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NADH Nitrate Reductase and NAD(P)H Nitrate Reductase in Genetic Variants and Regenerating Callus of Maize

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ABSTRACT

Different organs of maize seedlings are known to contain different complements of NADH and NAD(P)H nitrate reductase (NR) activity. The study of the genetic programming that gives rise to such differences can be initiated by looking for genetic variants exhibiting different patterns of distribution of the above enzymes. We demonstrate in this work that scutella of very young maize seedlings contain NADH NR almost exclusively and that this activity is gradually replaced, as the seedling ages, with NAD(P)H NR. Leaves in the seedlings contain exclusively the NADH NR activity. A genetic variant is described that contains much reduced levels of NAD(P)H NR activity but not of NADH NR activity in the scutellum. This same variant exhibits a relatively low level of NAD(P)H NR but normal NADH NR activity in seedling root tips. These observations suggest that the genetic program used to specify the scutellar complement of NR activity shares some common components with the genetic program used to determine the young root tip complement of NR activities. Parts of regenerating callus at different stages of differentiation were examined to determine when the differences in NR complement begin to appear. The same pattern of NADH NR and NAD(P)H NR activities was found in unorganized as well as in organized callus, in recognizable root-like and even in green shoot-like material, both activities being present in all these tissues. An examination of the NR complement in different organs of a number of siblings originating from a cross involving transposon Mu-containing parents and having different levels of leaf NADH NR activity shows that the leaf NADH NR activity content and the scutellum NAD(P)H NR activity content are relatively independent of each other, indicating that the genetic programs specifying the NR content of these organs are not tightly coupled, if at all.

Higher plants generally have more than one type of nitrate reductase (3). Dailey *et al.* (2) have demonstrated that there is a NADH and a NAD(P)H NR[1] in barley, the former being prevalent in leaves, the latter being found in leaves of mutants deficient in NADH NR activity. Shen *et al.* (9) found that nitrate induced a NADH NR in young rice seedlings, whereas chloramphenicol induced a NADPH NR activity. Orihuel-Iranzo and Campbell (6) observed both NADH and NADPH NR in soybean cotyledons, the latter activity predominating in older seedlings. Streit *et al.* (10) found three different forms of NR activity in soybeans, two that were constitutive and one that was inducible. One of the three used NADPH preferentially as electron donor.

Maize is no exception to the pattern just described. Campbell (1) found that scutella contained both NADH and NAD(P)H activities, the latter using NADPH preferentially as electron donor, whereas leaves had no NAD(P)H NR activity. Redinbaugh and Campbell (7) examined young maize roots and found both NADH and NAD(P)H NR activities. On the basis of similarities in electron donor specificity, pH optimum, Stoke's radius, and K_m for nitrate, these investigators concluded that the root NADH activity was identical with the leaf activity. Comparisons with NR activities in the scutellum were incomplete and revealed differences that were hard to interpret.

Wells and Hageman (12) pointed out that phosphatases can complicate studies on the pyridine nucleotide specificity of NR because they can catalyze the conversion of NADPH to NADH. When studying NADH and NAD(P)H NR it is therefore necessary either to separate the enzymes physically, purify them away from contaminating phosphatases, inactivate the phosphatases, or continuously eliminate NADH from a NADPH dependent assay, in order to interpret the results in terms of the NADH or the NAD(P)H specific enzyme activity.

In this study we show that scutella in very young maize seedlings have chiefly a NADH NR, and that this activity is gradually replaced almost entirely by an NAD(P)H NR activity. The latter enzyme is present in relatively low concentration in scutellar extracts of a genetic variant of maize. This same variant also has a relatively low NAD(P)H NR activity in extracts of root tips, suggesting a genetic coupling between the corresponding root and scutellum activities.

Maize callus contains both NADH and NAD(P)H NR activities. During the early stages of differentiation of organs from the callus, there does not seem to be any dramatic change in the relative tissue content of NADH and NAD(P)H NR.

