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ACTIVITY OF NITROGEN ASSIMILATION ENZYMES IN LEAVES OF YOUNG PLANTS OF SUNFLOWER *(Helianthus annuus L.)*

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ABSTRACT

The results of a biochemical and genetic research showed that there were significant differences in the examined biochemical parameters (enzyme activity and protein content) among the tested sunflower genotypes and their F_1 hybrids.

The inheritance of the examined characters was controlled by both additive and non–additive gene actions, as indicated by the results of variance analysis for general (GCA) and special combining ability (SCA). These results were in accordance with the results obtained for inheritance mode when the combinations were observed separately, where intermediate inheritance, full or partial dominance or heterosis occurred.

The genotype SNRF–4 having the highest mean values of enzyme activity (NR, GS, GOGAT and GDH) and leaf protein content was also the best general combiner. It is, therefore, recommended to be a parent in hybridization for high protein content.

There was a positive correlation between the enzyme activity (NR, GS, GOGAT and GDH) and leaf protein content.

Key words: *Helianthus annuus* L., nitrate reductase–glutamine synthetase, glutamate synthase, glutamate dehydrogenase, leaf soluble proteins, diallel crosses, inheritance, gene action, combining ability, early selection.

INTRODUCTION

Sunflower is a popular oil crop which is also cultivated because of its high protein content.

Most of biochemical research on sunflower has been carried out on oil and its compositions in different cultivars. However, since sunflower is also rich in proteins, it could also serve as a source of proteins, which is very important because today the importance of plant proteins in human and animal nutrition is becoming clear (Gašić 1984).

Nitrate reductase (NR, EC 1.6.6.1.) is an enzyme responsible for protein synthesis, therefore, it is applied recently more and more in plant breeding. Nitrate is a primary nitrogen form available to plants. Nitrate reductase is the first enzyme initiating the reduction of nitrate. This enzyme plays an important role in the regulation of nitrogen metabolism (Beevers and Hageman, 1969; Goodman *et. al.*., 1974), is labile *in vivo* under stress of environment (Mattas and Pauli, 1965), variable during day, sensitive to in-

hibitors of protein synthesis (Singh *et al.,* 1976), dependent on plant genetic composition (Duffield *et al.*, 1972), and so on.

Plant ammonium assimilation comprises the primary and secondary assimilation of ammonium ions. The primary ammonium assimilation encompasses the ammonium which is derived from symbiotic nitrogen fixation, absorbed directly from soil or reduced from nitrate. The secondary ammonium assimilation involves the ammonium released from organic nitrogen compounds (glycine, asparagine and arginine) during their metabolism (Miflin and Lea, 1980). The pathways of ammonium assimilation are controlled by a series of enzymatic systems of which some are as follows: glutamate dehydrogenase (GDH, EC 1.4.1.2–4), alanine dehydrogenase (EC 1.4.1.1), asparagine synthetase (EC 6.3.1.1), glutamine synthetase (GS, EC 6.3.1.2) and glutamate synthase (GOGAT, EC 1.4.1.13–14) (Miflin, 1974; Miflin and Lea, 1976; Lea and Miflin, 19 4; Fowden, 1976). Alanine dehydrogenase and asparagine synthetase, in contrast to the remaining enzymes, have not a significant role in ammonium assimilation. Their role is mainly related to the metabolism of amino acids (Lea *et al., 1980).* The remaining 3 enzymes (GDH, GOGAT and GS) are highly significant for ammonium assimilation (Tempest et al., 1970; Dougall, 1977; Lea and Thurman, 1972) so that they are also taken as parameters in the study of protein biosynthesis.

The subject of the present work was to determine activity of primary nitrogen assimilation enzymes (NR, GS, GOGAT and GDH) and leaf soluble protein content in young plants of sunflower, to investigate the correlation between the enzyme activity and the leaf soluble proteins content, and to determine the inheritance mode of the above characters in F_1 hybrids of sunflower.

MATERIAL AND METHODS

The inbred lines of sunflower (SNRF–1, SNRF–2, SNRF–3, SNRF–4 and SNRF–5) and their F_1 hybrids were used in this experiment.

The plants were grown in sterile sand in a greenhouse under controlled conditions and supplied with Hogland nutrient solution modified with 10 m mole/l NO_3^-.

Young sunflower plants at the stage of first leaf were used for the experiment.

