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Distribution of Wheat Germ Agglutinin in Young Wheat Plants[1, 2]

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ABSTRACT

A liquid phase, competition-binding radioimmunoassay for wheat germ agglutinin, with a detection limit of 10 nanograms, was developed in order to determine the distribution of this lectin in young wheat plants. Affinity columns for wheat germ agglutinin removed all antigenically detectable activity from crude extracts of wheat tissue; thus, the antigenic cross-reactivity detected by the assay possesses sugar-binding specificity similar to the wheat germ-derived lectin. The amount of lectin per dry grain is approximately 1 microgram, all associated with the embryo. At 34 days of growth, the level of lectin per plant was reduced by about 50%, with approximately one-third in the roots and two-thirds in the shoot. The data also indicate that actively growing regions of the plant (the bases of the leaves and rapidly growing adventitious roots) contain the highest levels of lectin. Half of the lectin associated with the roots could be solubilized by washing intact roots in buffer containing oligomers of *N*-acetylglucosamine, whereas the remainder is liberated only upon homogenization of the tissue.

Despite wide interest and much research (see ref. 12 for review), the lectins of higher plants remain, to a large extent, proteins in search of functions. As part of the effort to determine the possible functions of these proteins, their distribution in the various tissues of the plant during its life cycle has been investigated. Such studies of lectin distribution, however, have been applied mainly to the legume lectins. Interest has focused on the legumes because of the hypothesis that lectins play a role in the recognition of rhizobial symbionts. However, since the bulk of the lectin activity in numerous legumes has been shown to be localized in the cotyledons, with little if any present in the root tissues (*e.g.* 24), the role of lectins in rhizobial interactions remains unclear.

In contrast to the legumes, there has been little investigation of the tissue distribution of lectins from nonleguminous plants. An understandable cause for this lack of interest is the absence in many plants of an interaction potentially mediated by lectins as dramatic as that between rhizobia and legumes. This emphasis on the legume lectins, however, has in some respects precluded a broader understanding of lectin function. A survey of the tissue distribution of a nonlegume lectin would help fill this void.

Conspicuous among the nonlegume seed lectins is WGA.[6] Its physical characteristics are known in detail, and it has been widely used as a probe for the study of cell surfaces (see ref. 12 for review). Its role in wheat plants, however, has been virtually ignored. Contrary to the belief (or hope) of some, WGA did not evolve in wheat for the sole purpose of serving as a diagnostic tool in the study of mammalian cell surfaces. Rather, its properties of binding to chitin (a component of fungal cell walls), of inhibiting the growth of fungal hyphae (20), and of binding to the polymer of bacterial cell walls (1) make WGA a candidate for a role in the recognition or control of various pathogens. Because of this interest, we describe here the development of a competitive-binding, liquid phase RIA for WGA and its application in studies on the localization of WGA in the plant.

MATERIALS AND METHODS

Grain. *Triticum aestivum* L. cv "Era" was purchased from Olds Seed Company, Madison, Wisconsin. "Argee," "Titan," "Potomac," and "Little Club" varieties were obtained from sources at the University of Wisconsin, Madison. The "Era" cultivar was used in all experiments unless otherwise indicated.

Production of CH. A preparation of oligomers of GlcNAc was produced by hydrolyzing chitin as described by Rupley (27). The oligomer preparation used was that obtained by eluting the charcoal-celite with 60% ethanol after extensive washing with water and 10% ethanol. We determined that the mixture was composed of monomers, dimers, trimers, tetramers, and higher weight oligomers by paper chromatography using a 4:1:1 1-butanol-ethanol-water solvent system. This heterogeneous mixture was used in the preparation of the affinity ligand for the purification of WGA, as an inhibitor of WGA-sugar interactions in hemagglutination assays and RIA, in the elution of [^{125}I]WGA from affinity columns, and to wash WGA from roots.