MATERIALS AND METHODS

Plant Material. Maize inbreds H99 and W182BN were from the collection of Dr. E. Earle (Department of Plant Breeding, Cornell University, Ithaca, NY). Strain 905 comes from a line that is homozygous for purple aleurone, that contains transposon Mu, and is maintained by repeated backcrossing to a standard inbred in Dr. D. Robertson's laboratory (Department of Genetics, Iowa State University, Ames, IA). The stock (905) used in this investigation is seed from a selfing of the above line. Except for all seed being homozygous purple aleurone, the line has no known genetic defects.

Strain 609 comes from a Mu-containing line from Dr. Robertson's laboratory that has been maintained by repeated backcrossing (for more than 8 generations) to a standard inbred from the same laboratory, designated Q60. It has no known genetic markers. The stock used in this work is seed from a selfing of progeny from one of the above backcrosses to Q60. Strains Ha, like 609 and 905, come from a Mu-containing line that is maintained in Dr. Robertson's laboratory by repeated backcross-

[1] Abbreviations: NR, nitrate reductase; FAD, flavin adenine dinucleotide; MDH, malic dehydrogenase.

ing to Q60.

The progeny from such a backcross were selfed and the seed from one such selfing planted in our greenhouse and screened for leaf NADH NR activity. One of the seedlings that had very low leaf NADH NR activity was grown to maturity and selfed. The progeny was selfed once more in Hawaii by Dr. D. Walden (Department of Plant Sciences, University of Western Ontario, London, Ontario). The resulting seed is called Ha by us. There are no other known genetic markers in this strain.

Growth Conditions. Seeds were planted in a vermiculite and sand mixture (1:1 w/w) and watered with 1/10 Hoagland solution containing 4 g/L ammonium tartrate as nitrogen source. Seedlings were grown in an incubator at 28°C with a 16 h light and 8 h darkness schedule at approximately 200 μE m^{-2} s^{-1} for the desired time and then preinduced, 18.5 h before extraction, by watering with 1/10 Hoagland solution containing 4 g/L ammonium nitrate as nitrogen source. The seedlings were finally induced for 2.5 h prior to extraction by watering with 1/10 Hoagland solution containing 5 g/L potassium nitrate as nitrogen source.

Tissue Culture. Maize tissue cultures were derived from the scutellum of 1.0 to 2.0 mm immature embryos, taken from ears harvested 14 d after pollination. Ears were surface-sterilized in 30% Clorox + 0.5% PEX detergent. Embryos were cultured, scutellar side up, in the dark at 25°C, on medium containing MS (4) salts, 0.4 mg/L thiamine, 100 mg/L *myo*-inositol, 3% sucrose, 5 mg/L 2, 4-D and sometimes 20 mM L-proline, solidified with 0.22% Gel-Rite (Kelco, San Diego, CA). Organized growth from the scutellum was excised and subcultured. The W182BN culture used in this study had been grown without proline for 2 years; it had a mixed morphology, with some embryogenic areas and tiny leaves. The 12 H99 cultures used had been grown for 10 weeks on medium containing proline. These cultures consisted of unorganized callus with superficial organized (mostly embryogenic) areas.

Extraction and Assays. Leaves and root tips were severed from the seedlings with scissors, washed with H_2O, and ground in an ice-cold mortar and pestle with extraction buffer and alumina as abrasive (0.5 ml buffer per 4–6 cm of leaf tip or first leaf; 0.15–0.20 ml buffer per scutellum; 0.25 ml buffer per 10 root tips).The brei was then centrifuged in a microfuge for 10 min and the supernatant assayed immediately in the case of NR activity, or frozen at −18°C to be assayed later for malate dehydrogenase activity. Scutella were dissected away from the rest of the seed and extracted and treated as the other organs. The extraction buffer was a modified version of that of Dailey *et al.* (2) and contained 0.25 M Tris HCl (pH 8.5), 1 μM Na_2MoO_4, 1 mM Na_2EDTA, 0.3 mM DTT, 2% (w/v) casein (Hammerstein, BDH) and 0.2% (v/v) ethanol saturated with phenylthiocarbamide, to prevent polyphenol oxidase from tanning the extract proteins.

