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COMPARISONS OF PLASMA MEMBRANE POLYPEPTIDES FROM SOYBEAN AND ALFALFA

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Key Word Index—*Glycine max*; soybean; *Medicago sativa*; alfalfa; Leguminosae; plasma membrane proteins; 2D-electrophoresis.

Abstract— Polypeptides were solubilized with sodium dodecyl sulfate from plasma membrane vesicles of eight varieties of soybean roots [*Glycine max* (L.) Merr.] and of cultured alfalfa cells (*Medicago sativa* L.). The solubilized polypeptides were analysed by 2D-polyacrylamide gel electrophoresis. Apparent isoelectric point and MW values were obtained for 80 soybean plasma membrane polypeptides and 44 alfalfa plasma membrane polypeptides. From these data composite distribution patterns were constructed, which are representative of the soybean or alfalfa 2D-gels, respectively. The results showed that the general polypeptide staining patterns were similar for all the soybean varieties, but some minor differences were evident. The alfalfa electrophoretograms differed markedly from the soybean electrophoretograms in specific details, though some general pattern similarities were noted. The data are discussed in terms of a physiological role for the integral plasma membrane polypeptides and in terms of the potential for distinguishing among soybean varieties and between species at the plasma membrane polypeptide level.

INTRODUCTION

Integral plasma membrane proteins were first defined as such by Singer and Nicolson in 1972 [1], with the publication of the fluid mosaic model of biological membrane structure. They have been widely studied in viral and mammalian systems and have been found to function as membrane enzymes, as major structural components and as receptor or recognition sites for hormones and other extracellular signals [2, 3]. Little is known of the nature or function of the plasma membrane proteins of higher plants.

One approach toward knowledge of the plasma membrane proteins in higher plants is to determine whether the complement of plasma membrane proteins exists in a dynamic condition related to the physiological status of the cell. The presence of that condition would infer a role for particular integral proteins of the plasma membrane during specific stages of development of the plant tissue. A second approach is to determine if plants differing in both genetic determinants and physiological characters also differ in the complement of their plasma membrane proteins. The first hypothesis was confirmed in an earlier report from this laboratory [4]. There we documented both qualitative and quantitative changes in the complement of polypeptides solubilized from the plasma membrane of the developing soybean root. The second hypothesis is the subject of this communication. Here we report the characterization of the integral polypeptide complement of the plasma membrane from the roots of eight varieties of soybean by 2D-polyacrylamide gel electrophoresis. The solubilization procedure included a prewashing of the isolated membrane vesicles with 200 mM sodium chloride, necessary for maximum resolution in the second dimension [4], thus precluding evaluation of the peripheral or extrinsic membrane proteins [1].

Some differences in specific polypeptides among the eight soybean varieties were documented. However, the overall patterns on the second dimension gels were similar for all varieties. The results were used to construct a 'typical' distribution pattern for soybean root plasma membrane proteins (polypeptides). A similar composite drawing was constructed for the plasma membrane proteins of cultured alfalfa cells for comparison. The data are discussed in terms of a physiological role for the integral plasma membrane polypeptides and in terms of the potential for distinguishing among soybean varieties at the plasma membrane polypeptide level.

RESULTS

Variety comparison

Integral plasma membrane polypeptides from roots of eight varieties of soybean were solubilized with sodium dodecyl sulfate (SDS) and electrophoresed in two dimensions. Gels representative of the results are shown for four varieties in Fig. 1(a–d). The general staining patterns are very similar for each variety, however, close examination of the gels also revealed some differences in the polypeptide complement of each variety. To emphasize similarities between the gels a composite drawing was prepared by combining major polypeptides from all eight varieties (Table 1) on a single diagram (Fig. 2). Figure 2 is representative of the typical plasma membrane polypeptide complement of the gels in Fig. 1 as well as those gel