The activities of *in vitro* NR, synthetic GS, NADH–dependent GOGAT and GDH were measured in a common extract from leaves of sunflower plants according to the methods described by Coombs and Hall (1982). The extract was prepared by homogenizing 1.0 g fresh leaf in 10 ml extraction buffer (containing 50 mmole/l imidazole, 5 mmole/l 2–mercaptoethanol and 0.5 mmole/l EDTA, pH 7.2), followed by centrifuging at 10,000 x g for 15 min.

Leaf soluble proteins were determined by the method of Lowry *et al.* (1951).

General (GCA) and special combining ability (SCA) were estimated by the method 2 model 1 of Griffing (1956).

RESULTS

a) Nitrate reductase activity

Variability and mode of inheritance. The genotype with the highest NR activity was SNRF–2 ($\overline{X} = 6.21$) and with the lowest SNRF–5 ($\overline{X} = 3.94\,\mu$ moles $NO_2^-\,g^{-1}$fr. wt. h^{-1}).

In the F_1 hybrids, the NR activity varied from $\overline{X} = 10.53$ in the cross SNRF–3 x 4 to $\overline{X} = 2.91\,\mu$ mole $NO_2^-\,g^{-1}$ fr. wt. h^{-1} in SNRF–1 x 2 (Table 1a).

The inheritance of NR activity depended on cross combination. In the crosses of SNRF–1 x 5 and SNRF–2 x 3, intermediate inheritance occurred. Heterosis was observed in 3 crosses (SNRF–1 x 2, SNRF–1 x 4, and SNRF–3 x 4). In the other crosses, full dominance was present.

Table 1a. Mean values for NR activity (μ mole $NO_2^-\,g^{-1}$ fr. wt. h^{-1}) in diallel sunflower crosses (parents and F_1), $LSD_{0.05} = 0.52$; $LSD_{0.01} = 0.58$

Parent	(1) SNRF–1	(2) SNRF–2	(3) SNRF–3	(4) SNRF–4	(5) SNRF–5
(1) SNRF–1	5.70	2.91^{h-}	5.36^{d+}	4.08^{h-}	4.72^{i}
(2) SNRF–2		6.21	5.60^{i}	5.28	6.85^{d+}
(3) SNRF–3			4.29	10.53^{h+}	4.53^{d+}
(4) SNRF–4				6.13	6.04^{d+}
(5) SNRF–5					3.94

Table 1b. ANOVA of combining ability for NR activity

Source of variation	DF	MS	Fe	LSD 0.05	LSD 0.01
GCA	4	2.09	95.69^{**}	2.69	4.02
SCA	10	3.42	156.22^{**}	2.16	2.98
E	28	0.021			

Combining ability. The highly significant variances of GCA and SCA for NR activity show that this character was under control of additive and non–additive gene action, with the preponderance of non–additive gene action (Table 1b).

The best general combiner for the examined character was the genotype SNRF–4, the poorest SNRF–1 (Table 1c).

The best combination was SNRF–4 x 3, the cross of a good and a poor general combiner (Table 1d).

Table 1c. GCA values for NR activity

Parent	GCA	Rank	SE	LSD 0.05	LSD 0.01
SNRF–1	–0.62	5			
SNRF–2	0.02	3			
SNRF–3	0.24^{*}	2	0.05	0.16	0.37
SNRF–4	0.75^{**}	1			
SNRF–5	–0.40	4			

Table 1d. SCA values for NR activity

Parent	(2) SNRF–2	(3) SNRF–3	(4) SNRF–4	(5) SNRF–5	SE	LSD 0.05	LSD 0.01
(1) SNRF–1	–0.87	1.39**	–0.34	1.31**			
(2) SNRF–2		1.05**	0.28	2.87**			
(3) SNRF–3			5.34**	0.36	0.12	0.39	0.90
(4) SNRF–4				1.42**			

b) Glutamine synthetase activity

Variability and mode of inheritance. GS activity in the examined sunflower inbred lines varied from $\overline{X} = 335.12$ in SNRF–2 to $\overline{X} = 121.83$ μ mole, γ –GH g^{-1} fr. wt. h^{-1} in SNRF–5 (Table 2a).

In the F_1 hybrids, the highest GS activity was found in the combination SNRF–1 x 2 ($\overline{X} = 97.35$), the lowest in SNRF–3 x 4 ($\overline{X} = 369.33$).

