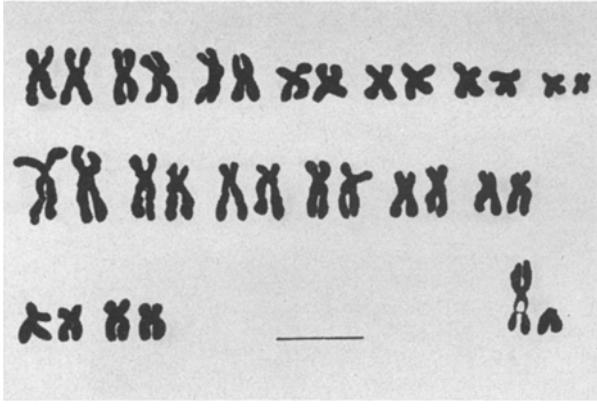
respectively, but because no females and only 1 male was examined, this identification is only tentative. The fundamental number of autosomal arms (FN) is 60.

Only 2 subgenera of ground squirrels include species that have a diploid number of 32 and an FN of 60. These are *Spermophilus* (*Ictidomys*) *spilosoma*, which also has 14 metacentric and 16 submetacentric autosomes[8], *S.* (*Spermophilus*) *columbianus*, which has 18 metacentric and 12 submetacentric autosomes[9], and *S.* (*Spermophilus*) *undulatus*, whose karyotype VORONTSOV and LYAPUNOVA[10] described as similar to that of *S. columbianus*. *Spermophilus spilosoma* and *S. columbianus* have minute *Y* chromosomes, whereas this element is a larger acrocentric in *S. undulatus* and *S. adocetus*. Both species of otospermophiles, *S. beecheyi* and *S. variegatus*, that have been examined[4,11] are characterized by a diploid number of 38, which consists of 22 metacentric autosomes, 14 submetacentric autosomes, a metacentric *X* chromosome, and an acrocentric *Y* chromosome. Therefore, the autosomal portion of the karyotype of *S. adocetus* is most like that of *S. spilosoma*, similar to that of *S. columbianus* and *S. undulatus*, but unlike that of members of the subgenus *Otospermophilus*. The *Y* chromosome of *S. adocetus* is simliar to that of *Otospermophilus* and *S. undulatus* but dissimilar from that of *S. spilosoma* and *S. columbianus*. However, the other two species in the subgenus *Ictidomys* (*S. mexicanus* and *S. tridecemlineatus*) and many other species of the subgenus *Spermophilus* are characterized by acrocentric *Y* chromosomes[8,9].



Karyotype of a male *Spermophilus adocetus adocetus* from Xalitla, Guerrero. The line represents a scale 10 μm in length.

Neither BRYANT[3] nor BLACK[12] considered members of the subgenus *Otospermophilus* to be closely related to those of either *Spermophilus* or *Ictidomys* on the basis of morphological data and the fossil record, and we do not suggest that here. However, although BRYANT[3] did not consider ground squirrels of the subgenera *Otospermophilus* and *Notocitellus* sufficiently different to warrant separate subgeneric recognition, we have found that the karyotype of *S. adocetus* is more like that of species within the subgenera *Ictidomys* and *Spermophilus* than like that of members of the subgenus *Otospermophilus*.

The interspecific relationships of *S. adocetus* should be reevaluated. BRYANT[3] listed examples of *S. annulatus* in several sections of specimens examined, but nowhere can we find reference to specimens of *S. adocetus* that were included in his study. Perhaps *S. adocetus* is not closely related to *S. annulatus* and should be placed in the subgenus *Ictidomys*, or less likely in the subgenus *Spermophilus*, or possibly both species should be placed in one of these subgenera. Other possibilities that might explain the incongruities observed have occurred to us, but additional speculation without knowing the karyotype of *S. annulatus* would seem premature.

Resumen. En este trabajo se encontró que el cariotipo de *Spermophilus adocetus* está compuesto de 32 cromosomas que pueden dividirse en 14 autosomas metacéntricos, 16 autosomas submetacéntricos, un cromosoma *X* grande metacéntrico, y un cromosoma *Y* pequeño acrocéntrico. *Spermophilus adocetus* esta en la actualidad colocado en el subgénero *Otospermophilus* pero su cariotipo indica una relación con el subgénero *Ictidomys* o posiblemente con el subgénero *Spermophilus*.

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[8] C. F. NADLER and C. E. HUGHES, J. Mammal. *47*, 46 (1966).
[9] C. F. NADLER, J. Mammal. *47*, 579 (1966).
[10] N. N. VORONTSOV and E. A. LYAPUNOVA, Dokl. biol. Sci. *187*, 644 (1969).
[11] C. F. NADLER and D. A. SUTTON, Proc. Soc. exp. Biol. Med. *110*, 36 (1962).
[12] C. C. BLACK, Bull. Mus. comp. Zool., Harvard Univ. *130*, 109 (1963).