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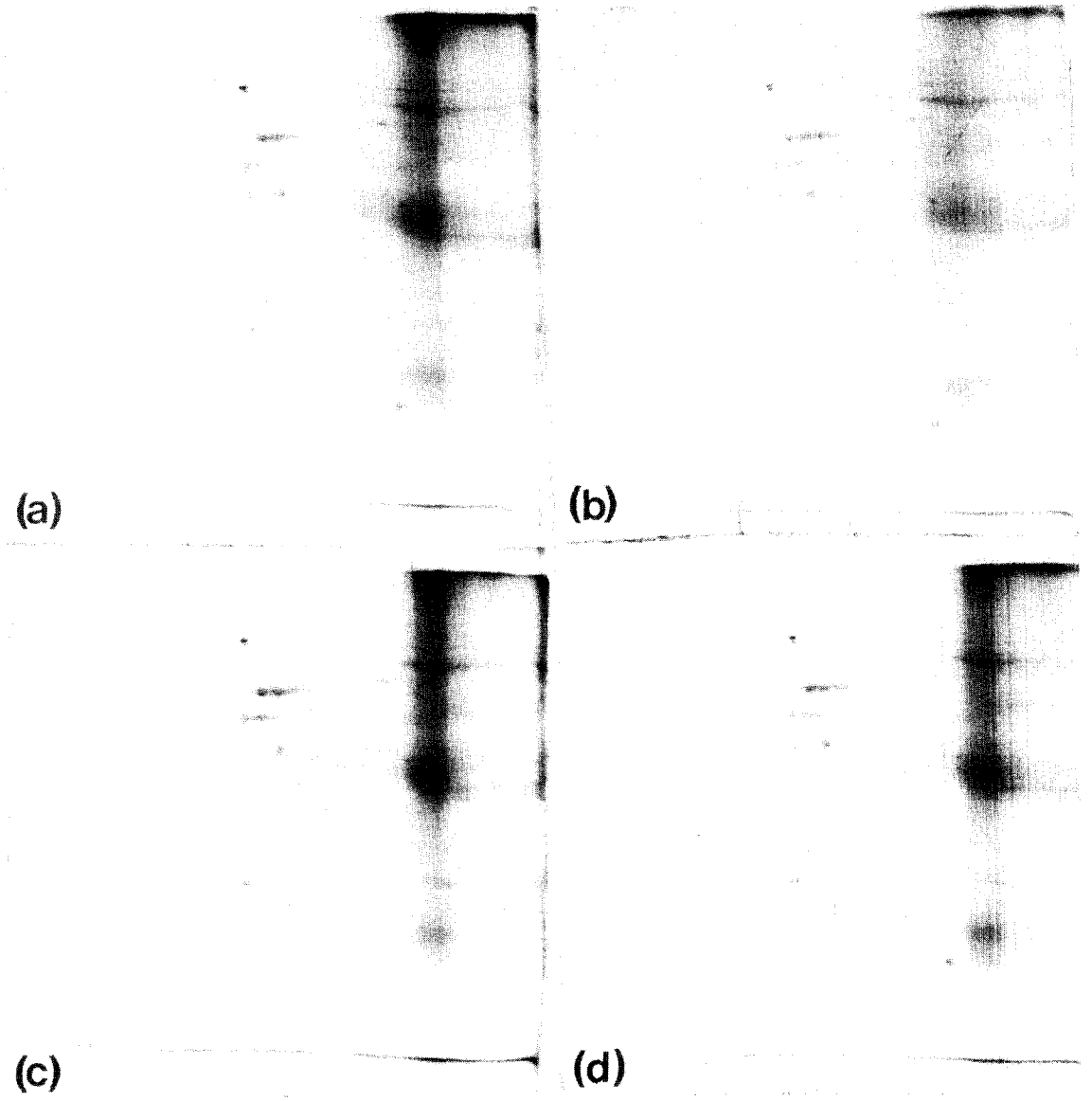

Fig. 1. Two-dimensional electrophoretograms of integral plasma membrane polypeptides of roots of four varieties of soybean. (a) Davis, (b) Wells II, (c) Tracy, (d) Essex.

staining patterns reported earlier [4, 5]. It should be emphasized that not every polypeptide from each gel was included on the composite drawing. Several spots were purposely omitted, as they were faintly stained or difficult to distinguish because of background staining. Close examination of the varietal gels and of gels from earlier studies revealed *ca* 90 identifiable polypeptides on typical whole root gels [4, 5].