Intermediate inheritance occurred in the crosses SNRF–1 x 3, SNRF–1 x 5, SNRF–2 x 3, SNRF–3 x 5, and SNRF–4 x 5. Heterosis was present in 3 hybrids (SNRF–2 x 5, SNRF–3 x 4, and SNRF–3 x 5), whereas in the remaining combinations the inheritance mode was full or partial dominance.

Combining ability. The results on the variance analysis of combining ability for GS activity show that the quadratic means of both GCA and SCA, with the preponderance of the latter, were significant, indicating that the character was governed mainly by non–additive, and less by additive gene action (Table 2b).

The genotypes SNRF–2 and SNRF–4 having the highest GS activity were also the best general combiners for high enzyme activity whereas the genotype SNRF–1 was the poorest for the character (Table 2c).

The combinations SNRF–3 x 4 and SNRF–2 x 5 had the highest GCA and SCA values. These crosses included one high GCA and one low GCA parent, which was a common case in other studies (Singh and Gupta, 1969; Kraljevic–Balalic *et al.*, 1983). The cross SNRF–4 x 5 had a good SCA (Table 2d).

Table 2a. Mean values for GS activity (μ mole γ–GH g^{-1} fr. wt. h^{-1}) in diallel sunflower crosses (parents and F_1), $LSD_{0.05} = 10.09$; $LSD_{0.01} = 11.14$

Parent	(1) SNRF–1	(2) SNRF–2	(3) SNRF–3	(4) SNRF–4	(5) SNRF–5
(1) SNRF–1	264.02	97.35^{h-}	207.97^{i}	143.66^{h-}	180.84^{i}
(2) SNRF–2		335.12	230.86^{i}	194.70^{h-}	364.03^{d+}
(3) SNRF–3			154.79	369.33^{h+}	177.59^{i}
(4) SNRF–4				315.35	268.45^{i}
(5) SNRF–5					121.83

Table 2b. ANOVA of combining ability for GS activity

Source of variation	DF	MS	Fe	LSD	
				0.05	0.01
GCA	4	5664.20	470.00**	2.69	4.02
SCA	10	8037.59	666.94**	2.16	2.98
E	28	12.05			

Table 2c. GCA values for GS activity

Parent	GCA	Rank	SE	LSD	
				0.05	0.01
SNRF–1	–29.32	5			
SNRF–2	25.14**	2			
SNRF–3	–9.68	3	1.17	3.80	8.70
SNRF–4	34.81**	1			
SNRF–5	–20.94	4			

Table 2d. SCA values for GS activity

Parent	(2) SNRF–2	(3) SNRF–3	(4) SNRF–4	(5) SNRF–5	SE	LSD	
						0.05	0.01
(1) SNRF–1	–77.50	63.60**	–39.64	46.31**			
(2) SNRF–2		38.82**	–36.27	163.84**			
(3) SNRF–3			168.83**	25.88**	3.03	9.31	21.31
(4) SNRF–4				77.80**			

c) Glutamate synthase activity

Variability and mode of inheritance. GOGAT activity in the examined sunflower inbred lines varied from $\overline{X} = 7.81$ in SNRF–5 to $\overline{X} = 22.07\,\mu$ mole prod. g^{-1} fr. wt. h^{-1} in SNRF–2 (Table 3a).

In the F_1 generation, the highest GOGAT activity was found in the combination of SNRF–2 x 5 ($\overline{X} = 22.81$), the lowest in SNRF–1 x 2 ($\overline{X} = 5.86\,\mu$mole prod. g^{-1} fr. wt. h^{-1}).

Intermediate inheritance was found in the combinations of SNRF–1 x 3, SNRF–1 x 5, and SNRF–2 x 3. Negative heterosis occurred in 3 hybrids (SNRF–1 x 2, SNRF–1 x 4, and SNRF–2 x 4) and positive in one hybrid (SNRF–3 x 4). In the remaining combinations the inheritance mode was full dominance of the parents with higher mean values (Table 3a).

Combining ability. The variance analysis of combining ability for GOGAT activity show that the quadratic means of both GCA and SCA were highly significant with the preponderance of SCA, indicating that the character was governed mainly by the non–additive gene action (Table 3b).

The genotypes SNRF–2 and SNRF–4 having the highest GOGAT activity were also the best general combiners for high enzyme activity whereas SNRF–5 was the poorest for the character (Table 3c).

Good SCA was found in all 6 hybrids (Table 3d).

