NITRATE-REDUCTASE ACTIVITY AND SOLUBLE PROTEIN CONTENT IN LEAVES OF YOUNG SUNFLOWER PLANTS

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INTRODUCTION

In Yugoslavia, sunflower is grown mostly for oil. However, sunflower is also rich in proteins, especially if we take into consideration the importance of plant proteins in human and animal nutrition (Gašić, 1984).

It is possible to estimate protein content in the seeds of F_1 plants by analysing certain biochemical parameters of young plants (21 days after emergence) (Kraljević-Balalić et al., 1983). Many researchers have found that the intensity of protein biosynthesis in plants may be followed on the basis of the activity of nitrate-reductase (Beevers and Hageman, 1969) which is capable of nitrate reduction and is thus considered a natural regulator in the metabolism of nitrate reduction.

That enzyme is induced by a substrate (NO_3^-) (Beevers et al., 1965 ; Ingle et al., 1966 ; Goodman et al., 1974), observed to be unstable *in vivo* under the environmental stress (Mattas and Pauli, 1965), sensitive to protein synthesis inhibitors (Singh and Wort, 1969), and found to be dependent on the genetic composition of plants (Duffield et al., 1972) as well as on the intensity of photosynthesis and respiration (Gašić, 1984).

Our earlier studies on wheat showed that the activity of nitrate-reductase is dependent on genotype and that it is correlated with protein content, i.e., that it is an operative biochemical criterion applicable in wheat breeding (Gašić et al., 1981 ; Kraljević-Balalić et al., 1983). Similar results were obtained for sunflower and maize cultivated in different nutrition conditions. The nitrate-reductase activity was found to be dependent on nitrogen concentration in the nutritive solution (Kaiser and Lewis, 1984).

The objectives of this study were to determine the mode of inheritance of NR activity and soluble protein content in leaves as determined from crosses of seven sunflower inbreds, and to examine the correlation between NR activity and soluble protein content in order to find good combining parents for high sunflowers yields.

MATERIALS AND METHODS

The experimental material consisted of seven sunflower inbreds, SNRF-44, SNRF-70, SNRF-86, SNRF-4, SNRF-115, SNRF-150 and SNRF-138 which differed in the total protein content in the seeds of F_1 plants. We examined also 21 F_1 hybrids (diallel crosses excluding reciprocals) whose parents were the above inbreds.

Nitrate-reductase and soluble proteins in leaf (21 day old) were extracted basically after the method of Sherrard and Dalling (1979). Plant materials (1.00 g) were homogenized with 5 cm^3 of semi-frozen extraction medium (containing 25 mmoles Na_2HPO_4, 5 mmoles ethylenedinitrilotetraacetic acid disodium salt-dihidrat and 5 mmoles cysteinhydrochloride with limit pH 7.50) by stirring them in a mixer for one minute. The homogenate was filtered through four layers of cotton and centrifuged at 25,500 g, for 15 minutes, at 4°C. NR activity and soluble protein content were determined in the supernatant obtained after centrifuging.

NR activity was analysed by the method of Schrader et al. (1968). Enzymic extracts (0.2 cm^3) were incubated for 15 minutes at 30°C with 1.8 cm^3 of reaction medium containing: 50 mmoles Na_2HPO_4 pH 7.50, 20 mmoles KNO_3 and 0.4 mmoles dihydronicotinamide adenine dinucleotide disodium salt (NADH-Na_2). The reaction was stopped with 1 cm^3 of 0.5% sulphanylamide in HCI concentration 1.5 moles/dm^3 and 1 cm^3 of 1.0% N-(1-Naphthyl)-ethylenediammonium dichloride. The amounts

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of reaction products were determined on the basis of absorbance readings at 540 nm after 15 minutes.

NR activity was expressed in μmoles of reaction product per hour per gram of fresh material.

Soluble protein content in leaves was determined by the method of Lowry et al. (1951) and expressed in mg/g fresh weight.

Combining ability was analysed after Griffing (1956), model 2, method I.

RESULTS

Nitrate-reductase activity. Table 1 a shows the mean values of the NR activity in the examined inbreds and their hybrids.

Table 1 a

Nitrate-reductase activity (μmoles NO_3^- reduced/hr per g fresh weight) in diallel crosses of seven sunflower inbreds

Parents	(1) SNRF-44	(2) SNRF 70	(3) SNRF-86	(4) SNRF-4	(5) SNRF-115	(6) SNRF-150	(7) SNRF-138
SNRF-44	1.50	5.69h	4.30h	5.17h	5.58h	8.81h	2.76i
SNRF-70		2.57	1.48h	5.75h	3.66h	4.93h	4.99h
SNRF-86			3.43	2.87	1.53h	2.11i	0.22h
SNRF-4				3.24	6.37h	1.97d	2.62h
SNRF-115					2.24	3.23h	0.05h
SNRF-150						1.72	0.06h
SNRF-138							3.08

LSD 0.05 = 0.22.
LSD 0.01 = 0.29.
i — intermediate.
h — heterosis.