WGA Purification. During the early part of the study, WGA was purified from raw wheat germ by the method of Bouchard *et al.* (4), except that powdered chitin (Sigma), prepared as described by Bloch and Burger (3), was used as the affinity ligand. During the latter part of the study, WGA was purified by the method of Uy and Wold (31) on a column of epoxide-activated Sepharose 4B to which was linked the chitin hydrolysate described above. Complete *N*-acetylation of the bound oligomers was ensured by reacetylation of the beads as described by Desai and Allen (8). The $E_{280}^{1\%,\,1\ cm}$ of 15 reported by Nagata and Burger (22) was used to determine the WGA concentration of pure solutions.

Hemagglutination Assay. Rabbit red blood cells were trypsinized as described by Lis and Sharon (17) and then fixed with glutaraldehyde by the method of Turner and Liener (30). The cells were stored at 4 C as a 20% suspension (packed cells to total volume) in PBS containing 0.02% sodium azide. For the hemagglutination assay, 10-μl aliquots of sample serially diluted in 2-fold increments were placed in conical microtiter wells followed by 100 μl of a 1% suspension of red blood cells in PBS. To check whether hemagglutinating activity was inhibitable by sugars that bind to WGA, CH at 0.5 mg ml^{-1} was included in the red blood cell suspension. End points, expressed as the reciprocal of the

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[6] Abbreviations: WGA, wheat germ agglutinin; RIA, radioimmunoassay; CH, chitin hydrolysate; GlcNAc, *N*-acetylglucosamine; PBS, phosphate-buffered saline.

greatest dilution that yielded positive hemagglutination, were read after a 1-h incubation at 4 C. In our assay, a solution of WGA at 10 μg ml^{-1} generated a titer of 4.

Antibody Preparation and Immunological Techniques. Rabbits received an injection intramuscularly at multiple sites of a total of 0.5 mg WGA in complete Freund's adjuvant. Two weeks later, a second injection of 0.5 mg in incomplete adjuvant was given in the same fashion as the first. After another 10 days, the rabbits were bled. This sequence was repeated with the WGA dose reduced to 0.1 mg and with incomplete Freund's adjuvant after the rabbits had rested for 14 days or more. A partially purified immunoglobulin fraction was obtained by adding a volume of 80% saturated ammonium sulfate to an equal volume of crude serum and precipitation overnight at 4 C. The precipitate was collected by centrifugation at 10,000g for 10 min, washed twice with 40% saturated ammonium sulfate, and resuspended in a volume of PBS equal to that of the initial aliquot of serum. It was then dialyzed extensively against distilled H_2O and, finally, PBS containing 0.02% sodium azide. Immunoglobulin fractions prepared in this way from antisera, nonimmune sera, and goat anti-rabbit-IgG sera were used in the RIA.

Double diffusion was performed in 0.5% agarose gels prepared with barbital buffer (pH 8.6) to which was added GlcNaC at 50 mM to inhibit the formation of sugar-lectin precipitin bands. Immunoelectrophoresis was performed in 1.5% agarose gels prepared in 20 mM Tris-acetate buffer (pH 7.4). This, rather than the standard pH 8.6 barbital buffer, was used because, at the higher pH, the lectin does not move from the origin (pI isoelectric point of WGA $\cong$ 8.7 [25]). Gels, prepared by pouring 7 ml molten agar into a 3- × 5-inch glass slide, were run for 90 min at 5 mamp/gel. A single precipitin band was seen in both double diffusion and immunoelectrophoresis (Fig. 1) when antiserum diffused against either purified WGA or crude wheat germ extract.

RIA. During the early part of the investigation, [^{125}I]WGA was prepared by the chloramine-T method as described by Burridge (6), except that the incubation time in chloramine-T was reduced to 5 min. During the latter part of the investigation, the insoluble catalyst, Iodo-gen (Pierce Chemical Company), was used to perform the radioiodination. In this procedure, 100 μg WGA in 0.1 ml PBS with GlcNac at 40 mg ml^{-1} were added to 1 mCi of carrier-free Na^{125}I (New England Nuclear), which in turn was transferred to a tube coated with 4 μg Iodo-gen as described by Markwell and Fox (18). After gentle agitation in an ice-water bath for 5 min, 50 μl of 10 mM tyrosine was added, and the tube was agitated for another min. The reaction mixture was then diluted with 3 ml unlabeled 0.25 M NaI in PBS. The contents of the reaction tube were then applied to a small column of powdered chitin that had been equilibrated with 0.25% BSA in PBS. The column was washed with PBS until fewer than 4 × 10^4 cpm ml^{-1} were detected in the effluent. The labeled lectin was eluted with PBS containing 10 mg ml^{-1} CH and then dialyzed against PBS to remove the large amounts of inhibitory sugar.