NR activity was assayed in a reaction mixture containing 0.14 M K-phosphate (pH 7.4), 30 mM potassium nitrate, 1.4 μM FAD, and either 0.2 mM NADH or 0.2 mM NADPH plus 1.4 mM oxaloacetic acid and cell free extract, in a final volume of 0.45 ml. The reaction was incubated for 1 h at 28°C as described by Dailey *et al.* (2). One unit of activity is defined as the production

Table I. *NADH and NADPH Nitrate Reductase in Seedling Organs*

Seedlings were grown as described in "Materials and Methods" (in the light) or covered with aluminum foil (in the dark). Dissections, extractions, and assays were as described in "Materials and Methods." Each value in the table is the average of two measurements with at least 90% agreement, except for NR activities below 0.02, where agreement was approximately 50–70%.

Organ	Line	Seedling Treatment[a]	NADH-NR nmol/h·μl[b] (1)	(1)/(3)	NADPH-NR nmol/h·μl (2)	(2)/(3)	MDH μmol/h·μl (3)	(1)/(2)
Leaf	W182BN	(8 d L)	3.3	110.0	0	0	0.03	
		(8 d D)	0.5	45.0	0	0	0.03	
Leaf	H99	(8 d L)	2.0	33.0	0.22	3.7	0.06	9.1
		(8 d D)	0.3	10.0	0.05	1.2	0.04	8.0
Leaf	609	(8 d L)	2.0	40.0	0	0	0.05	
		(8 d D)	0.3	6.0	0	0	0.05	
Leaf	905	(8 d L)	1.4	47.0	0	0	0.03	
		(8 d D)	0.5	10.0	0	0	0.05	
Scutellum	W182BN	(8 d L)	3.5	1.30	3.5	1.30	2.7	1.0
		(8 d D)	3.7	1.37	4.1	1.50	2.7	0.9
		(3 d L)	2.4	0.83	1.9	0.66	2.9	1.3
Scutellum	H99	(8 d L)	1.1	0.46	0.8	0.33	2.4	1.4
		(8 d D)	0.7	0.29	0.5	0.21	2.4	1.4
		(3 d L)	1.9	0.79	1.3	0.54	2.4	1.5
Scutellum	609	(8 d L)	1.5	1.00	0.4	0.26	1.5	3.7
		(8 d D)	0.8	0.57	0.4	0.28	1.4	2.0
		(3 d L)	2.8	1.03	0.5	0.18	2.7	5.6
Scutellum	905	(8 d L)	1.7	1.31	1.7	1.31	1.3	1.0
		(8 d D)	2.9	1.61	3.3	1.83	1.8	0.9
		(3 d L)	2.9	1.16	2.1	0.84	2.5	1.4
Root	W182BN	(3 d L)	0.058	1.9	0.033	1.0	0.03	1.7
Root	H99	(3 d L)	0.047	2.3	0.022	1.1	0.02	2.1
Root	609	(3 d L)	0.044	0.7	0.011	0.2	0.06	4.0
Root	905	(3 d L)	0.072	2.4	0.050	1.8	0.03	1.4

[a] d L, number of days in light; d D, number of days in darkness. [b] μl extract.

