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INHERITANCE OF NITRATE REDUCTASE ACTIVITY IN SEEDLINGS OF SPRING BARLEY (*HORDEUM VULGARE* L.)[1]

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Summary. Four cultivars of spring barley differing by nitrate reductase (NR) activity were crossed in a complete diallel fashion. The NR activity was studied in the leaves and roots of seedlings of F_1 and F_2 hybrids and their parental forms growing under controlled conditions. From the performed studies it follows that the NR activity is determined by both additive nonadditive gene action. It has been found that there occurs domination towards a low activity of the studied enzyme. Besides that, a statistical analysis showed the occurrence of maternal effects in the both studied generations.

Nitrate reductase (NR) is the main enzyme in nitrogen metabolism in plants and is directly associated with protein biosynthesis. Despite many studies performed on NR in higher plants, the genetic control of that enzyme is still little known. The inheritance of NR activity was studied chiefly in maize (Schrader et al. 1966, Warner et al. 1969) and wheat (Sherrad et al. 1976, Deckard, Busch 1978, Gallagher et al. 1980, Gašić et al. 1981, Kraljević-Balalić et al. 1983). Investigation concerning genetic control of NR activity in barley are few and are dealing first of all with mutants (Tokarev, Shumny 1981).

The purpose of the present paper was to know the inheritance mode of NR activity in spring barley seedlings.

MATERIAL AND METHODS

The studying material consisted of four cultivars of spring barley: Diva, Lubuski, Menuet and Union, differing by nitrate reductase activity in leaves and roots (Lubuski and Union — high activity, Diva and Menuet — low activity). These cultivars were crossed in a complete diallel design. During 1984-85 parental forms and F_1 and F_2 hybrids were studied.

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The NR activity was determined in the leaves and roots of 6-day-old seedlings growing in water cultures, under controlled conditions. From the moment of grain sterilization until the obtaining of seedlings the procedure followed Smith and Thompson (1971), with some modifications. The details are given in an earlier paper (Krzywański, Skoczek 1985).

The NR activity was determined by in vivo method on the basis of the procedure applied by Buczek (1976, 1979), which was slightly modified (Krzywański, Skoczek 1985). After incubation, a certain amount of the solution was taken to analyse it for the contents of nitrite on the basis of method given by Sanderson and Cocking (1964). The enzyme activity was expressed in nmoles of NO_2 — released through 100 mg of fresh tissue during 1 hour.

The NR activity was determined in 3-4 experiments for each variety or hybrid. Each experiment had 4-5 replications for both leaves and roots. The experiments were carried out each time from late January till late May.

Statistic calculations of diallel crosses were performed by the method of Griffing (1956) and Dobek et al. (1977, 1978).

RESULTS

Results showing the NR activity in the leaves and roots of the parental forms and F_1 and F_2 hybrids are summarized in Table 1. A comparison of the cultivars and their hybrids in respect of the studied enzyme activity is presented by Tables 2 and 3. From that comparison it follows that the cultivars Union and Lubuski distinguished by significantly higher NR activity than Menuet and Diva in both leaves and roots. The F_1 hybrids displayed a lower activity of the studied enzyme as compared to the parents, in both leaves and roots. However, not all the differences were statisti-

Table 1. Nitrate reductase (NR) activity in the leaves and roots of barley seedlings

Cultivars and hybrids	NR activity (nmole NO_2/100 mg of fresh matter/h)			
	leaves		roots	
	F_1	F_2	F_1	F_2
Union (U)	181.4	181.9	149.4	145.1
Lubuski (L)	179.3	143.1	142.9	145.8
Menuet (M)	115.3	105.0	77.3	73.0
Diva (D)	123.1	116.1	88.8	105.1
U×L	174.6	179.4	134.9	135.5
U×M	142.6	185.7	100.7	140.3
U×D	106.4	132.8	98.0	145.0
L×U	161.2	162.3	99.0	142.8
L×M	130.5	121.9	94.7	138.2
L×D	128.1	141.0	84.8	124.1
M×U	129.8	170.7	97.7	134.2
M×L	142.3	156.3	98.9	119.9
M×D	113.1	118.5	77.8	99.6
D×U	143.6	176.0	110.6	123.8
D×L	146.7	141.1	111.6	122.1
D×M	106.3	116.3	77.7	111.5