Table 1 lists the isoelectric points and MWs of the 80 polypeptides shown in the composite drawing and notes the varieties in which each polypeptide occurred. Those polypeptides which showed varietal specificity are listed in Table 2. Varietal specificity is most evident for polypeptide 4 which appeared on every gel of varieties W, WII and D, and was absent from every gel of the remaining five varieties. Thus, polypeptide 4 represents a significant varietal difference among the varieties compared. The significance of the varietal differences among the remaining polypeptides listed in Table 2 is less clear. Those polypeptides noted as present (column 2) appeared on more than half of the gels for the variety listed. Those noted as absent (column 4) did not appear on any of the replicate gels for that variety. Polypeptides listed as unclear (column 3) appeared on less than half of the replicate gels. These results were obtained even when a large amount of membrane protein (*ca* 400 μg) was applied to the top of the first dimension tube gel.

Genus comparison

Plasma membrane polypeptides of cultured alfalfa cells were also extracted with SDS and electrophoresed in two dimensions [4]. A composite diagram of 44 polypeptides common to the alfalfa electrophoretograms was con-

Table 1. Integral polypeptides of soybean root plasma membrane vesicles

Spot No.	pI	MW × 10^{-3}	Varieties
1	4.78 ± 0.19	148 ± 26	—
2	5.65 ± 0.09	124 ± 23	All
3	5.90 ± 0.10	120 ± 18	W, WII
4	4.85 ± 0.09	117 ± 19	W, WII, D*
5	5.06 ± 0.09	114 ± 27	All
6	6.20 ± 0.10	113 ± 20	W, WII
7	5.07 ± 0.09	109 ± 25	All
8	5.31 ± 0.13	100 ± 23	W,WII, T, E†
9	6.04 ± 0.13	100 ± 23	W, B, D
10	4.68 ± 0.07	97 ± 29	E†, D‡, ‖
11	4.79 ± 0.08	91 ± 10	—
12	5.06 ± 0.06	91 ± 12	—
13	6.26 ± 0.10	85 ± 23	All
14	4.81 ± 0.05	82 ± 17	—
15	5.11 ± 0.12	82 ± 17	—
16	5.15 ± 0.15	82 ± 12	—
17	5.35 ± 0.19	77 ± 24	All
18	4.60 ± 0.08	70 ± 20	T, D‡, L‖, ¶
19	5.53 ± 0.08	70 ± 25	—
20	5.63 ± 0.10	70 ± 27	All
21	5.70 ± 0.11	70 ± 23	—
22	5.79 ± 0.11	70 ± 27	All
23	4.75 ± 0.12	67 ± 7	—
24	4.65 ± 0.08	66 ± 24	—
25	5.08 ± 0.12	66 ± 21	All
26	5.19 ± 0.24	66 ± 22	All
27	5.10 ± 0.12	63 ± 18	All
28	5.09 ± 0.10	61 ± 18	W, WII, D, E†
29	5.37 ± 0.07	61 ± 10	—
30	5.51 ± 0.11	61 ± 18	W, WII, T, D, E†
31	4.74 ± 0.21	59 ± 21	All
32	5.39 ± 0.10	57 ± 21	All
33	5.66 ± 0.15	56 ± 19	W, WII, T, D‡, E
34	6.26 ± 0.12	56 ± 12	—
35	5.14 ± 0.11	55 ± 21	W, WII, T, D
36	5.17 ± 0.07	55 ± 17	W, WII, T
37	6.28 ± 0.13	55 ± 9	—
38	5.37 ± 0.08	54 ± 18	—
39	5.54 ± 0.07	53 ± 13	W, WII, T, D‡
40	6.41 ± 0.09	53 ± 16	W§, WII, T, D
41	5.88 ± 0.16	51 ± 15	W§, WII, T, D‡
42	4.97 ± 0.13	49 ± 17	—
43	4.89 ± 0.12	49 ± 23	—
44	6.33 ± 0.07	45 ± 12	—
45	5.41 ± 0.10	45 ± 14	W, WII, T, D, E
46	5.52 ± 0.07	45 ± 14	All
47	5.93 ± 0.07	45 ± 6	—
48	6.14 ± 0.13	44 ± 19	All
49	6.34 ± 0.08	44 ± 6	—
50	5.64 ± 0.08	42 ± 13	All
51	5.90 ± 0.14	42 ± 14	—
52	5.64 ± 0.10	40 ± 11	—
53	6.10 ± 0.18	40 ± 11	WII, T, D‡, E, L
54	6.23 ± 0.15	39 ± 11	WII, T, D, E‖
55	5.36 ± 0.12	38 ± 12	T, D‡, E†
56	6.06 ± 0.13	38 ± 13	T, D, E
57	5.27 ± 0.10	37 ± 17	All
58	7.23 ± 0.05	36 ± 10	—
59	6.24 ± 0.15	34 ± 14	All
60	5.17 ± 0.10	34 ± 15	All
61	6.10 ± 0.15	34 ± 14	W§, WII, T, D, E†, E
62	6.85 ± 0.13	34 ± 7	—
63	7.10 ± 0.12	34 ± 7	—
64	7.27 ± 0.10	34 ± 17	All
65	4.82 ± 0.13	32 ± 15	All
66	5.25 ± 0.09	31 ± 18	W, WII, T, E†
67	5.95 ± 0.14	31 ± 14	W, WII, T, D‡, E¶
68	6.41 ± 0.13	31 ± 7	W§, WII**
69	5.48 ± 0.24	30 ± 13	All
70	6.12 ± 0.14	29 ± 4	—
71	4.68 ± 0.14	29 ± 14	—
72	5.88 ± 0.09	28 ± 8	—
73	6.19 ± 0.17	28 ± 2	—
74	5.59 ± 0.11	28 ± 8	W§, WII, T, D, E†, ¶
75	6.19 ± 0.13	24 ± 4	—
76	6.36 ± 0.11	24 ± 6	All
77	5.68 ± 0.09	23 ± 6	—
78	4.87 ± 0.17	21 ± 2	—
79	6.15 ± 0.16	21 ± 3	D, T, E†, L
80	6.40 ± 0.09	18 ± 6	—

