolytic degradation origin for the 12 polypeptides appeared in S-Rt and also detected in S-Rs.

Newly synthesized proteins (13) and quantitative changes in protein patterns have already been mentioned in response to osmotic shock (3) or nonphysiological desiccation stress (5). However, changes under progressive dehydration have been reported only one other time, in the leaves of *Xerophyta villosa*, a desiccation tolerant Angiosperm, but the qualitative or quantitative nature of these modifications remains unclear (26).

Under physiological conditions of drought, we have found qualitative and quantitative changes in the protein patterns of *B. napus* var. *oleifera* roots. Moreover, the release of drought conditions leads to a return to the control pattern within the whole root system (N Vartanian, unpublished data). Although the physiological significance of proteins induced in response to altered environmental conditions (heat-shock, osmotic stress, anoxia) is not yet always clearly understood, their synthesis appears related with the acquisition of a better resistance to the adverse inductive factor (17, 19, 21, 24). With regards to the adaptive value of drought rhizogenesis, it might be suggested that the modifications we observed have the same significance.

LITERATURE CITED

² Abbreviations: W-Rt, S-Rt, SW-Rt, control, water-stressed, and rehydrated tap roots, respectively; S-Rs, short tuberized roots.

1. BALESTRINI S, N VARTANIAN 1983 Rhizogenic activity during water stress-induced senescence in *Brassica napus* var. *oleifera*. *Physiol Veg* 21: 269-277

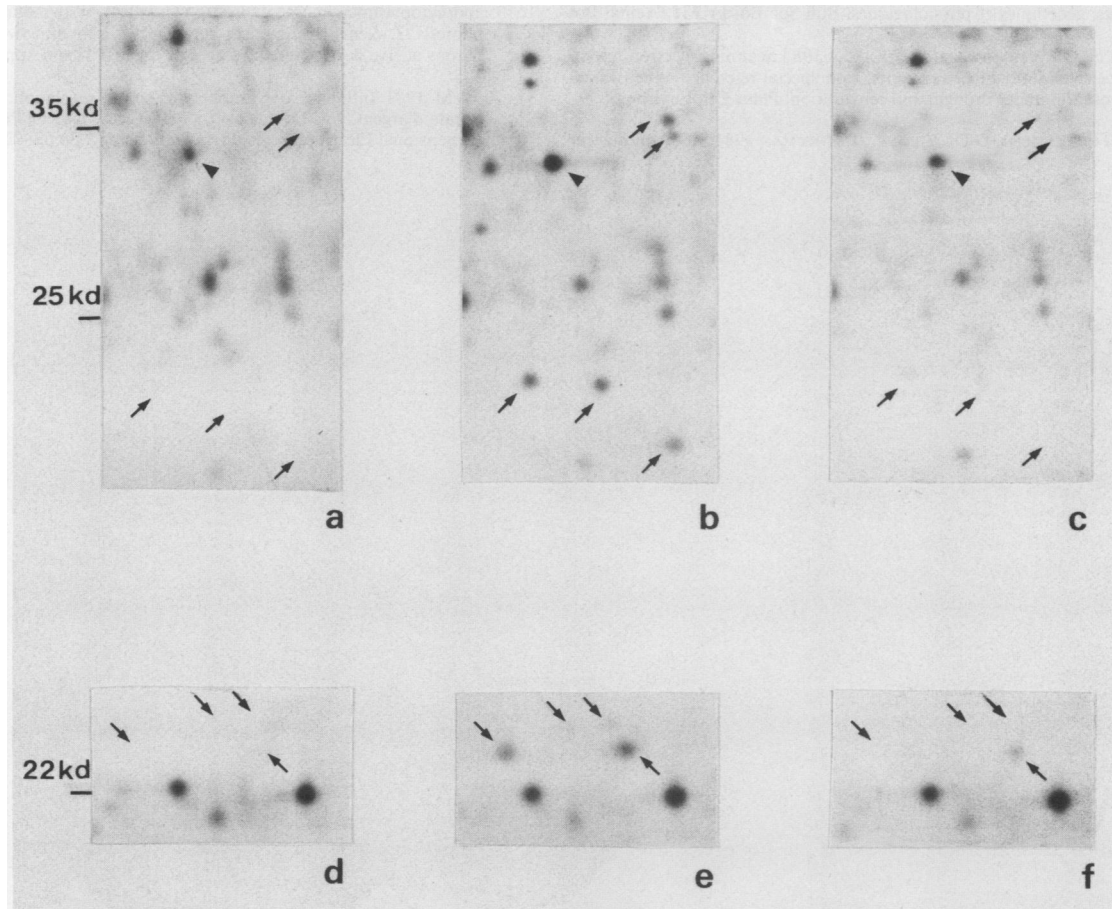


FIG. 1. Detailed portions of two-dimensional gels from control watered tap roots (a, d), water-stressed tap roots (b, e) and rehydrated tap roots (c, f). The arrows point to spots appearing in water-stressed tap roots and the black triangle to spot increasing in intensity under drought (molecular mass in kilodaltons [kd] is indicated at the left).