Table 2. A comparison of barley cultivars and hybrids in respect of nitrate reductase activity in the seedling leaves

Contrast	Contrast value		F calculated	
	F_1	F_2	F_1	F_2
Union – Lubuski	2.1	38.8	0.05	16.93
Union – Menuet	66.1	76.9	47.16	66.69
Union – Diva	58.3	65.8	36.68	48.76
Lubuski – Menuet	64.0	38.2	44.14	16.41
Lubuski – Diva	56.2	27.0	34.03	8.23
Menuet – Diva	−7.8	−11.2	0.66	1.40
$U \times L - \frac{U+L}{2}$	−2.9	8.4	0.47	3.49
$U \times M - \frac{U+M}{2}$	−2.9	21.1	0.48	21.92
$U \times D - \frac{U+D}{2}$	−23.0	−8.1	30.35	3.21
$L \times U - \frac{L+U}{2}$	−9.6	−0.1	5.26	0.00
$L \times M - \frac{L+M}{2}$	−8.4	−1.1	4.06	0.06
$L \times D - \frac{L+D}{2}$	−11.6	5.7	7.70	1.58
$M \times U - \frac{M+U}{2}$	−9.3	13.7	4.98	9.16
$M \times L - \frac{M+L}{2}$	−2.5	16.1	0.36	12.76
$M \times D - \frac{M+D}{2}$	−3.1	3.9	0.55	0.78
$D \times U - \frac{D+U}{2}$	−4.3	13.5	1.08	8.94
$D \times L - \frac{D+L}{2}$	−2.3	5.8	0.30	1.63
$D \times M - \frac{D+M}{2}$	−6.5	2.9	2.42	0.41
$F_{0.05}$			4.04	4.15
$F_{0.01}$			7.19	7.50

cally significant. A significant reduction of the NR activity in the leaves was detected for five hybrids, whereas that in the roots was detected for nine hybrids. The activity of the studied enzyme in the leaves and roots of F_2 hybrids was in most cases on the level of the parental mean. A significantly higher NR activity in both leaves and roots was revealed only in hybrids of Union × Menuet and Menuet × Union, whereas that in root — in hybrids of Union × Diva, Lubuski × Menuet and Menuet × Union, and that only in leaves — in hybrids of Menuet × Lubuski and Diva × Union. Thus, the F_2 hybrids as compared to the F_1 hybrids distinguished by a higher NR activity in relation to the parental forms.

A diallelic analysis of variance (Table 4) for the F_1 and F_2 generations showed the significance of variance of the general specific and combining ability and the significance of the maternal effects for the NR activity in both leaves and roots. The portion of the general combining ability variance in the NR activity variation for leaves and roots was several fold higher than that of the specific combining ability

Table 3. A comparison of barley cultivars and hybrids in respect of nitrate reductase activity in the seedling roots

Contrast	Contrast value		F calculated	
	F_1	F_2	F_1	F_2
Union – Lubuski	6.5	−0.7	2.41	0.01
Union – Menuet	72.1	72.1	299.51	85.69
Union – Diva	60.7	40.0	211.79	26.44
Lubuski – Menuet	65.7	72.8	248.15	87.44
Lubuski – Diva	54.2	40.8	168.98	27.42
Menuet – Diva	−11.5	−32.0	7.58	16.93
$U \times L - \frac{U+L}{2}$	−5.7	−4.9	9.82	2.10
$U \times M - \frac{U+M}{2}$	−6.3	15.6	12.26	21.51
$U \times D - \frac{U+D}{2}$	−10.6	9.9	34.18	8.76
$L \times U - \frac{L+U}{2}$	−23.6	−1.3	171.12	0.16
$L \times M - \frac{L+M}{2}$	−7.7	14.4	18.32	18.16
$L \times D - \frac{L+D}{2}$	−15.5	−0.7	74.07	0.04
$M \times U - \frac{M+U}{2}$	−7.8	12.6	18.71	13.86
$M \times L - \frac{M+L}{2}$	−5.6	5.3	9.72	2.43
$M \times D - \frac{M+D}{2}$	−2.6	5.3	2.09	2.45
$D \times U - \frac{D+U}{2}$	−4.3	−0.7	5.55	0.04
$D \times L - \frac{D+L}{2}$	−2.1	1.7	1.39	0.25
$D \times M - \frac{D+M}{2}$	−2.7	11.3	2.19	11.12
$F_{0,05}$			4.04	4.15
$F_{0,01}$			7.19	7.50