The 80 polypeptides shown in Fig. 2 are listed above. The isoelectric point (pI) and MW (MW × 10^{-3}) values are those from which the composite drawing was constructed. Polypeptides which appeared in over half of the gels of a particular variety are listed. W, Wells; WII, Wells II; D, Davis; T, Tracy; E, Essex; B, Bragg; L, Lee 74; F, Forest. Those polypeptides which were visible in all eight varieties are also indicated. Polypeptides which appeared on less than half of the gels of any of the varieties are not indicated in the variety column. Values following pI and MW designation are s.e.'s.

*Present on all gels of these varieties. Not present on any gels of all other varieties.

†Present on two of four gels of Essex.

‡Present on two of four gels of Davis.

§Present on two of four gels of Wells.

‖Not present on any gels of Wells.

¶Not present on any gels of Bragg.

**Not present on any gels of Essex.

structed in the same way as the soybean composite (Table 3 and Fig. 3). Although plasma membrane polypeptides from two different kinds of tissue were compared, the differences in tissue type (roots vs cultured cells) were not relevant here, as gels of plasma membrane polypeptides of cultured soybean cells showed the same staining pattern as those of soybean roots (results not shown). The composite drawings of the alfalfa plasma membrane polypeptides (Fig. 3) and the soybean plasma membrane polypeptides (Fig. 2) show some similarities. For example, soybean composite polypeptides 17, 25 and 31 stained intensely and formed a pattern similar to alfalfa composite polypeptides 9, 10, 16 and 17. However, the corresponding polypeptides differ considerably in pI and MW. Also, the region between pH 6.0 and 6.3 on both composite drawings contains a complex pattern of polypeptides of both high and low MWs, further confirming

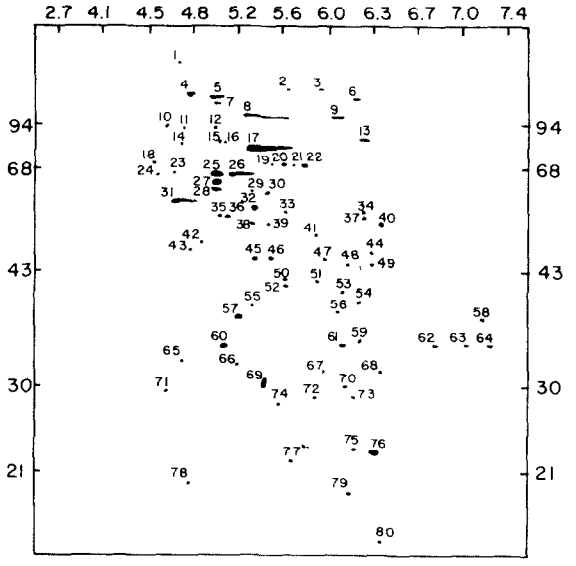

Fig. 2. Composite distribution pattern of soybean root plasma membrane polypeptides, drawn from the data in columns 2 and 3 of Table 1. The pH gradient is on the horizontal axis, and the MW values $\times 10^{-3}$ are along the vertical axis. The polypeptide numbers refer to the polypeptides in Table 1.