2. BEWLEY JD 1981 Protein synthesis. In LC Paleg, D Aspinall, eds, *Physiology and Biochemistry of Drought Resistance in Plants*. Academic Press, New York, pp 261-282
3. BEWLEY JD, KM LARSEN, JET PAPP 1983 Water stress-induced changes in the pattern of protein synthesis in maize seedling mesocotyls: a comparison with the effects of heat shock. *J Exp Bot* 34: 1126-1133
4. BURKE JJ, JL HATFIELD, RR KLEIN, JE MULLETT 1985 Accumulation of heat shock proteins in field-grown cotton. *Plant Physiol* 78: 394-398
5. CLOUTIER Y 1983 Changes in the electrophoretic patterns of the soluble proteins of winter wheat and rye following cold acclimation and desiccation stress. *Plant Physiol* 71: 400-403
6. DAMERVAL C, D DE VIENNE, M ZIVY, H THIELLEMENT 1986 Technical improvements in two-dimensional electrophoresis increase the level of genetic variation detected in wheat-seedling proteins. *Electrophoresis* 7: 52-54
7. DAMERVAL C, M LE GUILLOUX, J BLAISONNEAU, D DE VIENNE 1987 A simplification of the Heukeshoven and Dernick's silver staining of proteins. *Electrophoresis*. In press
8. DHINDSA R, CD TSAI, L LALONDE 1986 Protein changes during oat leaf senescence. *Plant Physiol* 80: S-32
9. DUNGEY NO, DD DAVIES 1982 Protein turn-over in the attached leaves of non-stressed and stressed barley seedlings. *Planta* 154: 435-440
10. GARRELS JI 1979 Two-dimensional electrophoresis and computer analysis of proteins synthesized by clonal cell lines. *J Biol Chem* 254: 7961-7977
11. GEAY A, N VARTANIAN, O QUEIROZ 1984 Variations des teneurs en polyamines et leur précurseurs au cours de l'adaptation morphogénétique du colza, *Brassica napus* L. var. *oleifera* à la sécheresse. *Bull Soc Bot Fr*, 131 (Actual Bot 1): 99-111
12. GRANIER F, D DE VIENNE 1986 Silver staining of proteins: standardized procedure for two-dimensional gels bound to polyester sheets. *Anal Biochem* 155: 45-50
13. HEIKKILA JJ, JET PAPP, GA SCHULTZ, JD BEWLEY 1984 Induction of heat shock protein messenger RNA in maize mesocotyls by water stress, abscisic acid, and wounding. *Plant Physiol* 76: 270-274
14. HEUKESHOVEN J, R DERNICK 1985 Simplified method for silver staining proteins in polyacrylamide gels and the mechanism of silver staining. *Electrophoresis* 6: 103-112
15. KIMPEL JA, JL KEY 1985 Heat shock in plants. *Trends Biochem Sci* 10: 353-357.
16. KIMPEL JA, JL KEY 1985 Presence of heat shock mRNAs in field grown soybeans. *Plant Physiol* 79: 672-678
17. MATTERS GL, JC SCANDALIOS 1986 Changes in plant gene expression during stress. *Dev Genet* 7: 167-175
18. O'FARRELL PH 1975 High resolution two-dimensional electrophoresis of proteins. *J Biol Chem* 250: 4007-4021
19. OUGHAM HJ, JL STODDART 1986 Synthesis of heat-shock protein and acquisition of thermo-tolerance in high-temperature tolerant and high-temperature susceptible lines of *Sorghum*. *Plant Sci* 44: 163-167
20. SABATIER G, N VARTANIAN 1983 Cinétique de la rhizogenèse adaptative à la sécheresse. Relation avec l'évolution des paramètres hydriques et morphologiques chez le *Sinapis alba* à deux niveaux d'énergie lumineuse. *Physiol Plant* 59: 501-507
21. SACHS MM, T-H D Ho 1986 Alteration of gene expression during environmental stress in plants. *Annu Rev Plant Physiol* 37: 363-376
22. SCHOLANDER PF, ED HAMMEL, ED BRADSTREET, EA HEMMINGSEN 1965 Sap pressure in vascular plants. *Science* 148: 339-346
23. SCHUSTER AM, E DAVIES 1983 Ribonucleic acid and protein metabolism in pea epicotyls. II. Response to wounding in aged tissue. *Plant Physiol* 73: 817-821
24. SINGH NK, AK HANDA, PM HASEGAWA, RA BRESSAN 1985 Proteins associated with adaptation of cultured tobacco cells to NaCl. *Plant Physiol* 79: 126-137
25. THIELLEMENT H, N BAHRMAN, C COLAS DES FRANCS 1986 Regulatory effects of homeologous chromosome arms on wheat proteins at two developmental stages. *Theor Appl Genet* 73: 246-251
26. TYMMS MJ, DF GAFF, ND HALLAM 1982 Protein synthesis in the desiccation tolerant angiosperm *Xerophyta villosa* during dehydration. *J Exp Bot* 33: 332-343
27. VARTANIAN N 1981 Some aspects of structural and functional modifications induced by drought in root systems. *Plant Soil* 63: 83-92
28. VARTANIAN N 1984 Un modèle de processus adaptatif à la sécheresse: aspects

- phylétiques, génétiques et physiologiques. Bull Soc Bot Fr 131 (Actual Bot 1): 59-67
29. VARTANIAN N, DS WERTHEIMER, H COUDERC 1983 Scanning electron microscopic aspects of short tuberized roots, with special reference to cell rhizodermis evolution under drought and rehydration. Plant Cell Environ 6: 39-46
30. ZIVY M, H THIELLEMENT, D DE VIENNE, JP HOFFMAN 1984 Study on nuclear and cytoplasmic genome expression in wheat by two-dimensional electrophoresis. 2. Genetic differences between two lines and two groups of cytoplasm at five developmental stages or organs. Theor Appl Genet 78: 335-345
31. ZIVY M 1981 Influence des ampholytes sur la révélation des protéines au nitrate d'argent. In MM Galteau, G Siest, eds, Recent Progresses in Two-Dimensional Electrophoresis. Press Univ Nancy, pp 69-72