Table. 3a. Mean values for GOGAT activity (μ mole NADH g^{-1} fr. wt. h^{-1}) in diallel sunflower crosses (parents and F_1), $LSD_{0.05} = 1.09$; $LSD_{0.01} = 1.20$

Parent	(1) SNRF–1	(2) SNRF–2	(3) SNRF–3	(4) SNRF–4	(5) SNRF–5
(1) SNRF–1	19.06	5.86^{h-}	15.69^{i}	9.05^{h-}	12.90^{i}
(2) SNRF–2		22.07	17.95^{i}	14.48^{h-}	22.81^{d+}
(3) SNRF–3			10.63	22.73^{h+}	11.60^{d+}
(4) SNRF–4				20.53	20.24^{d+}
(5) SNRF–5					7.90

Table 3b. ANOVA of combining ability for GOGAT activity

Source of variation	DF	MS	Fe	LSD	
				0.05	0.01
GCA	4	21.34	195.80^{**}	2.69	4.02
SCA	10	38.87	338.29^{**}	2.16	2.98
E	28	0.11			

Table 3c. GCA values for GOGAT activity

Parent	GCA	Rank	SE	LSD	
				0.05	0.01
SNRF–1	–1.68	5			
SNRF–2	1.69^{**}	2			
SNRF–3	–0.59	3	0.11	0.36	0.82
SNRF–4	2.02^{**}	1			
SNRF–5	–1.43	4			

Table 3d. SCA values for GOGAT activity

Parent	(2) SNRF–2	(3) SNRF–3	(4) SNRF–4	(5) SNRF–5	SE	LSD	
						0.05	0.01
(1) SNRF–1	–6.37	5.45^{**}	–3.47	3.40^{**}			
(2) SNRF–2		4.75^{**}	–0.99	10.35^{**}			
(3) SNRF–3			9.25^{**}	1.14	0.28	0.88	2.02
(4) SNRF–4				7.49^{**}			

d) Glutamate dehydrogenase activity

Variability and mode of inheritance. The mean values of GDH activity in the examined sunflower inbred lines ranged from 37.75 in SNRF–2 to 14.66 μmole prod. g^{-1} fr. wt. h^{-1} in SNRF–5 (Table 4a).

In the F_1 generation GDH activity varied from $\overline{X} = 42.72$ in the combination SNRF–3 x 4 to $\overline{X} = 11.09$ μmole prod. g^{-1} fr. wt. h^{-1} in the hybrid SNRF–1 x 2 (Table 4a).

Intermediate inheritance occurred in the combinations SNRF–1 x 5 and SNRF–2 x 3. Positive or negative heterosis was found in 6 hybrids. Partial dominance was present in the hybrid SNRF–1 x 3 (Table 4a).

Combining ability. The quadratic means of GDH activity for GCA and SCA were highly significant, with the preponderance of SCA, indicating that the character was governed mainly by non–additive gene, and less by additive gene action (Tab. 4b).

The best general combiners for GDH activity were the genotypes SNRF–4 and SNRF–2, the poorest was SNRF–1 (Table 4c).

All 6 hybrids had good SCA for this character (Table 4d).

Table 4a. Mean values for GDH activity (μ mole NAD^+ g^{-1} fr. wt. h^{-1}) in diallel sunflower crosses (parents and F_1), $LSD_{0.05}$ = 1.56; $LSD_{0.01}$ = 1.73

Parent	(1) SNRF–1	(2) SNRF–2	(3) SNRF–3	(4) SNRF–4	(5) SNRF–5
(1) SNRF–1	33.33	11.09^{h-}	30.13^{pd}	17.06^{h-}	24.80^{i}
(2) SNRF–2		37.76	30.88^{i}	26.56^{h-}	41.60^{h+}
(3) SNRF–3			18.45	42.72^{h+}	22.39^{h+}
(4) SNRF–4				34.66	33.70^{h-}
(5) SNRF–5					14.66

Table. 4b. ANOVA of combining ability for GDH activity

Source of variation	DF	MS	Fe	LSD	
				0.05	0.01
GCA	4	49.55	238.79^{**}	2.69	4.02
SCA	10	113.49	546.89^{**}	2.16	2.98
E	28	0.20			

Table 4c. GCA values for GDH activity

Parent	GCA	Rank	SE	LSD	
				0.05	0.01
SNRF–1	–2.59	5			
SNRF–2	2.53^{**}	2			
SNRF–3	–0.69	3	0.15	0.49	1.14
SNRF–4	3.06^{**}	1			
SNRF–5	–2.29	4			