Table 4. A diallel analysis of variance of nitrate reductase activity in the leaves and roots of barley seedlings

Variation source	Generation	Degrees of freedom	Mean square	
			leaves	roots
General combining ability (*GCA*)	F_1	3	2383.738**	1875.257**
	F_2	3	2555.087**	1427.696**
Specific combining ability (*SCA*)	F_1	6	149.808**	666.446**
	F_2	6	237.993**	221.795**
Maternal effect	F_1	3	186.420*	1036.228**
	F_2	3	205.898**	108.508**
Effects of interaction of maternal cytoplasm with father's genes	F_1	3	190.027	1023.148
	F_2	3	389.413	61.086
Error	F_1	48	46.326	8.684
	F_2	32	44.393	22.729

* significance at $\alpha = 0.05$

** significance at $\alpha = 0.01$

variance. This indicates the predominance of the additive gene action over the non-additive.

From the estimates of the general combining ability (*GCA*) effects presented in Table 5 it follows that the cultivars Menuet and Diva with a low NR activity showed negative *GCA* effects for the activity of the studied enzyme in both roots and leaves. The cultivars Union and Lubuski characterizing by a high NR activity in the leaves and roots distinguished by high positive effects of the general combining ability.

Table 5. Effects of the general combining ability for nitrate reductase activity in the leaves and roots of barley cultivars seedlings

Cultivars	Effects of general combining ability			
	leaves		roots	
	F_1	F_2	F_1	F_2
Union	13.61	24.58	9.10	13.62
Lubuski	16.23	1.77	16.48	8.91
Menuet	−14.62	−11.83	− 9.48	−14.18
Diva	−15.22	−14.52	−16.10	− 8.35
Standard error of parameter	2.084	2.040	0.902	1.460
Standard error of difference	3.403	3.331	1.473	2.384

Effects of the specific combining ability (*SCA*) are presented in Table 6. Hybrids obtained from crosses of varieties with a high NR activity (Union × Lubuski) showed negative *SCA* effects in the F_1 and F_2 generations, particularly in the case of NR activity in the roots. Significantly negative effects of the specific combining ability were noted also for the F_1 hybrids of Union × Diva, whereas effects significantly higher than zero were noted for the F_2 hybrids of Union × Menuet. The *SCA* effects of hybrids with a low NR activity did not differ significantly from zero, except the NR activity in the roots of the F_1 hybrids.

Table 6. Effects of the specific combining ability for nitrate reductase activity in the leaves and roots of barley seedlings

Hybrids	Effects of specific combining ability			
	leaves		roots	
	F_1	F_2	F_1	F_2
Union × Lubuski	−0.96	−2.26	−5.86	−8.66
Union × Menuet	−1.81	18.69	2.34	12.43
Union × Diva	−12.41	−2.42	−30.44	3.75
Lubuski × Menuet	−4.23	2.41	−7.44	8.93
Lubuski × Diva	−2.63	7.04	0.59	−2.85
Menuet × Diva	0.52	−3.01	6.09	2.69
Standard error of parameter	3.805	3.725	1.647	2.665
Standard error of difference for half-siblings	5.894	5.770	2.552	4.129
Standard error of difference for not related progenies	4.813	4.711	2.084	3.371

Estimates of the maternal effects, the occurrence of which was detected on the basis of the analysis of variance, are presented in Table 7. Significantly positive maternal effects for the NR activity in the leaves were detected in the both generations for Diva, while significantly negative effects — for the variety Lubuski. In the case of the cultivars Union and Menuet these effects did not differ significantly from

Table 7. Estimates of maternal effects for nitrate reductase activity in the leaves and roots of barley cultivars seedlings

Cultivars	Maternal effect			
	leaves		roots	
	F_1	F_2	F_1	F_2
Union	−1.38	−1.39	−7.84	2.53
Lubuski	−5.48	−6.45	−8.36	3.43
Menuet	0.73	2.70	0.16	−4.54
Diva	6.13	5.14	16.04	−1.41
Standard error of parameter	2.084	2.040	0.902	1.460
Standard error of difference	3.403	3.331	1.473	2.384

zero. For the NR activity in the roots of the F_1 hybrids, the maternal effects were positive for the cv. Diva and negative for the cv. Union and Lubuski. In the F_2 generation, however, other results were obtained: the cv. Lubuski displayed significantly positive maternal effects, whereas the cv. Menuet — significantly negative. In view of the fact that the maternal effect estimates were not clear, particularly in the case of roots, the pursuit of further studies in this direction seems reasonable.