Significant differences were found among the examined cultivars concerning the mean values of NR activity. The parent with the highest NR activity was SNRF-86 (X = 3.43) ; SNRF-44 had the lowest NR activity (X=1.50).

The NR activity was intermediate in two combinations (SNRF-44 × SNRF-138) and (SNRF-86 × SNRF-150). There was dominance for the lower activity in the combination SNRF-4 × SNRF-150. In the other hybrids there occurred either positive or negative heterosis (Table 1 a).

The analysis of variance for combining ability revealed that there were highly significant differences for both GCA and SCA indicating that both additive and nonadditive effects were important in the inheritance of these characters.

The estimates of the GCA effects of the parents showed that SNRF-44 had the best combining ability for NR activity. SNRF-70 and SNRF-4 had lower but still significant GCA effects (Table 1 b).

Table 1 b

GCA effect for nitrate-reductase activity

Parents	GCA effect	Rank
(1) SNRF-44	+1.00	1
(2) SNRF-70	+0.59	2
(3) SNRF-86	—0.76	6
(4) SNRF-4	+0.55	3
(5) SNRF-115	—0.15	4
(6) SNRF-150	—0.19	5
(7) SNRF-138	—1.04	7

SE = 0.037 ;
LSD 0.05 = 0.074.
LSD 0.01 = 0.098.

The highest SCA value was found in the hybrid combination SNRF-44 × SNRF-150. High SCA values were also found in the combinations SNRF-4 × SNRF-115, SNRF-70 × SNRF-138, SNRF-70 × SNRF-4, SNRF-70 × SNRF-150 and SNRF-44 × SNRF-115. The results obtained indicate that the crosses with high SCA usually had one parent with high GCA and another parent with low GCA (Table 1 c).

Table 1 c

SCA effect for nitrate-reductase activity

Parents	(2) SNRF-70	(3) SNRF-86	(4) SNRF-4	(5) SNRF-115	(6) SNRF-150	(7) SNRF-138
SNRF-44	+0.81	+0.78	+0.33	+1.44	+4.71	—0.48
SNRF-70		—1.63	+1.32	—0.07	+1.24	+2.16
SNRF-86			—0.20	—0.38	—0.22	—1.25
SNRF-4				+2.69	—1.67	—0.17
SNRF-115					+0.29	—2.04
SNRF-138						—1.99

SE = 0.09 ; LSD 0.05 = 0.19 ; LSD 0.01 = 0.26.

It ensues that the combining ability of a sunflower inbred is valid only for a particular combination ; if combined with a third line, the inbred may change in a better or poorer combiner for NR activity.

Leaf protein content. Table 2 a shows the mean values of soluble proteins in the examined inbreds and their hybrids.

The SNRF-138 and SNRF-86 had the highest protein contents in leaf ; SNRF-70, SNRF-4 and SNRF-115 had medium content and SNRF-150 and SNRF-44 had the lowest content (Table 2 a). The protein content was intermediate in the crosses SNRF-44 × SNRF-138 and SNRF-4 × SNRF-150. The high level was dominant in the crosses SNRF-70 × SNRF-138, SNRF-86 × SNRF-4 and SNRF-4 × SNRF-138. Positive or negative heterosis occurred in the other combinations.

Table 2 a

Content of soluble protein in leaf (mg/g fresh weight) in diallel crosses of seven sunflower inbreds

Parents	(1) SNRF-44	(2) SNRF-70	(3) SNRF-86	(4) SNRF-4	(5) SNRF-115	(6) SNRF-150	(7) SNRF-138
SNRF-44	23.25	30.66h	29.05h	32.08h	32.31h	35.52h	25.43i
SNRF-70		25.06	23.45h	33.74h	29.00h	27.65h	27.67d
SNRF-86			27.55	26.84h	23.43h	24.47d	23.22h
SNRF-4				25.95	35.11h	24.74i	25.39h
SNRF-115					24.98	28.21h	19.30h
SNRF-150						23.83	22.62h
SNRF-138							28.00

LSD 0.05 = 0.8.
LSD 0.01 = 1.06.
i — intermediate.
h — heterosis.
d — dominant.

GCA and SCA were highly significant for protein content in leaf. Thus both additive and nonadditive genetic effects determined the protein content in the leaf. The highest GCA for soluble proteins was found in SNRF-44 (Table 2 b). SNRF-4 also had high GCA for that character. SNRF-70 was in the third place. The other inbreds had a poor combining ability for this character.