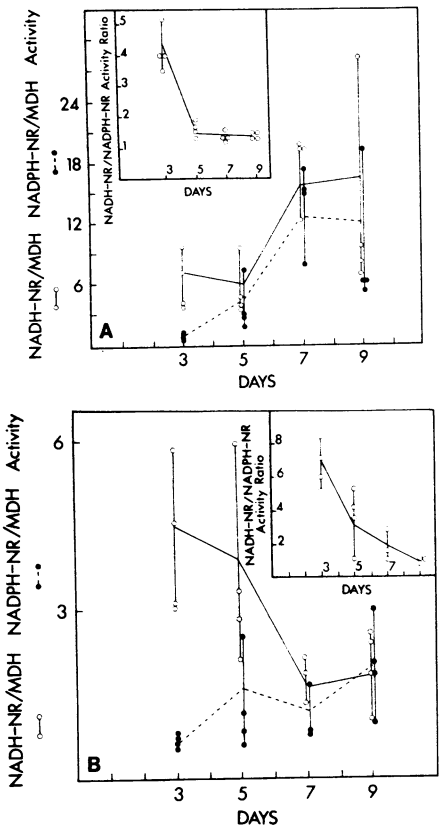

FIG. 1. Relative activities of NADH and NADPH nitrate reductase in scutella of different ages. Scutella were dissected away from nitrate-induced seedlings, extracted and assayed for NADH and NADPH nitrate reductase and malic acid dehydrogenase activity as described in "Materials and Methods." Each point represents a duplicate sample of an extract of a single scutellum. Agreement between measurements was within 10% except at levels of NR below 0.1. A, line 905; B, line 609.

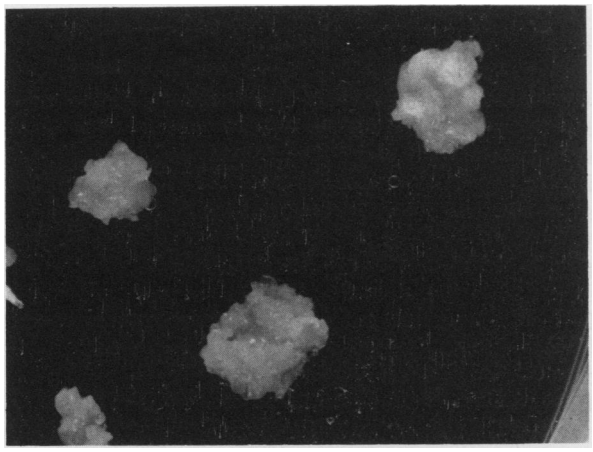

FIG. 2. 'Unorganized' sectors of H99 callus culture 123, grown for 11 d on medium lacking 2,4-D. This tissue looked similar on medium containing 5 mg/L 2,4-D (×2).

of 1 nanomol nitrite/h in the above assay. Because there was so much casein in the extraction buffer it was considered meaningless to express the NR specific activity as units/mg protein. Instead, the activity is expressed as a function of the concentration of malic acid dehydrogenase activity in the same extract, *i.e.* the 'relative activity' being the number of units of NR per μl of extract divided by the number of units of malic acid dehydrogenase per μl of extract.

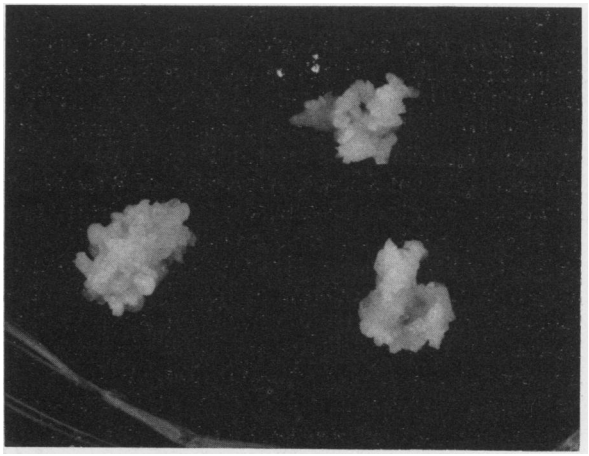

FIG. 3. 'Organized' sectors of H99 culture 123 grown for 11 d on medium containing 5 mg/L 2,4-D (×2).

FIG. 4. 'Organized' sectors of H99 culture 123 grown for 11 d on medium lacking 2,4-D. Note roots and white scutellar like-areas (×2).

Malic acid dehydrogenase was measured by a modified version of the procedure of Thorne and Kaplan (11). One to 3 μl of undiluted or diluted extract was added to a 0.90 ml reaction mixture containing 0.1 M K-phosphate (pH 7.4), 0.125 mM NADH and 1 mM oxaloacetic acid and the decrease in A at 340 nm followed with time in a Perkin Elmer recording spectrophotometer, in a water jacketed cuvette kept at 30°C. The decrease in absorbance was calibrated with a standard curve of NADH. One unit of activity is defined as the oxidation of 1 μmol NADH/min in the above assay.