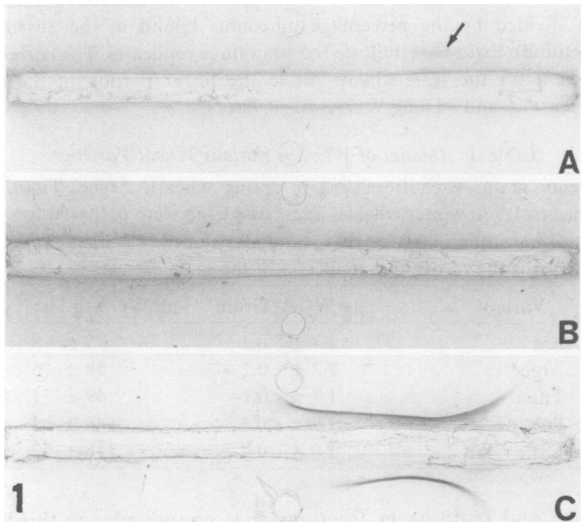

FIG. 1. Immunoelectrophoresis of purified WGA and crude wheat germ extract. GlcNAc (100 mM) was included in the gels in B and C. Purified WGA (~30 μg) was added to each upper well, and 30 μl of a wheat germ extract, obtained by homogenizing 5 g of wheat germ in 25 ml 0.05 N HCl followed by centrifugation and dialysis against distilled H_2O, was added to each lower well. After electrophoresis (as described in the text), either nonimmune (A and B) or immune (C) IgG fractions were added to the center trough and allowed to diffuse for 48 h at 4 C. The gels were stained with phenol red and dried (11) before being photographed through a green filter. Note that the diffuse precipitin band (in A; arrow) does not form in the presence of 100 mM GlcNAc (B).

Radioiodination by either method yielded [^{125}I]WGA with a specific activity of 10^8 to 10^9 cpm mg^{-1} WGA. The labeled lectin was found to be nonspecifically precipitated by nonimmune serum unless it was repurified after the iodination procedure by affinity chromatography. In addition, if affinity chromatography repurified [^{125}I]WGA was stored at 1 × 10^8 cpm ml^{-1} (the concentration of the lectin in the effluent of the affinity column), nonspecific precipitation would return within 7 days. However, we found that the labeled WGA would remain satisfactory for the RIA for at least 30 days if it was diluted 100-fold in PBS containing 0.25% BSA immediately after affinity column repurification and stored either at 4 or −20 C.

Radioactivity was determined by liquid scintillation counting in a 1:1 Triton X-100-toluene cocktail containing 6 g PPO and 0.25 g POPOP/liters. (Sigma).

Because the surveys in this study involved numerous samples, a rapid and efficient assay system was devised. It was found that, if large immunoprecipitates were formed, the supernatant fractions could be easily removed for counting without disturbing the pellet. This was accomplished by diluting the antiserum in nonimmune serum that had been diluted to an $A_{280}^{1\,cm}$ of 0.1.

The RIA protocol was as follows: to 1.5-ml polypropylene centrifuge tubes were added, in order, 10 μl PBS containing 4 mg ml^{-1} CH, 100 μl sample or standard, 250 μl nonimmune serum or 6,000-fold diluted antiserum, and 50 μl [^{125}I]WGA (1 ng μl^{-1}) in PBS containing 0.25% BSA. After incubation for 1 h at 4 C, 20 μl of 2-fold diluted goat antirabbit IgG was added. After storage at 4 C for 18 to 24 h, the immunoprecipitate was pelleted by centrifugation for 10 min in an Eppendorf microfuge, and 350 μl supernatant fraction were removed for counting.