Table 4d. SCA values for GDH activity

Parent	(2) SNRF–2	(3) SNRF–3	(4) SNRF–4	(5) SNRF–5	SE	LSD	
						0.05	0.01
(1) SNRF–1	–10.88	11.02^{**}	–5.33	7.09^{**}			
(2) SNRF–2		7.28^{**}	–0.32	19.44^{**}			
(3) SNRF–3			18.65^{**}	3.02	0.39	1.22	2.79
(4) SNRF–4				11.04^{**}			

e) Leaf protein content

Variability and mode of inheritance. The highest leaf protein content was found in the genotype SNRF–4 ($\overline{X}$ = 24.05), the lowest in SNRF–5 ($\overline{X}$ = 9.75 mg protein g^{-1} fr. wt.).

In the F_1 generation, leaf protein contents ranged from $\overline{X}$ = 41.06 in the combination SNRF–3 x 4 to $\overline{X}$ = 5.05 mg protein g^{-1} fr. wt. in the hybrid SNRF–1 x 2 (Table 5a).

In the hybrids SNRF–1 x 5 and SNRF–3 x 5, full dominance occurred in the inheritance of leaf protein content. In the hybrids SNRF–1 x 3 and SNRF–4 x 5, the inheritance was intermediate, while in the combination SNRF–2 x 3 partial dominance was present. Positive heterosis was found in two and negative in three combinations (Table 5a).

Combining ability. The results of variance analysis of combining ability for leaf soluble proteins show that the quadratic means of GCA and SCA were highly significant, with the preponderance of SCA variance (Table 5b).

The genotype SNRF–4 having the highest leaf protein content was also the best combiner for high protein content (Table 5c).

The highest value of SCA was found in the cross SNRF–3 x 4 in which one high GCA and one low GCA parent was included. Other combinations also showed good SCA (Table 5d).

Table 5a. Mean values for leaf soluble protein content (mg protein g^{-1} fr. wt.) in diallel sunflower crosses (parents and F_1), $LSD_{0.05}$ = 1.83; $LSD_{0.01}$ = 2.46

Parent	(1) SNRF–1	(2) SNRF–2	(3) SNRF–3	(4) SNRF–4	(5) SNRF–5
(1) SNRF–1	19.82	5.05^{h-}	17.77^{t}	9.75^{h-}	17.01^{d+}
(2) SNRF–2		21.99	19.07^{pd}	17.66^{h-}	25.35^{h+}
(3) SNRF–3			12.57	41.06^{h+}	12.57^{d+}
(4) SNRF–4				24.05	17.33^{i}
(5) SNRF–5					9.75

Table 5b. ANOVA of combining ability for leaf soluble protein content

Source of variation	DF	MS	Fe	LSD	
				0.05	0.01
GCA	4	48.37	120.76^{**}	2.69	4.02
SCA	10	81.36	203.12^{**}	2.16	2.98
E	28	0.40			

Table 5c. GCA values for leaf soluble protein content

Parent	GCA	Rank	SE	LSD	
				0.05	0.01
SNRF–1	–2.72	5			
SNRF–2	0.39	3			
SNRF–3	1.03^{*}	2	0.21	0.69	1.58
SNRF–4	3.65^{**}	1			
SNRF–5	–2.36	4			

Table 5d. SCA values for leaf soluble protein content

Parent	(2) SNRF–2	(3) SNRF–3	(4) SNRF–4	(5) SNRF–5	SE	LSD 0.05	LSD 0.01
(1) SNRF–1	–7.09	5.06**	–5.24	7.28**			
(2) SNRF–2		3.62*	0.07	12.88**			
(3) SNRF–3			22.76**	–0.45	0.55	1.69	3.88
(4) SNRF–4				2.02**			

f) Correlation between nitrogen metabolism enzyme activity and leaf soluble protein content

There were significant positive correlations between the activity of the examined nitrogen metabolism enzymes (NR, GS, GOGAT and GDH) and the content of leaf soluble proteins in the tested sunflower lines and their hybrids. All coefficients of correlation were significant ($r = 0.979$ for NR, $r = 0.605$ for GS, $r = 0.849$ for GOGAT and $r = 0.658$ for GDH). This means that leaf protein content in young plants of sunflower lines and their hybrids could be judged on the basis of enzyme activity.