The Localization of Water-Soluble Proteins in the Wheat Endosperm as Revealed by Fluorescent Antibody Techniques

Extraction experiments[1] and amino acid analyses[2] have suggested that a high proportion of the water-soluble, cytoplasmic, or non-gluten-forming proteins are associated with the starch granules in the mature wheat endosperm cell. The in situ demonstration of the distribution of this group of proteins is of interest and possible importance in relation to the vitreosity and milling properties of the grain. We now report the successful application of fluorescent antibody techniques[3,4] to this problem.

Antisera were prepared by injection of dialyzed and freeze-dried aqueous buffer extracts (pyrophosphate, 0.01 *M*; pH 7.0) of wheat flour (cv. Timgalen) into New Zealand white rabbits[5]. The primary immunizing dose (10 mg) was emulsified with Freund's complete adjuvant and given i.m., while boosting doses (10 mg) in buffered saline (0.02 *M* phosphate buffer, 0.15 *M* NaCl, pH 7.2)

[1] R. W. JONES and R. J. DIMLER, Cereal Chem. *39*, 336 (1962).
[2] D. J. STEVENS, E. E. MCDERMOTT and J. PACE, J. Sci. Fd. Agric. *14*, 284 (1963).
[3] A. H. COONS, H. J. CREECH, R. N. JONES and E. BERLINER, J. Immun. *45*, 159 (1942).
[4] R. C. NAIRN, *Fluorescent Protein Tracing* (E. and S. Livingstone Ltd., Edinburgh, London 1969), 3rd Ed.
[5] P. G. H. GELL and R. R. A. COOMBS *Clinical Aspects of Immunology* (Blackwell, Oxford 1963).

were given i.v. The animals were bled 7 and 9 days after the last injection. The resulting antisera were tested by micro-immunoelectrophoresis[6] and double immunodiffusion[7] and were shown to contain antibodies with specificities for water-soluble wheat proteins.

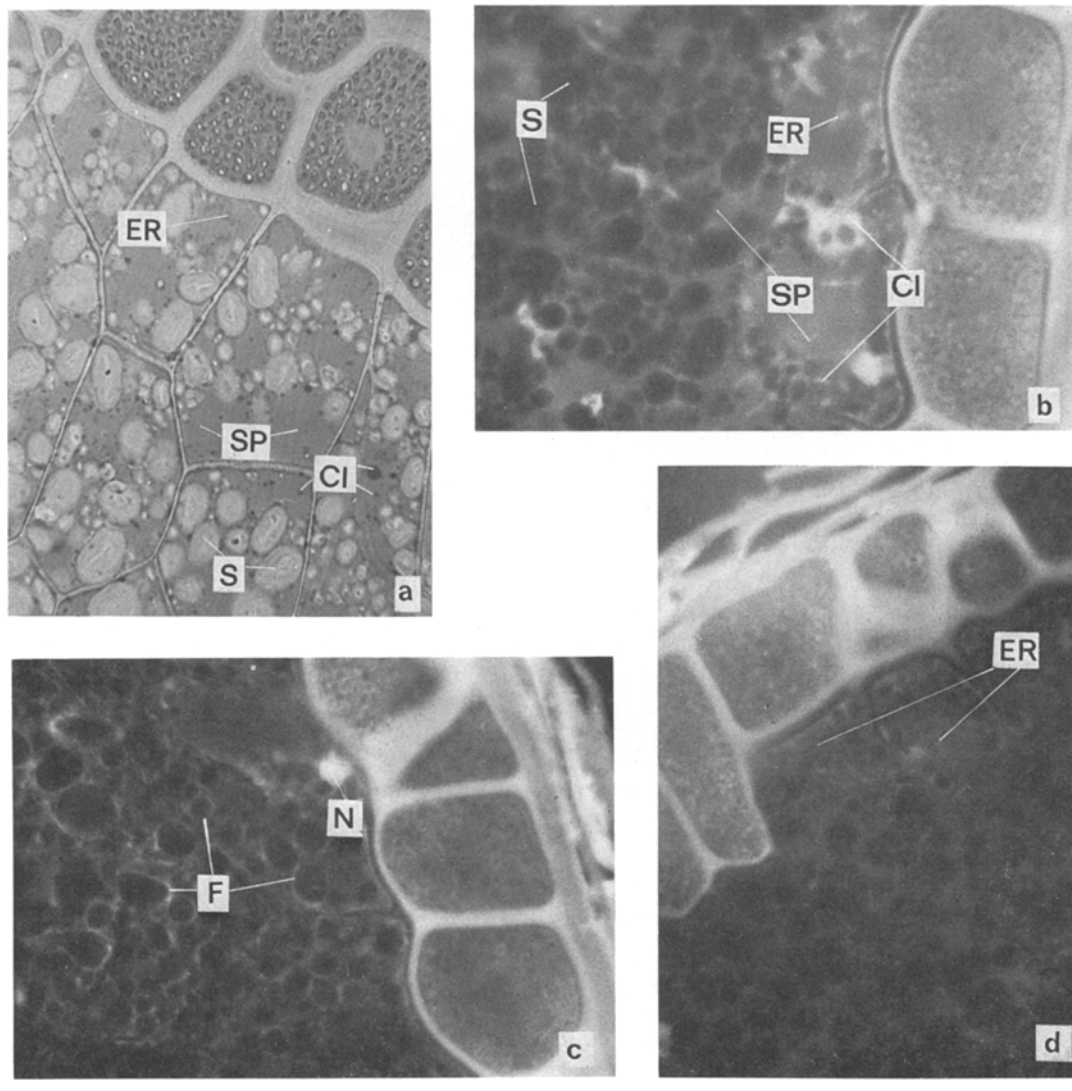
Slices of Timgalen endosperm with aleurone cells attached were fixed for 12 h in glutaraldehyde (3% in 0.025 *M* phosphate buffer, pH 6.8), and washed for 24 h in the same buffer before being frozen for sectioning on the cryostat microtome. Sections (6 µm thick) were cut and allowed to stand overnight prior to fluorescent antibody (FA) staining. This was carried out by incubating with the rabbit antisera at room temperature for 60 min. After washing in phosphate buffered saline (PBS; 7.1) for 60 min the sections were incubated with fluorescein-conjugated sheep anti-rabbit globulin for 60 min. They were then washed for 60 min. in PBS and rinsed in distilled water, after which they were allowed to air dry before being mounted in phosphate-buffered glycerol (pH 8.6). They were viewed under a Zeiss fluorescence microscope using primary filter UG 1 and secondary filter 41.