RESULTS

Initial observations confirmed Campbell's report (1) that leaves and scutella had different complements of NADH and NAD(P)H NR activity. The following procedure was devised to avoid the problem of phosphatases (12): 1.5 mM oxaloacetate was added to the NAD(P)H NR assay mixture to remove NADH as it was formed. Within 1 min more than 1.5 mM NADH was removed from the assay mixture by endogenous malic acid dehydrogenase; the assay on the other hand lasted 1 h.

NADH and NAD(P)H NR Activities in Different Seedling Organs. Using the assays described in "Materials and Methods" and above, the NR activity content of different organs of seedlings from four different lines of maize was measured. The results in Table I show that leaves contain little or no NADPH NR in all but inbred H99, which contain a very small amount of the enzyme activity. All lines had relatively high levels of leaf NADH

Table II. *NADH and NADPH Nitrate Reductase in Callus and Regenerating Tissue*

Tissue was grown, induced, extracted, and assayed for enzyme activities as described in "Materials and Methods." Each value is the average of a duplicate sample with at least 90% agreement, except for nitrate reductase activities below 0.1 nmol/h, where agreement was between 50 and 70%.

Experiment No.	Line	Type of Callus/Tissue	Treatment		NADH-NR nmol/h·μl[a] (1)	(1)/(3)	NADPH/NR nmol/h·μl (2)	(2)/(3)	MDH μmol/h·μl (3)	(1)/(2)
			2,4-D	Light						
1	H99	U[b]	+	12 d D	0.94	5.9	0.67	4.2	0.16	1.4
		U	−	12 d D	1.33	5.5	0.78	3.2	0.24	1.7
		O	+	12 d D	3.61	15.0	1.94	8.1	0.24	1.9
		O	−	12 d D						
		a. Scu			4.17	19.0	2.33	10.6	0.22	1.8
		b. root			0.13	4.3	0.06	2.0	0.03	2.2
2	W182BN	O	+	5 d D	4.44	8.7	3.06	6.0	0.51	1.4
		O	−	5 d D	5.28	25.1	4.17	19.9	0.21	1.3
3	H99	U	+	12 d D, 1 d L[c]	1.00	6.7	0.44	2.9	0.15	2.3
		U	−	12 d D, 1 d L	1.22	4.7	0.44	1.7	0.26	2.8
		O	+	12 d D, 1 d L	4.33	11.1	2.44	6.2	0.39	1.8
		O	−	12 d D, 1 d L						
		a. U sector			0.78	6.0	0.28	2.1	0.13	2.8
		b. Scu			1.22	8.0	2.56	17.1	0.15	0.5
		c. green			1.00	11.1	0.83	7.5	0.09	1.2
		d. root			0.22	2.7	0.13	1.6	0.08	1.7
		e. mixed O			4.56	13.8	3.33	10.1	0.33	1.4
4	W182BN	O	+	5 d D, 1 d L	8.3	21.2	5.0	12.8	0.39	1.7
		O	−	5 d D, 1 d L	18.9	43.9	7.2	16.7	0.43	2.6
5	H99	U	+	12 d D, 2 d L	0.78	6.5	0.50	4.2	0.12	1.6
		O	+	12 d D, 1 d L	4.89	9.8	3.44	6.9	0.50	1.4
		O	−	12 d D, 1 d L						
		a. U sector			0.67	4.2	0.33	2.1	0.16	2.0
		b. Scu			2.22	13.9	0.89	5.6	0.16	2.5
		c. leaf			0.67	18.6	0.94	26.1	0.036	0.7
		d. root			0.56	7.5	0.22	2.9	0.09	2.5
		e. mixed O			1.44	19.2	0.56	7.5	0.075	2.6

[a] μl extract. [b] U, Unorganized; O, organized; Scu, scutellar area. [c] 12 d D, 1 d L, 12 days in the dark and the last day in the light.