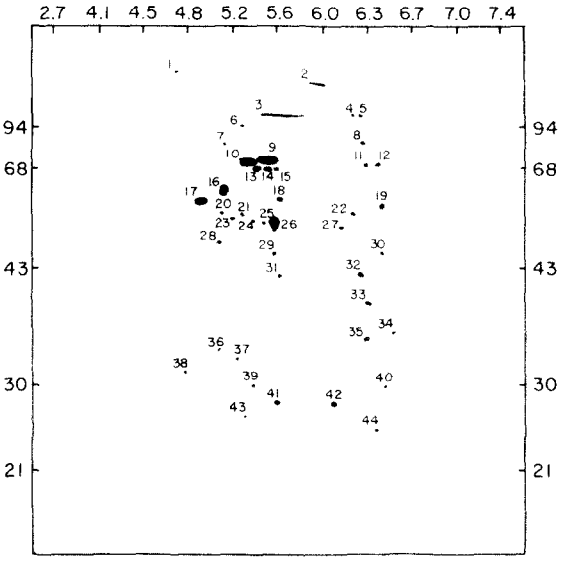

Fig. 3. Composite distribution pattern of plasma membrane polypeptides of alfalfa tissue culture cells, drawn from the data of columns 2 and 3 in Table 3. The pH gradient is on the horizontal axis, and the MW values $\times 10^{-3}$ are along the vertical axis. The polypeptide numbers refer to the polypeptides in Table 3.

the similarity of the distribution patterns. Several polypeptides from both composites have very close p*I*'s and MWs (Table 4). Polypeptides 3 and 2, 16 and 7, 36 and 20, 50 and 31, 60 and 36, and 74 and 41, of soybean and alfalfa composites respectively, have almost identical p*I* and MW values and may represent the same polypeptide in each case.

Even though the staining patterns for the two genera show some similarities, there are numerous differences between the two patterns. The details of the distribution patterns differ, both in location of the polypeptides and in the intensity of staining of particular polypeptides (alfalfa gels not shown, compare Figs. 2 and 3).

DISCUSSION

Electrophoretic analyses of polypeptides solubilized from plasma membranes of roots from eight soybean varieties revealed slight, but consistent, variation in the protein complement among varieties. A single polypeptide (spot 4, Fig. 2) appeared on every gel from three of the varieties tested and was absent from the remaining varieties in every case. Several other polypeptides appeared to show some variation among varieties (Table 2), but the lack of absolute reproducibility for those polypeptides on the replicate gels of each variety make it difficult to state with certainty whether any of those polypeptides represented specific varietal differences.

In an earlier report [4] we noted that such factors as chemical modification (e.g. glycosylation), high background staining in the high MW–high pH region of the 2D-gels, and difficulties in resolving equivalent levels of protein on replicate gels may have affected the resolution of the plasma membrane polypeptides. Those factors may have also affected the results presented here, especially in the case of polypeptides which were not consistently

Table 2. Varietal differences among integral plasma membrane polypeptides of soybean root

Polypeptide No.*	Present†	Unclear‡	Absent§
4 (13)	W, WII, D	—	B, T, E, F, L
10 (10)	—	E, D	W
18 (12)	T, L	D	W, B
54 (15)	WII, T, D	E	W
67 (17)	W, WII, T, E	D	B
68 (10)	WII	W	E
74 (13)	WII, T, D	W, E	B

Abbreviations as in Table 1.

*No. in parentheses is No. of gels on which polypeptide appeared.

†Appeared on over half of the gels of the variety cited.

‡Appeared on half of the gels of the variety cited.

§Appeared on none of the gels of the variety cited.