Table 2 b

GCA effect for soluble protein content in leaf

Parents	GCA effect	Rank
(1) SNRF-44	+1.64	1
(2) SNRF-70	+0.61	3
(3) SNRF-86	—1.24	6
(4) SNRF-4	+1.45	2
(5) SNRF-115	+0.06	4
(6) SNRF-150	—0.64	5
(7) SNRF-138	—1.90	7

SE = 0.13.
LSD 0.05 = 0.25.
LSD 0.01 = 0.34.

High SCA for soluble protein content was found in the crosses SNRF-44 × SNRF-150, SNRF-4 × SNRF-115, SNRF-70 × SNRF-4, SNRF-44 × SNRF-115, SNRF-44 × SNRF-4, SNRF-70 × SNRF-138 and SNRF-116 × SNRF-150.

The crosses with high SCA for soluble protein content have almost regularly one parent with a high GCA and another with a low GCA for that character. The only exception was SNRF-70 × SNRF-4 where both parents had a high GCA (Table 2 c).

Relationship between nitrate-reductase activity and leaf protein content. The correlation between nitrate-reductase activity and soluble protein content in leaf was found to be positive and highly significant (r=0.974).

Table 2 c

SCA effect for soluble protein content in leaf

Parents	(2) SNRF-70	(3) SNRF-86	(4) SNRF-4	(5) SNRF-115	(6) SNRF-150	(7) SNRF-138
SNRF-44	+1.31	+1.56	+1.89	+3.51	+7.43	—1.40
SNRF-70		—3.01	+4.58	+1.23	+0.59	+1.87
SNRF-86			—0.46	—2.48	—0.71	—0.72
SNRF-4				+6.50	—3.11	—1.29
SNRF-115					+1.70	—5.95
SNRF-150						—1.92

SE = 0.36.
LSD 0.05 = 0.72.
LSD 0.01 = 0.95.

DISCUSSION

It is very important to have information about the genetic basis of the expression of the main characters. The breeder should know what portion of the total variation among plants is based on genetic differences and the contributions of additive or nonadditive gene effects.

The differences in the mean values of NR activity and soluble protein content in leaf of the sunflower inbred lines and their hybrids were highly significant.

The analysis of our data revealed that both additive and nonadditive genetic effects were important in the inheritance of NR activity and soluble protein content in sunflower leaf. Over dominance was observed for both characters in the majority of the hybrids. SNRF-44 was the most promising parent in the crossing programme. It had the highest GCA values for both characters.

Hybrids with good performance, i.e. obtained from parents with good SCA, may be produced

by crossing two lines of which at least one should have a high GCA for the desired character, e.g., SNRF-44.

The obtained results indicate the existance of a significant correlation ($r = 0.974$) between NR activity and soluble protein content in leaf of the sunflower inbred lines and their hybrids. It means that an intensified NR activity makes the reduction of nitrates more efficient which in turn intensifies the biosynthesis of soluble proteins in leaf.

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ACTIVITÉ DE LA NITRATE-RÉDUCTASE ET LA TENEUR EN PROTÉINES SOLUBLES DES FEUILLES JEUNES DE TOURNESOL

Résumé

L'activité de la nitrate-réductase (NR) et la teneur en protéines solubles ont été étudiées chez sept lignées de tournesol et chez les hybrides résultés de ces lignées. Les valeurs NR ont varié de 0,05 à 8,81 (μ moles NO_3^- réduites par heure, par g de matière fraîche) et les valeurs des protéines solubles ont varié entre 19.30 et 35,52 (mg/g de matière fraîche).

L'analyse de la variance pour la valeur combinative a montré que la variance de la capacité générale et spécifique de combinaison a été significative en tant que l'activité de la nitrate-reductase et la teneur en protéines solubles.

Les résultats ont mis en évidence la corrélation positive ($r = 0,974$) entre la teneur en protéines solubles et l'activité de la nitrate-réductase chez les lignées de tournesol étudiées et leur hybrides.

LA ACTIVIDAD DE LA NITRAT-REDUCTÁSIS Y EL CONTENIDO DE PROTEÍNAS SOLUBLES EN LAS HOJAS DE LAS PLANTAS JÓVENES DE GIRASOL

Resúmen

La actividad de la nitrat-reductásis (NR) y el contenido de protéinas solubles fueron estudiados en siete líneas de girasol y en los híbridos resultados de estas líneas. Los valores NR variaron desde 0,05 hasta 8,81 (μ moles NO_3^- reducidos per hora, per g de sustancia fresca) mientras que los valores de las proteínas solubles variaron entre 19,30 y 35,52 (mg/g sustancia fresca).

El análisis de la variación para el valor combinativo mostró que la variación de la capacidad general y específica de combinación fue sustancial en cuanto a la actividad nitrat-reductásis y del contenido en proteínas solubles.

Los resultados evidenciaron la correlación positiva ($r = 0,974$) entre el contenido de proteínas solubles y la actividad nitrat-reductásis en las líneas de girasol estudiadas y sus híbridos.