DISCUSSION

The obtained results show that the examined sunflower lines and F_1 hybrids differed significantly in the activity of NR, GS, GOGAT and GDH, and leaf protein content. Therefore, for development of high protein F_1 hybrids, a possibility should be considered of using the activity of NR and other nitrogen metabolism enzymes as a "biochemical" criterion in selecting inbred lines for hybridization. NR activity is one of many similar "biochemical" criteria used at the seedling stage in order to facilitate an early identification of parents in programs of hybridization (Hageman and Flesher, 1960; Hageman *et al.*, 1967; Eilrich and Hageman, 1973; Johnson *et al., 1969; Croy and Hageman, 1970; Duffield et al.,* 1972; Dalling and Loyn, 1977; Hewitt, 1979).

The rating of the parents on the basis of their GCA effects shows that the inbred line SNRF–4 was the best combiner for all examined characters (NR, GS, GOGAT, GDH activity and leaf soluble protein content), and thus should be used for hybridization. The cross which showed highly significant values for the examined characters was SNRF–4 x 3 , where the best general combiner SNRF–4 was included. The tested charachters were under control of both additive and non–additive gene action.

Furthermore, the results obtained indicated the existence of a significant correlation ($r = 0.979$) between NR activity and leaf soluble protein content in the young plants of sunflower lines and their F_1 hybrids. Apparently, as the intensified NR activity makes nitrate reduction more efficient, this in turn intensifies the biosynthesis of leaf soluble proteins. The correlations between GS, GOGAT, GDH activity and leaf soluble protein content were also highly significant.

Since this experiment was conducted under controlled (greenhouse) conditions, it remains to be studied whether the activity of NR and other nitrogen metabolism enzymes (GS, GOGAT and GDH) is also indicative for protein content in the leaf and grain and NHI (nitrogen harvest index) under field conditions.

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ACTIVIDAD DE ENZIMAS DE ASIMILACION DE NITROGENO EN HOJAS DE PLANTAS JOVENES DE GIRASOL (*Helianthus annuus* L.)

RESUMEN

Los resultados de una investigación bioquímica y genética mostró que existian diferencias significativas en los parámetros bioquímicos examinados (actividad enzimática y contenido de proteína) entre los genotipos testados y sus híbridos F_1.

La herencia de los caracteres examinados estuvo controlada por acciones genéticas aditiva y no–aditivas, como indicaron los resultados del análisis de la varianza para aptitud combinatoria general (ACG) y especifica (ACE). Estos resultados estuvieron de acuerdo con los resultados obtenidos para el modo de herencia cuando las combinaciones fueron observadas separadamente, donde la herencia intermedia, dominancia completa o parcial o la heterosis tuvieron lugar.

El genotipo SNRF–4 mostrando los valores medios mas altos de actividad enzimática (NR, GS, GOGAT y GDH) y el contenido proteínico de la hoja, fue también el mejor combinador general.

Existió una correlación positiva entre la actividad enzimática (NR, GS, GOGAT y GDH) y contenido de proteína de la hoja.

ACTIVITÉ DES ENZYMES D'ASSIMILATION DE L'AZOTE DANS LES FEUILLES DE JEUNES PLANTES DE TOURNESOL

RÉSUMÉ

Les résultats d'une recherche biochimique et génétique montre qu'il y a des différences significatives pour les paramètres biochimiques examinés (activité enzymatique et teneur en protéine) entre génotypes de tournesol testés et leurs hybrids F1.

Le mode transmission héréditaire des caractères examinés est controlé par des actions géniques additives et non additives, comme le montrent les résultats d'une analyse de variance pour l'aptitude générale (AGC) et spécifique (ASC) à la combinaisonon. Ces résultats concordent avec ceux qui sont obtenus lorsque les combinaisons sont étudiées séparément, où des modes de transmission intermédiaires, complètement ou partiellement dominant, ou reflétant un hétérosis, sont enregistrés. Le génotype SNRF - 4 qui exhibe les plus fortes valeurs pour les activités enzymatiques (NR, GS, GOGAT et GDH) et pour la teneur en protéine des feuilles, es également celui qui a la meilleure aptitude générale à la combinaison. Il est donc recommandé comme parent pour obtenir en hybridisation une forte teneur en protéine.

Il y a une corrélation positive entre l'activité enzymatique (NR, GS, GOGAT et GDH) et la teneur en protéine des feuilles.