Table 3. Integral plasma membrane polypeptides of cultured alfalfa cells

Spot No.	p*I*	MW × 10^{-3}
1	4.76 ± 0.076	137 ± 16
2	5.84 ± 0.018	125 ± 8
3	5.45 ± 0.083	100 ± 11
4	6.19 ± 0.018	100 ± 12
5	6.24 ± 0.029	100 ± 12
6	5.29 + 0.011	94 ± 6
7	5.15 ± 0.032	82 ± 19
8	6.27 ± 0.029	81 ± 9
9	5.54 ± 0.061	73 ± 9
10	5.31 ± 0.047	70 ± 5
11	6.32 ± 0.018	70 ± 12
12	6.40 ± 0.018	70 ± 11
13	5.42 ± 0.022	67 ± 6
14	5.50 ± 0.022	67 ± 4
15	5.56 ± 0.018	67 ± 6
16	5.14 ± 0.018	62 ± 9
17	4.97 ± 0.029	59 ± 16
18	5.62 ± 0.018	59 ± 8
19	6.43 ± 0.014	57 ± 15
20	5.14 ± 0.040	56 ± 4
21	5.29 ± 0.040	56 ± 11
22	6.18 ± 0.025	56 ± 14
23	5.21 ± 0.061	55 ± 12
24	5.39 ± 0.018	54 ± 10
25	5.48 ± 0.022	54 ± 11
26	5.55 ± 0.004	52 ± 10
27	6.09 ± 0.025	52 ± 10
28	5.12 ± 0.014	48 ± 13
29	5.56 ± 0.011	46 ± 11
30	6.44 ± 0.022	46 ± 11
31	5.60 ± 0.00	42 ± 12
32	6.23 ± 0.025	42 ± 12
33	6.31 ± 0.022	39 ± 10
34	6.51 ± 0.014	35 ± 7
35	6.31 ± 0.018	35 ± 6
36	5.12 ± 0.032	33 ± 9
37	5.28 ± 0.025	32 ± 11
38	4.87 ± 0.032	31 ± 8
39	5.38 ± 0.018	29 ± 6
40	6.45 ± 0.00	29 ± 5
41	5.58 ± 0.018	28 ± 9
42	6.04 ± 0.025	28 ± 7
43	5.33 ± 0.007	26 ± 7
44	6.38 ± 0.083	25 ± 7

The 44 polypeptides shown in Fig. 3 are listed below. Isoelectric point (p*I*) and MW (MW × 10^{-3}) values are those from which the composite drawing was constructed. Values following p*I* and MW designations are s.e.'s.

resolved. It is also possible that variety-specific proteins may have remained bound to the insoluble material at the origins of the gels. The application of a large amount of membrane protein to the top of the first dimension tube gel (400 μg) provided little difference in resolution of minor membrane polypeptides. However, the reproducible presence or absence of polypeptide 4 suggests that it is variety specific.

It should be emphasized that only integral polypeptides were considered in this study. The peripheral proteins were removed by treating the membrane vesicles with 200 mM sodium chloride prior to protein solubilization. This was included to obtain good resolution in the second dimension electrophoresis [4]. Thus, the possibility of variety-specific peripheral polypeptides was not tested.

It is likely that variation in plasma membrane polypeptides among cultivars relates to variation in physiological function. The observation that the plasma membrane functions in pathogen recognition [6–8] has led to the suggestion that elicitors of pathogen origin have specific receptors on the surface of plant cells [6, 9]. A plasma membrane protein has been identified in sugar cane which binds the toxin produced by *Helminthosporium sacchari*. Resistant lines contain a similar protein, differing by a few amino acids, but do not bind the toxin [10].

While we have no direct evidence that any of the polypeptides reported here relate to disease resistance in soybean, it is interesting to note that certain polypeptides (e.g. 18, 67 and 74) appeared on varieties which carry one or more genes for resistance to *Phytophthora* (see Experimental) and were absent from the variety Bragg which carries the *rps* gene for susceptibility. Similarly, polypeptide 54 appeared on Wells II, a variety resistant to many of the races of *Phytophthora*, yet was absent from Wells. That is interesting because Wells and Wells II carry two different alleles for resistance in the *Rps* gene.

It is possible that further evaluation of the varieties in relation to known membrane functions, e.g. permeability relationships, enzyme activities, ion uptake rates or specificity, etc. may provide a further link between membrane protein composition and physiological function.