Standard curves were performed for each experiment with samples assayed in triplicate. Unknowns were run at multiple dilutions so that at least two determinations occurred within the linear portion of the standard curve. The curves generated when crude extracts of each lectin-containing tissue were assayed at various dilutions were superimposable with curves generated with WGA purified from wheat germ.

Plant Growth Conditions and Tissue Preparation. Washed grain was imbibed for 8 to 10 h in distilled H_2O after surface sterilization in 15% bleach. Imbibed grain was placed on top of cheesecloth that had been stretched over a beaker maintained full with one-quarter-strength Hoagland solution. The grain was then germinated in a growth chamber maintained at 25 C on a 16:8 light-dark cycle. After 1 day, those seeds that had not germinated (less than 10%) were discarded.

After dissection, the tissue was ground to a fine powder in liquid N_2, homogenized in 0.05 N HCl, filtered through two layers of cheesecloth, centrifuged at 10,000g for 10 min, and dialyzed against PBS. For all tissues tested, homogenization in 0.05 N HCl solubilized more WGA than did similar homogenization in PBS, so this procedure was used routinely. HCl solubilizes approxi-

mately 50% of the protein as does PBS, so the values reported for WGA as percentage of total protein represent an overestimation of at least 50%.

Protein was estimated by the method of Bradford (5) using BSA as a standard.

RESULTS

Development of the RIA. A potential problem in developing an RIA for WGA was that the sugar-binding site on the lectin might interact with either GlcNAc or sialic acid (2, 10) that might be present on the immunoglobulins and thereby obscure the antibody-antigen interaction. Bouchard *et al.* (4) reported that, when WGA is allowed to diffuse against crude nonimmune rabbit serum, a diffuse precipitin band is formed. We found similar bands in both double diffusion and immunoelectrophoresis, but contrary to the results reported by Bouchard *et al.*, we were unable to prevent their formation by use of a partially purified immunoglobulin fraction instead of crude serum. Interference from sugar-lectin interactions was also found in immunoprecipitation. Using incubation conditions described under "Materials and Methods," we found nonimmune serum to precipitate 20% of the added [^{125}I]WGA. Inclusion of 40 μg CH in the incubation mixture decreased the level of nonspecific precipitation to 10%. Additional CH (up to 80 μg) did not result in a further decrease. At this level of nonspecific precipitation, however, a sufficiently sensitive and reproducible standard curve could be generated. Alternate means of removing this interference, such as treatment of the serum with neuraminidase, were not attempted.

When a standard curve is plotted (Fig. 2a) as percentage of [^{125}I]WGA precipitated *versus* the amount of unlabeled WGA added, a sigmoid curve results. To determine the WGA content of crude extracts as well as the assay variability, a linear transformation of the sigmoid curve, the logit function, was employed routinely to plot standard curves (Fig. 2b). An assay was repeated if the correlation coefficient for the linear least squares fit of the logit transformation was below 0.995. Samples to be directly compared were run in the same assay.

Evidence to support the claim that the antigenic activity detected by the RIA was in fact WGA was obtained in two ways. Leaf bases from 125 plants were prepared as described below. The extract obtained from this material was concentrated on an Amicon ultrafiltration apparatus fitted with a PM 10 filter so that the WGA could be detected by hemagglutination. Within the accuracy of the hemagglutination assay, the level of lectin estimated by the two methods was identical. A second check on the identity of the immunologically defined WGA was to pass crude extracts of leaf bases and roots through an affinity column for WGA before assay. This procedure removed 94% of the antigenically detectable lectin from leaf base extracts and reduced the levels of WGA in root extracts to below the sensitivity of the RIA.

An estimation of the efficiency of recovery of WGA during tissue preparation for the RIA was made by adding 200 ng [^{125}I]-WGA to the tissue just prior to homogenization. After filtration, centrifugation, and dialysis, 40 to 60% of the counts added to leaves, leaf bases, roots, or grain was recovered. Despite these results, lectin levels are reported uncorrected for yield. Since discussion of absolute amounts of lectin will depend only on order-of-magnitude differences, corrections based on yield would be insignificant.