NR. The above pattern was not changed qualitatively by incubation of the seedlings in the dark, but the levels of the leaf enzyme activities were lowered by this treatment.

Scutella of all lines had high levels of both NADH and NADPH NR activities, differing markedly from leaves in this respect, as reported by Campbell (1). Line 609 stands out in having a relatively low level of NADPH NR activity (Table I).

Roots of all lines had both NADH and NADPH NR activities, line 609 again being noticeably different from the others in having a relatively low NADPH NR activity (Table I).

This suggests that there is a genetic coupling between root and scutellar NAD(P)H NR. Many seedlings of each of strain 609 and 905 were tested. The difference between them was completely consistent under a variety of environmental conditions indicating that the strain difference is genetic in nature.

Effect of Seedling Age on the Scutellar NR Content. Three-d-old seedlings contain a relatively high scutellar NADH NR content and a relatively low scutellar NADPH NR content (Fig. 1). The NADH NR activity drops over the next 2 d, and the scutellar NADPH NR content rises. The pattern observed in lines 609 and 905 differs markedly beyond d 5: the NADPH NR and NADH NR activity of line 905 rise and remain at a relatively constant ratio to each other, whereas in line 609 the NADH NR drops continuously and the NADPH activity rises only slightly (Fig. 1). Once again the difference between strains 609 and 905 was consistent over the seedilng ages tested, indicating that these differences are stable and not due to microenvironmental or physiological differences.

Lines W182BN and H99 have not been studied in the same detail as 609 and 905, but they appear to resemble the latter more than they do the former (Table I). On this basis one is tempted to suggest that line 905 is representative of the wild type situation. Taken together, the above results suggest that scutellar NADH NR is gradually partly replaced by NAD(P)H NR as the seedling increases in age and that line 609 is partially deficient in scutellar NAD(P)H NR activity.

Effect of Callus Redifferentiation on NADH and NAD(P)H NR Activities. Different seedling organs have been shown to contain different complements of NADH and NAD(P)H NR activity (see previous sections). At what stage during the appearance of these organs do the differences manifest themselves? We decided to examine this question in callus at different stages of redifferentiation. Two tissue cultures were used, one (H99) of recent origin and the oher (W182BN) 2 years old. When grown in the presence of 2,4-D, these cultures consist of undifferentiated tissue plus either nodular embryoidal organized areas (H99) or small leafy areas (W182BN). Removal of 2,4-D from the medium usually results in rapid formation of recognizable shoots, roots and/or scutellar-like structures.

H99 callus growing on MS medium containing 5 mg/L 2,4-D

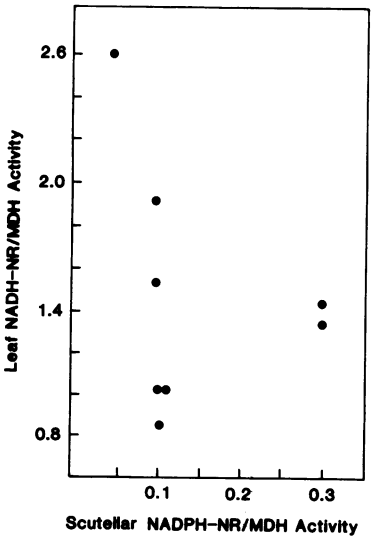

FIG. 5. Ratio of NADH to NADPH NR activity in extracts of scutella from progeny of an Ha × Ha selfing. Line Ha was selfed and individual progeny seed grown for 10 d on vermiculite watered with 1/10 Hoagland solution with ammonium tartrate (4 g/L) as nitrogen source. The seedlings were watered with the same solution but containing 4 g/L ammonium nitrate 16 to 19 h before extraction, and with the same solution but containing 5 g/L potassium nitrate 2.5 h before extraction. The scutella were then dissected away from the rest of the seedling and the level of NADH and NADPH nitrate reductase measured in cell free extracts, as described in "Materials and Methods." Points are from duplicate measurements as in Figure 1, and represent the NR activity/μl extract.