Non-specific staining was limited as much as possible, by absorbing both antiserum and conjugate with a liver homogenate. The Table summarizes the controls used in the experiment to establish the specificity of staining.

In each case, the results were unequivocal and are illustrated in Figure 1.

The appearance of starch and protein (both water-soluble and storage types) in the mature wheat endosperm cell, after staining with fast green, is illustrated in the photomicrograph of Figure 1a. This also shows the appearance of various cellular inclusions under these conditions. These organelle remnants show a bright yellow autofluorescence, storage protein a deep blue, while the starch granules are non-fluorescent under ultraviolet light. The aleurone cell walls show a very bright blue autofluorescence which is particularly difficult to quench through the filter system used. This is illustrated in Figure 1b. The

[6] J. J. SCHEIDEGGER, Int. Archs Allergy appl. Immun. *7*, 103 (1955).
[7] O. OUCHTERLONY, Prog. Allergy *6*, 30 (1962).



Localization of water-soluble proteins in wheat endosperm (cv. Timgalen) by FA staining. a) Transverse section stained with fast green. b) Autofluorescence observed through exciter filter UG 1 and barrier filter 41. c) Specific serum-labelled antibody treated section. Green fluorescence (F) is observed around each starch granule. d) Control Section. Storage protein (SP), starch granules (S), nuclear remnant (N), endoplasmic reticulum (ER), and various cellular inclusions (CI).

autofluorescence of these cellular components may be clearly distinguished under the microscope, or in color photographs, from the pale green fluorescence due to the labelled antibody-antigen complex. The latter, as shown in Figure 1c, occurs specifically around each starch granule, thus demonstrating the presence of water-soluble proteins in high concentration in this area. The cross sections used as controls (see Table), emitted no fluorescence other than that due to autofluorescence of the grain, as shown in Figure 1d.

Controls used for establishing specificity of staining by the indirect FA procedure

Stain A	Stain B	Result
Saline	Labelled antibody	No green fluorescence
Non-immune serum	Labelled antibody	No green fluorescence
Pre-immune serum	Labelled antibody	No green fluorescence
Specific serum	Labelled antibody	Green fluorescence located around each starch granule
Specific serum absorbed with antigen	Labelled antibody	Green fluorescence markedly reduced

The proteins localized by this technique are probably enzymes associated with starch granule synthesis in the developing grain. It is likely that residues of similar water-soluble, enzymically active proteins, originally associated with storage protein synthesis are located in the matrix between the starch granules. The fact that they have not been detected does not exclude their presence, but suggests that they occur in much lower concentration than those surrounding the starch granules.