Lectin Distribution in Dry and Imbibing Grain. A survey was made to determine whether there are major differences in the WGA content of various commercially available wheat varieties. The amount of lectin, expressed on either a per grain or per gram dry weight basis, is relatively constant among four of the varieties tested (Table I). Little Club, a variety maintained as a host for many fungal pathogens (*e.g.* 28), has somewhat higher levels.

To determine the location of WGA in dry and imbibing grain, embryo and endosperm fractions that contained one-third and two-thirds of the grain weight, respectively, were obtained. As shown in Table II, all of the lectin is located in the embryo portion. During the first 2 weeks of growth, small amounts of lectin (10–30 ng/plant) could be detected in pericarps and remaining endosperm which were removed from the plant axis with a forceps. Older pericarps yielded no lectin.

Because soybean lectin is released into the surrounding medium during early growth (9), we determined if similar release occurs in wheat. Two hundred grains were added to a 50-ml Erlenmeyer flask containing 20 ml distilled H_2O. The flask was shaken continuously at room temperature and the water replaced at the intervals

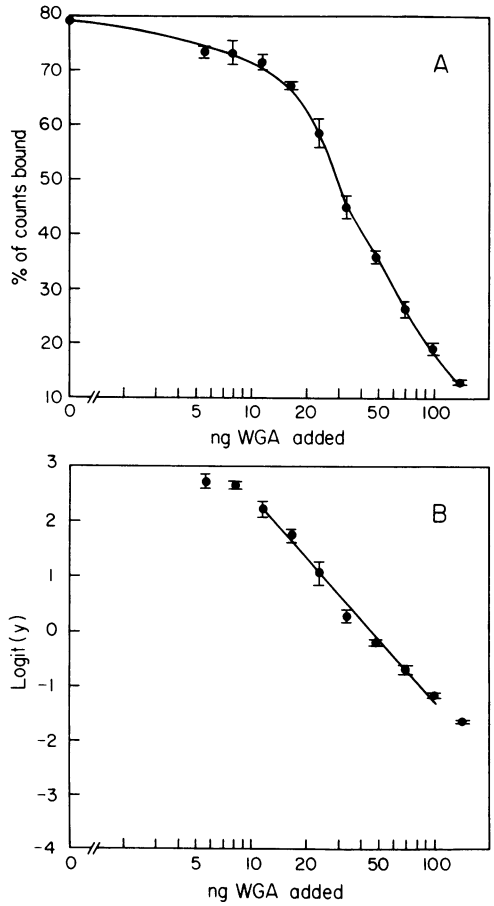

FIG. 2. Standard curves for the RIA plotted as (A) percentage of counts precipitated as a function of added, unlabeled WGA and as (B) logit (y) as a function of added, unlabeled WGA (logit [y] = ln y[1 − y]$^{-1}$, where y = percentage of counts bound in the presence of competing unlabeled WGA divided by the percentage of counts bound in the absence of competition). Error bars indicate the SD of three replicates. The correlation coefficient for the least squares fit to the linear portion of the curve (between 11.5 and 98.0 ng WGA) is −0.996.

Table I. *Amount of WGA in Various Wheat Varieties*

Twenty grains each from Era (a spring wheat), Argee, Titan, and Potomac (soft red winter wheats) and Little Club were prepared for assay by homogenization in 0.05 N HCl and dialysis of the clarified extract into PBS. Error is the SD of three replicates of the same extract.

Variety	μg WGA/Grain	μg WGA/g Dry Grain
Era	1.7 ± 0.2	75 ± 8
Argee	2.3 ± 0.2	58 ± 5
Titan	1.7 ± <0.1	49 ± 1
Potomac	1.9 ± <0.1	49 ± <1
Little Club	3.0 ± 0.4	118 ± 12

Table II. *WGA Distribution in Dry and Imbibed Grain*

Pooled embryo and endosperm fractions from 20 grains were obtained as described in the text and prepared for assay by homogenization in 0.05 N HCl, followed by dialysis of the clarified extract against PBS.