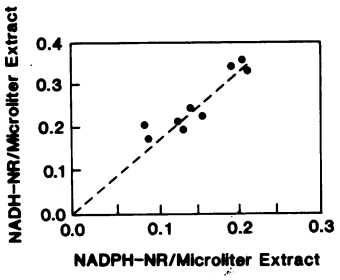

FIG. 6. Relationship between NADH NR in leaves and NADPH NR in scutella in individual progeny of an Ha × Ha selfing. The first leaf and scutellum of 9-d-old progeny seedling from an Ha × Ha selfing were dissected, and treated as described in the legend to Figure 1, were extracted and assayed for NR and malic acid dehydrogenase activity, as outlined in "Materials and Methods." Relative activity is expressed as units of NR/μl extract divided by the units of malic acid dehydrogenase activity/μl extract. Each point represents a duplicate sample, as described in the legend to Figure 1.

was separated (under a dissecting microscope) into unorganized and organized areas, and both types were transferred to the same medium ± 2,4-D. The W182BN callus was transferred to medium ± 2,4-D without separation into different types of tissue. These cultures were incubated at 25°C in the dark for 5 or 12 d. They were then preinduced by transfer to filter paper soaked with the same liquid culture medium containing 4 g/L ammonium nitrate as sole nitrogen source. The cultures were induced by transfer onto fresh discs soaked in the same liquid medium but containing 5 g/L potassium nitrate as nitrogen source. Preinduction and induction were done either in the light or in the dark, at 25°C, and lasted 24 and 2.5 h, respectively. All operations were done asceptically with sterile solutions.

The 'unorganized' H99 callus grew, but remained largely unorganized on medium ± 2,4-D (Fig. 2). The occasional organized area or root that formed was removed before assay of these samples. On medium containing 2,4-D, the 'organized' H99 material and the W182BN tissue grew and produced more small nodular/leafy structures (Fig. 3). In this case, only the organized areas were used for assay; any undifferentiated sections were discarded. When 2,4-D was omitted from the medium, both the organized H99 and W182BN tissue produced small leafy or scutellar structures and/or roots (Fig. 4). For some samples these different structures were dissected out and assayed separately. Exposure to light (77 $\mu E\ m^{-2}\ s^{-1}$) for 1 to 2 d resulted in greening of the leafy structures and some of the organized callus.

Neither the 2,4-D treatment nor the morphology of the tissue nor exposure to light affected the basic pattern observed with all the cultures: both NADH and NADPH NR activities were present at levels reminiscent of scutella (Table II). The two exceptions found (experiment 3b and 5c) to this pattern do not appear to reflect any biological pattern, as their analogous counterparts (experiment 5b and 3c) were not exceptional. The differences in relative levels of NADH and NADPH NR activity that we observed cannot be said to be significant, probably reflecting no more than uncontrolled variability.

Relationship between Leaf and Scutellum NR Activities. Individual progeny from selfed Mu (8)-containing line Ha display variable levels of leaf NADH NR activity (G Sorger, DO Gooden, ED Earle, J McKinnon, unpublished data; Fig. 5). If the scutellar NADH NR is genetically coupled to the leaf NADH NR one might see the variability reflected in the scutellum. Figure 6 shows that the ratio of NADH to NAD(P)H NR in 10-d-old scutella is unaffected by the variability in the leaf NADH NR activity. Figure 5 demonstrates that the level of NAD(P)H NR in 10-d-old scutella is independent of the level of NADH NR in the leaf.

Taken together, these results suggest that the level of NAD(P)H NR in scutella of 8- to 10-d-old seedlings is independent of the level of NADH NR of the leaf, and hence that their genetic regulation is different.