Our attempt to differentiate among varieties with the 2D-polyacrylamide gel electrophoresis procedure produced results difficult to interpret. No polypeptides clearly specific to only one variety were revealed. The problems with replication made the results more ambiguous with regard to this question. If one is trying to relate differences in polypeptide complement to differences in a specific physiological function, it may be possible to draw some tentative conclusions (see above discussion). However, the procedure, as conceived for plant root plasma membrane vesicles, cannot be used to 'fingerprint' a variety.

Our results do indicate that 2D-electrophoresis can differentiate between genera. The similarity of the two distribution patterns presented here suggests also that a general plasma membrane polypeptide distribution pattern may be recognized after further species are compared in this manner. Table 4 suggests that certain plasma membrane polypeptides are the same between these two genera, indicating that those polypeptides may have been conserved during species differentiation.

EXPERIMENTAL

Variety and genus comparison. The eight varieties of soybean and the sources of seed were: Wells and Wells II, northern cultivars from the Indiana Seed Improvement Association, and Tracy, Davis, Bragg, Essex, Lee 74 and Forest, southern varieties, obtained from Dr. Benjamin Beard of this department. Three of the varieties, Essex, Forest and Bragg, carry the *rps* gene, with no resistance to *Phytophthora*. The other five varieties carry three of

Table 4. Comparison of selected soybean and alfalfa plasma membrane polypeptides

Soybean			Alfalfa		
Spot No.	p*I*	MW $\times 10^{-3}$	Spot No.	p*I*	MW $\times 10^{-3}$
3	5.90	120	2	5.90	122
13	6.26	85	8	6.27	81
16	5.15	82	7	5.15	82
36	5.17	55	20	5.14	56
34	6.26	56	22	6.18	56
38	5.37	54	25	5.48	54
50	5.64	42	31	5.60	42
60	5.17	34	36	5.12	33
69	5.48	30	39	5.38	29
74	5.59	28	41	5.58	28

the five known genes for resistance and three of the alleles of one of the gene loci, each gene or allele conferring resistance to specific races of the pathogen: Wells, Rps_1, Wells II, Rps_1^c, Lee 74 Rps_1^c, Tracy, Rps_1^b, Rps_3 and Davis Rps_2. [Wilcox, J. R. and Hartwig, E. E., personal communications]. The varieties also differ in the character of time of pod maturity, Wells and Wells II being northern or early varieties, and the other six being southern or late varieties.

The cultured alfalfa cells were obtained from S. Stavarek of the laboratory of D. W. Rains of this department. Enough material was obtained for four 2D-slab gels.

Isolation of plasma membrane vesicles and 2D-electrophoresis. Plasma membrane vesicles were isolated from roots of 4-day-old dark-grown soybean seedlings [*Glycine max* (L.) Merr.] or from cultured cells of alfalfa (*Medicago sativa* L.), following the procedures developed for the isolation and solubilization of soybean root plasma membrane vesicles for 2D-electrophoresis [4]. The polypeptides were solubilized by SDS, as it has been shown to be most effective in this procedure [5]. 2D-electrophoresis was carried out as previously described [4]. Polypeptides from each variety were solubilized and electrophoresed 2–5 times. *Ca* 400 μg membrane protein was applied to the first dimension tube gel to determine any differences in the minor membrane protein constituents. Preliminary results showed that Na_2EDTA enhanced the polypeptide resolution more than EGTA–Tris. Thus Na_2EDTA was used. Protein concn was determined by the method of ref. [11].

Construction of composite drawings. 29 2D-electrophoretograms of soybean root plasma membrane polypeptides were analysed for the soybean composite distribution pattern drawing. These included 4 of variety Wells, 5 of Wells II, 3 of Tracy, 4 of Davis, 4 of Essex, 5 of Bragg, 2 of Lee 74 and 2 of Forest. Individual polypeptides were identified from slab to slab by comparing the relative intensity and the relative proximity of the spot in relation to nearby major spots. The gels were photocopied on a color Xerox and relative mobility measurements were made on the Xerox copies. Relative mobility values (R_m) for each polypeptide were averaged for both dimensions and the average values were converted to p*I* and MW values from standard curves as in ref. [4]. The p*I* and MW values $\pm$ s.e. were plotted to construct the composite drawings. Each polypeptide locus was drawn to resemble the corresponding polypeptide on the 2D-gels to enhance the comparison. For the alfalfa composite drawing, four 2D-slab gels were analysed in the same manner.

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