Sample	Age	ng WGA/Grain	WGA as % of Total Protein
	days		
Dry endosperm	0	<10	<0.02
Endosperm	1	<10	<0.02
Dry embryo	0	1,010 ± 40	1.2 ± 0.1
Embryo	1	870 ± 50	1.6 ± 0.1

Table III. *Release of WGA into Distilled H_2O during Imbibition*

Distilled H_2O in which 200 grains were imbibing was removed at the indicated times and concentrated for assay as described. Error represents the SD of three replicates of the same sample.

Time after Imbibition Begins	Protein Released per Grain	WGA Released per Grain	WGA as % of Total Protein
	μg	*ng*	
10 min	0.6	5.2 ± 0.2	1.0 ± <0.1
1 h	0.3	5.0 ± 0.5	1.8 ± 0.2
5 h	0.3	5.8 ± 0.5	1.7 ± 0.2
24 h	0.7	23.2 ± 2.0	3.4 ± 0.3

indicated in Table III. Prior to assay, the water was filtered through a glass fiber filter and concentrated to 2 ml on an Amicon ultrafiltration apparatus fitted with a PM 10 filter. Small amounts of lectin were recovered from the incubation medium during the first 24 h after the beginning of imbibition (Table III).

WGA Distribution during Early Growth. Since the observed pattern of lectin distribution is a function of the manner in which the plants are dissected, various preparation methods were tried before a satisfactory scheme was found. The trials demonstrated that after the first week of growth most of the lectin associated with the shoot could be extracted from the basal portion. Given this finding, plants were prepared for the time course study as follows: after the pericarp and endosperm were pulled from the plants with a forceps, the roots were cut where they emerged from the shoot and rinsed in distilled H_2O. Shoots of 3-day-old plants were harvested whole, whereas in older material, the shoot was cut 5 cm from its base, and the proximal and distal segments were pooled separately for assay. The proximal fraction, termed leaf base in Table IV, contained all of the meristematic tissue of the young shoot as well as some mature leaf tissue. The distal shoot fraction, defined as leaf apex in Table IV, contained only mature leaf tissue. The small size of the 3-day-old shoots (1 cm) precluded easy, mass dissection into meristematic and adult regions; however, because this fraction contained all of the intercalary meristems, it is included with the leaf base fraction in Table IV. After dissection all tissue was prepared for assay as described under "Materials and Methods."

At 34 days of growth, the plant still contains almost 50% of the levels of lectin found in the dry seeds, with approximately one-third in the roots and two-thirds in the shoot (Table IV). All of the lectin in the shoot appears in the basal fraction. Assayable levels of WGA never appeared in the leaf apices at any time during the time course. The limit of detectability per plant for this fraction increased with age due to the larger volume necessary to homogenize the increased amounts of leaf tissue. To ascertain an upper limit for the amount of lectin in the leaf apices, apices from 75 plants, 14 days old, were prepared for assay as described above, except that prior to assay, the extract was concentrated on an Amicon ultrafiltration apparatus fitted with a PM 10 filter. This extract, 10-fold more concentrated than those assayed for the time course study, yielded 5.9 ± 0.5 ng WGA/plant. The high level of protein in this concentrated extract might have introduced non-specific competition for antigenic sites in the RIA. Thus, the value obtained should be considered an upper limit rather than an absolute value for the level of WGA in leaf apices. We concluded, however, that less than 5% of the WGA in the shoot of 14-day-old plants is located in the apical portion.

Table IV. *Amount of WGA in Various Plant Parts during Early Growth*

For each time point, pooled tissue from 20 plants was prepared for assay as described. Error represents the SD of three replicates of the same sample.