DISCUSSION

According to Campbell (1) partially purified NAD(P)H NR from maize scutellum uses NADH approximately 60% as well as NADPH. In another report (7) the same group of investigators found that partially purified maize root NAD(P)H NR uses NADH 85% as efficiently as NAD(P)H. The partially purified NADH NR of maize root and leaf, on the other hand, use NADPH very inefficiently: 13 and 3% as efficiently as NADH, respectively (1, 7). One must keep in mind that the enzyme preparations discussed above were not completely pure, and that each was therefore slightly contaminated with the other. The fact that the root NADH enzyme could use NADPH more efficiently than the leaf enzyme may reflect the presence of more NAD(P)H NR in the root, and thus more contaminating NAD(P)H enzyme, than in the leaf, and not any important difference between the leaf and root NADH enzymes.

Keeping these reservations in mind, one could assume that if one found a ratio of NADH to NADPH NR activity of more than 0.85 in an extract, this would indicate the presence of both the NADH and the NAD(P)H specific enzymes. Our results indicate that fully differentiated leaf tissue in most cases contains exclusively the NADH specific NR. This agrees with Campbell's observations (1). Roots and scutella would appear to contain both types of activity, again in agreement with the observations of others (1, 7).

The difference between roots and scutella, on the one hand, and leaves, on the other, is not simply a matter of exposure to light, because light-exposed and light-deprived leaves were both deficient in NADPH NR activity. The one exception that had

some leaf NADPH NR activity had so in both the light and dark grown seedlings (H99, Table I). The presence of both NADH and NADPH NR in differentiating tissue was also no different in light-exposed and dark-grown material.

The fate of NADH NR activity in scutella of young line 609 seedlings is very interesting because it is not obscured by a high level of NADPH NR activity. The NADH NR appears to decrease rapidly during the first 5 d of seedling life, after which time even the low level of NAD(P)H activity obscures what happens to it. A drop in scutellar NADH specific NR activity is seen very early in the line 905 seedlings, but this is quickly obscured by the rapidly rising NAD(P)H NR activity. Taken together, these results suggest that initially wild type scutella contain predominantly the NADH specific NR and that this enzyme is mostly, but not completely, replaced by the NAD(P)H specific enzyme, as the scutellum ages.

Line Ha was selected in a large mutant hunt for seedlings defective in leaf NADH NR. The initially isolated line had very low leaf activity, but when grown to maturity and selfed, the progeny had variable levels of leaf NADH NR activity (results not shown). The ratio of NADH to NADH NR activity in scutella of individual progeny of the above selfed line was constant (Fig. 6) even though the ratio of leaf NADH NR to scutellum NADPH NR was not (Fig. 5). This observation suggests that the two activities, leaf NADH NR and scutellum NAD(P)H NR, are under different genetic regulation. Ten-d-old scutella contain very little NADH specific NR (Fig. 1); hence the relationship between the leaf and scutellar NADH specific activities has not been addressed by these experiments.

There does seem to be a possible genetic relationship between the NAD(P)H NR of the root and the scutellum, from the observation that the relative level of NADPH NR in seedlings of line 609 is low in both organs (Table I). Either the regulation or the enzyme itself is abnormal in this line. At present, we have no information to distinguish between these two possibilities. The fact that Campbell (1) and Redinbaugh and Campbell (7) found both NADH-NR and NAD(P)H NR in young roots and scutella, and that inbred lines H99 and W182BN also display both activities in these organs, suggests that this is probably the standard pattern, in which case strain 609 could be considered a variant, unlike its close relative 905 that should be thought of as standard. A more extensive survey of different strains is needed to establish what the 'Standard' pattern is, if one actually exists.

Nakagawa *et al.* (5) have found two forms of NADH NR in maize leaves. The authors concluded from their studies that the two forms reflected different states or conformations of the same enzyme. Our studies would not be concerned with differences of this nature.

All the maize callus material assayed had NR patterns similar to those of scutella and roots. This may reflect the tissue's initial origin from scutellum and its tendency to form roots more readily than shoots when 2,4-D is withdrawn. At the time of assay any leaves present were small, at most a few mm long. It woud be interesting to determine if/when leaves of plants regenerated from these cultures show typical leaf NR enzyme patterns.

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