DISCUSSION

From the performed studies it follows that the NR activity in the leaves and roots of barley seedlings is determined by both additive and nonadditive gene action, with the predominance of the additive action. Similar results were obtained by Gašić et al. (1981) as well as by Kraljević et al. (1983) for wheat.

Hybrids of the F_1 generation were characterized by a lower NR activity in the leaves and roots in comparison with the parental mean. This suggest the occurrence of domination towards a low activity of that enzyme. Though the statistic analysis has not provided a direct estimate of variance and parameters associated with domination, the significance of the specific combining ability variance supports in an indirect way the occurrence of dominance. Schrader et al. (1966), when studying the inheritance of the NR activity in maize, also found that the F_1 hybrids distinguished by a lower activity as compared to the parental forms. Hybrids of the F_2 generation compared to the F_1 hybrids distinguished by a higher NR activity in relation to the parental mean. Therefore, the domination towards a low activity of the studied enzyme, visible in the F_1 generation, was reduced in the F_2 generation, which is in agreement with the theoretically half-smaller portion of the domination effects in the mean of the F_2 generation (Mather, Jinks 1971).

From the estimates of the specific combining ability effects presented in this paper it follows that the *SCA* effects calculated on the basis of F_1 and F_2 hybrids are not always similar. As known (Griffing 1956, Dobek et al. 1978) the specific combining ability is a result of the nonadditive gene action, i.e. domination and nonallelic interaction. The portion of both domination and nonallelic interaction in the mean for the succeeding generations arisen due to self-pollination decreases (Mather, Jinks 1971, Kaczmarek et al. 1984), which may cause changes in the estimates of *SCA* effects.

The statistico-genetic analysis showed the occurrence of maternal effects, which made impossible a thorough genetic analysis. These effects were significant for both F_1 and F_2 generation for the NR activity in the leaves and roots. The estimates of the maternal effects for NR activity in the leaves were similar in the both analysed generations. In the case of roots no repeatable results were obtained in the F_1 and F_2 generations. For that reason, too, it seems necessary to conduct further studies in this respect.

From the performed studies it follows that the cv. Union would be a good component for crossing, as it possesses high positive effects of the general combining ability for NR activity in the leaves and roots of barley.

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DZIEDZICZENIE AKTYWNOŚCI REDUKTAZY AZOTANOWEJ W SIEWKACH JĘCZMIENIA JAREGO (*HORDEUM VULGARE* L.)

Streszczenie

Cztery odmiany jęczmienia jarego różniące się aktywnością reduktazy azotanowej (NR) skrzyżowano w pełnym układzie diallelicznym. Aktywność NR badano w liściach i korzeniach siewek form rodzicielskich oraz mieszańców pokolenia F_1 i F_2, rosnących w kontrolowanych warunkach. Z przeprowadzonych badań wynika, że aktywność NR jest uwarunkowana zarówno addytywnym, jak i nieaddytywnym działaniem genów. Stwierdzono występowanie dominowania w kierunku niskiej aktywności badanego enzymu. Ponadto analiza statystyczno-genetyczna wykazała występowanie efektów matecznych w obu badanych pokoleniach.

НАСЛЕДОВАНИЕ АКТИВНОСТИ НИТРАТНОЙ РЕДУКТАЗЫ В СЕЯНЦАХ ЯРОВОГО ЯЧМЕНЯ (*HORDEUM VULGARE* L.)

Резюме

Четыре сорта ярового ячменя, отличающиеся активностью нитратной редуктазы, были скрещены по полной диаллелической схеме. Активность нитратной редуктазы исследовалась в листьях и корнях сеянцев гибридов поколений F_1 и F_2, а также родительских форм, растущих в контролируемых условиях. Из проведённых исследований следует, что активность нитратной редуктазы обусловлена как аддитивным, так и неаддитивным действием генов. Обнаружено выступление доминирования в направлении низкой активности исследуемого энзима. Кроме того, статистическо-генетический анализ показал выступление материнских эффектов в обоих исследованных поколениях.