Sample	Age	WGA per Plant	WGA as % of Total Protein
	days	*ng*	
Leaf apex	9	<30	<0.1
	17	<50	<0.1
	23	<75	<0.1
	34	<120	<0.1
Leaf base	3	346 ± 70	1.8 ± 0.4
	9	156 ± <10	0.8 ± <0.1
	17	255 ± 43	0.8 ± 0.1
	23	381 ± 40	0.9 ± 0.1
	34	317 ± 36	0.6 ± 0.1
Root	3	244 ± 53	2.7 ± 0.6
	9	221 ± 46	0.9 ± 0.2
	17	116 ± 17	0.5 ± 0.1
	23	63 ± 4	0.2 ± <0.1
	34	146 ± 7	0.2 ± <0.1

Table V. *Amount of WGA in Rapidly-growing, Recently-emerged Adventitious Roots and in Older Roots*

Root systems were dissected as described in the text and prepared for assay as described. Error represents SD of three replicates of the same sample.

Root Sample	Age of Plant	WGA per Plant	μg WGA/g Fresh Weight	WGA as % of Total Protein
	days	*ng*		
Old	47	21 ± 3	0.2 ± <0.1	0.1 ± <0.1
Young		59 ± 5	5.0 ± 0.4	1.4 ± 0.1
Old	51	15 ± 3	0.1 ± <0.1	0.1 ± <0.1
Young		24 ± 1	4.4 ± 0.2	2.9 ± 0.1

Distribution of Lectin in the Roots. Given the heterogeneous distribution of lectin in leaves, we sought to determine whether a similar heterogeneity exists in roots. Two methods of dividing root tissues were employed. First, roots from 80 plants, 18 days old, were cut from the shoot axes. The proximal 2 cm were separated from the distal 14 cm, yielding fractions with 23 and 77% of the fresh weight of the roots, respectively. Each fraction was found to contain 30 to 40 ng WGA/plant. Thus, on a fresh weight basis, there was a 3-fold enrichment in WGA in the proximal fraction.

These results might indicate that, unlike the shoots, the older tissue of roots is enriched in WGA. An alternate explanation, however, is that the proximal 2 cm contain young, rapidly growing adventitious roots as well as older root tissue, and it is these adventitious roots that are enriched in WGA. This hypothesis was tested using older plants. After 30 days of growth, recently emerged adventitious roots are easily distinguished from older roots. These younger roots, with larger diameters and more abundant root hairs, were easily separated out during the dissection of the root system. The data in Table V demonstrate that the younger roots contain two-thirds to three-quarters of the lectin in the root

system. When expressed on a fresh weight or protein basis, the younger roots are enriched in WGA by more than an order of magnitude.

To determine whether the root lectin might be localized on the cell surface or in cellular membranes, roots were washed and homogenized. The homogenates were fractionated by differential centrifugation. While attached to the plant axes, the roots from 50 plants, 14 days old, were soaked at 4 C for 10 min in four washes of alternating PBS and PBS with 10 mg ml^{-1} CH. Although PBS alone solubilized some WGA, the addition of CH to the wash buffer increased this amount 3-fold (Table VI). Therefore, some of the lectin is available for removal from intact roots and might be bound through its sugar-binding sites. The second set of washes yielded decreased amounts of lectin; however, when the roots were homogenized, a considerable proportion of the lectin was solubilized (Table VI). Thus, close to 50% of the lectin associated with the roots is not available for solubilization until the roots are disrupted. The difference in specific activity between the lectin in the washes and the lectin in the homogenate is striking. If broken cells at the root surface were the source of the WGA solubilized during the washes, the more than an order of magnitude difference in specific activity would not be expected. Thus, some of the lectin associated with the root may be secreted and exposed at the root surface.

DISCUSSION

Our findings demonstrate that the pattern of WGA distribution is markedly different from that of the legume lectins. This difference is prominent even in the dry grain and seed. WGA is present at approximately 1 μg/dry grain whereas soybean lectin, for instance, is present at mg levels per dry seed (24). When the 7-fold difference in dry weight is accounted for, the three orders of magnitude difference in lectin levels is still substantial. It is interesting to note that, even though they are not embryologically homologous, the storage organ in wheat, the endosperm (which is not part of the embryo), is virtually devoid of WGA, whereas the storage organ in soybean, the cotyledon (which is part of the embryo), contains the highest levels of lectin in the seed (24).