Zusammenfassung. Die Anreicherung wasserlöslicher Proteine in Endospermzellen von Weizenkörnern, in der die Stärkegranula umgebenden Zone, konnten mittels der Fluoreszenz-Antikörper-Technik nachgewiesen werden.

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Glycine Seed Germination: Differential Response to Abscisic Acid

The naturally occurring plant hormone, abscisic acid (ABA), has been shown to be a potent inhibitor of numerous growth processes[1,2]. ABA is particularly inhibitory to germination of seeds of many species[2]. Contrary to the usual inhibitory effects, ABA has also been shown to promote several growth phenomena such as soybean cotyledon callus in the presence of kinetin[3], rooting of mung bean cuttings[5] and promotion of callus formation in citrus bud culture[4]. We now report on the differential seed germination responses of several *Glycine max* (L.) Merr. cultivars to ABA.

Methods. Seeds were imbibed in solutions of ABA for 16 h, transferred to water saturated vermiculite and germinated in the dark at 25 ± 1 °C. For each of 3 experiments 5 dishes containing 10 seeds each were used for each treatment and replicated 5 times. Percentage germination was determined at 24-h-intervals and the data expressed as Σ_3 values according to TIMSON[6].

Results. A differential response to ABA was observed. Seed germination was inhibited in 'Hood' and no significant effect was observed in 'Perry' (Table). In contrast, ABA stimulated seed germination in 'Bragg' at concentrations of $1.5\times10^{-7}M$ to $1.5\times10^{-5}M$ and did not inhibit germination at $1.5\times10^{-4}M$. ABA stimulation of seed germination in 'Bragg' at $1.5\times10^{-6}M$ equalled that observed with gibberellin (GA_3) at $1.5\times10^{-5}M$. ABA did not reverse cycloheximide (2 µg/ml) induced inhibition. Differences in germination cannot be attributed to differential water or ABA absorption since there were no differences in uptake of water or ^{14}C labelled ABA among the 3 cultivars during the imbibition period.

Differential response of *Glycine* seed to abscisic acid

Concentration (M)	'Bragg' Σ_3 [a]	% [b]	'Hood' Σ_3	%	'Perry' Σ_3	%
0	122	100	186	100	260	100
1.5×10^{-7}	220	180	110	59	272	105
1.5×10^{-6}	200	164	110	59	256	98
1.5×10^{-5}	168	138	116	62	248	95
1.5×10^{-4}	135	111	52	28	212	81

[a] Germination index according to TIMSON[6]. [b] Percent of control.

Discussion. SLOGER and CALDWELL[7] demonstrated differential sensitivity of *Glycine* cultivars to foliar applied ABA based on inhibition of leaf expansion, shoot extension and induced leaf senescence. We also have found a differential cultivar response to ABA based on seed germination, however, there was not always a strict relationship between the effects of ABA on seed germination and the reported growth effects[7]. While 'Hood' and 'Perry' were considered sensitive to foliar-applied ABA[7], seed germination was not affected in 'Perry' but was inhibited in 'Hood'. Of the non-responsive cultivars, Bragg, Clark, Kent, Semmes[7], ABA promoted seed germination only in 'Bragg'. To our knowledge this is the first report of ABA promotion of seed germination. Our data and those of SLOGER and CALDWELL[7] emphasize the difficulty in extrapolating ABA data from one cultivar to another, and further that the nature of the response for a given cultivar may be related to the parameter being observed[8].

Zusammenfassung. In Keimen von Glycinensamen wurden unterschiedliche Reaktionen der Kulturvarianten (Cultivaren) zur Abscisinsäure (ABA) beobachtet. Die Unterschiede im Keimem können nicht der unterschiedlichen Wasser- oder ABA-Absorption zugeschrieben werden.

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[1] F. T. ADDICOTT and J. L. LYON, A. Rev. Pl. Physiol. *20*, 139 (1969).
[2] B. V. MILBORROW, Sci. Prog., Lond. *57*, 533 (1969).
[3] A. BLUMENFELD and S. GAZIT, Pl. Physiol. *45*, 535 (1970).
[4] A. ALTMAN and R. GOREN, Pl. Physiol. *47*, 848 (1971).
[5] C. TING-YAN, H. M. MEYER and L. BEEVERS, Planta *88*, 192 (1969).
[6] J. TIMPSON, Nature, Lond. *207*, 216 (1965).
[7] C. SLOGER and B. E. CADWELL, Pl. Physiol. *46*, 634 (1970).
[8] We thank Dr. E. E. HARTWIG, U.S.D.A., A.R.S., Stoneville, Miss. for the gift of *Glycine* seed. Journal Article No. 5929 from the Michigan Agricultural Experiment Station.