After germination, the levels of WGA per wheat plant remain high for at least 34 days. In contrast, many legume lectins (*Phaseolus vulgaris* (19), *Dolichos biflorus* (29), *Lens culinaris* (15), *Arachis hypogaea* (23), *Pisum sativum* (26)) diminish in quantity to below the level of detectability during the first few weeks of growth. Material that cross-reacts with antibody to the seed lectin has been identified in leaves of *Phaseolous* (19) and *Dolichos* (29), but in neither case does the cross-reactive material possess the sugar-binding activity of the seed lectin.

In our study, lectin was present in both root and shoot preparations. Functional lectin has also been identified in adult roots of two legumes. Using fluorescent-labeled antibodies, Dazzo and Brill (7) showed that lectin is present on root hairs of clover; Hapner and Robbins (14) purified a lectin from 60-day-old sainfoin roots whose sugar-binding activity, mol wt, amino acid composition, and carbohydrate composition were identical to the lectin purified from the seeds of the same plant. Hamblin and Kent (13) provided indirect evidence for the presence of lectin in roots by showing that type A red blood cells bind to specific regions of *Phaseolus* roots. In wheat, the lectin detected in adult tissue possesses hemagglutinating and sugar-binding activity similar to that of the lectin purified from the dry grain. The CH-inhibitable hemagglutinating activity of the concentrated leaf-base extract and the ability of chitin affinity columns to remove antigenic activity from leaf-base and root extracts indicate that the material detected by RIA is structurally and functionally similar to the lectin isolated from wheat germ. It was recently reported that small quantities of lectin that both agglutinate red blood cells and cross-react with antibodies to the seed lectin were found in the roots of a nonlegume, *Datura stramonium* (16). Thus, roots as well as seeds may prove to be a common location for many lectins.

A striking aspect of the localization pattern is the association of WGA almost exclusively with rapidly growing tissue. Thirty-day-old plants possess lectin mainly in the basal portion of the shoot, a region that contains the basal intercalary meristems, and in rapidly growing adventitious roots. Immunocytochemical localization demonstrates that most of the WGA in the shoot region is associated with adventitious roots that have not yet emerged from the plant axis (Mishkind, Palevitz, and Keegstra, manuscript in preparation).

The observation that WGA is released during germination and that it can be washed from root surfaces suggests its presence in the rhizosphere of young plants. Our observation that CH is able to facilitate removal of WGA from root surfaces is similar to the finding of Dazzo and Brill (7) that 2-deoxyglucose, the sugar-inhibitor of clover lectin, can wash that lectin from clover roots. Whereas clover lectin is thought to function as a receptor for symbiotic rhizobia, we suggest that WGA in the rhizosphere of wheat may act as a fungastatic agent in accordance with the observation that it inhibits the elongation of fungal hyphae (20). It may also function in a defense system against fungal pathogens along with the chitinase recently purified from wheat germ (21).

Table VI. *Amounts of WGA Solubilized from Roots by Surface Washing and Differential Centrifugation of the Homogenate Obtained from the Washed Roots*

Roots were washed as described in the text, with the second and fourth wash containing 10 mg ml^{-1} CH. Washed roots were homogenized as described under "Materials and Methods" except that PBS was used instead of 0.05 N HCl. Error represents SD of three replicates of the same sample.

Fraction	Protein per plant	WGA per plant	% of Total WGA	WGA as % of Total Protein
	μg	ng		
Wash 1 (PBS)	0.1	13 ± 1	38	11.0 ± <0.2
Wash 2 (PBS + CH)	0.1	40 ± 3		40.0 ± 3.5
Wash 3 (PBS)	ND[a]	20 ± 1		
Wash 4 (PBS + CH)	ND	14 ± 1		
Cheesecloth particulate	5.0	22 ± 1	10	0.4 ± <0.1
4,000*g* pellet	0.9	7 ± <1	3	0.8 ± <0.1
100,000*g* pellet	0.9	6 ± <1	2	0.6 ± <0.1
PBS-soluble	40.8	109 ± 4	47	0.3 ± <0.1

[a] ND, none detected.

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