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1. Genetics of the meiotic mutant st_4 .^{*}

In 1968 Walter R. Fehr, Department of Agronomy, Iowa State University, observed sterile plants in a single plant progeny row of the cultivar 'Hark'. Further analyses by Fehr indicated that sterility existed in both sexes and that it was a recessive genetic trait (Fehr, personal communication, 1970).

The current research effort began in 1970. Fertile plants from segregating progenies were threshed individually in 1970, 1971, 1972 and 1973. These progenies were evaluated each succeeding year and data were recorded for number of segregating progenies (Table 1), ratio of fertile plants : sterile plants (Table 2), and seed set on the near-sterile plants (Table 3). The data in Tables 1 and 2 support the hypothesis that sterility is associated with the homozygous condition of a single recessive gene.

We were interested in the number of seeds produced on the near-sterile plants and in their chromosome constitution. There was considerable variation in seed set from year to year (Table 3). This was particularly evident in the number of seeds per near-sterile plant within line T258^{**} : 1 seed per 7.4 sterile plants in 1971; 1 seed per 22.2 sterile plants in 1972; 1 seed per 18.7 sterile plants in 1973. These differences are also reflected in the percent completely sterile plants for the three different years (Table 3). There was no noticeable difference in the number of flowers per plant in the three years.

Interestingly, two different F_2 populations grown in 1973 in which the st_4 ^{**} gene was segregating had fewer completely sterile plants than the original line (Table 3). Similarly, we observed 1 seed per 3.5 sterile plants and 1 seed per 4.5 sterile plants, respectively, for the two different

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^{**} Genetic type collection T-number and gene symbol assigned by Soybean Genetics Committee, January, 1974.

F₂ populations. The most likely explanation for the differences observed between the original line and the F₂ populations is genotype x environment interaction.

Pollen from fertile and sterile plants of T258 was classified for stainability using I₂KI. The percentage of plump and well-stained pollen grains from fertile and sterile plants was 97.3 and 2.7, respectively. Pollen grains from sterile plants varied greatly in size. Some grains were collapsed, devoid of starch and smaller than grains from fertile sibs; some were considerably larger.

Cytological observations were made of meiotic chromosomes in sterile plants. The earlier meiotic stages in soybeans are not amenable to detailed cytological study and presently we cannot distinguish between desynapsis and asynapsis of the chromosomes.

Two asynaptic mutants in soybeans, T241 and T242, were described by Hadley and Starnes (1964). Allelism tests were conducted with T241 and T258 (Table 4); and with T242 and T258 (Table 5). In Table 4, progenies in five F₂ families (PR 19-2, 25-2, 28-2, 29-1, and 29-4) represent a 3:1 population. The remaining five families represent a 9:7 population. If segregation for sterility were at two loci and if the steriles were phenotypically similar, the expected ratio would be 9 fertile : 7 sterile. Similarly in Table 5, the F₂ families fit into two discrete populations, one representing a 3:1 segregation, the other a 9:7 segregation. Therefore, from the data presented in Tables 4 and 5, we can conclude that st₄ is a different locus than st₂ and st₃.

T241 has white flowers (w) and tawny pubescence (T); T242 has purple flowers (W) and gray pubescence (t); and T258 has purple flowers and gray pubescence. Chi-square tests for independent assortment between st₄ and flower color, st₄ and pubescence color were calculated. The data gave no indication of linkage.

We are determining mitotic chromosome numbers of progeny produced by the homozygous st₄ plants. The diploid chromosome number of soybeans is 2N=40. Aneuploids between 40-45 chromosomes have been identified. Aneuploids are also found at the tetraploid level.

Table 1
Segregating and nonsegregating F_2 families in T258 for 1971,
1972, 1973 (expected ratio — 2 segregating : 1 nonsegregating)

Year	Segregating	Nonsegregating	χ^2	Probability
1971	95	42	0.4416	.75-.50
1972*	39	28	2.1567	.25-.10
1973	28	15	0.0465	.90-.75
Total	162	85	2.6448	
Pooled chi-square (1df)			0.1296	.75-.50
Homogeneity chi-square (2df)			2.5152	.50-.25

* One family had segregating ratios of 1 segregating : 7 nonsegregating progenies rather than the expected 2:1 ratio and is not included.

Table 2
Fertile and sterile plants in segregating F_2 families of
T258 and T258 crosses (expected ratio — 3 fertile : 1 sterile)

Year	Fertile	Sterile	χ^2	Probability
1971*	1099	353	0.3673	.75-.50
1972	1106	400	1.9557	.25-.10
1973	809	281	0.3535	.75-.50
1973**	390	128	0.0232	.90-.75
1973***	926	322	0.4274	.75-.50
Total	4330	1484	3.1271	
Pooled chi-square (1df)			0.85	.50-.25
Homogeneity chi-square (4df)			2.2771	.75-.50

* Includes only 66 of the 95 segregating families.

** Segregating F_2 families from crosses between homozygous fertile plants of T241 and heterozygous fertile plants of T258.

*** Segregating F_2 families from crosses between homozygous fertile plants of T242 and heterozygous fertile plants of T258.

Table 3
Number of seeds from st₄st₄ plants

Number of seeds per plant	Number of plants				
	1971*	1972*	1973*	1973**	1973***
0	519	385	267	100	261
1	58	13	13	23	55
2	11	1	1	2	4
3	0	1	0	2	1
4	0	0	0	1	0
5					0
6					1
<hr/>					
Total number of plants	588	400	281	128	322
Total number of seeds	80	18	15	37	72
% plants with no seeds	88	96	95	78	81

*Segregating F₂ families of T258.

**Segregating F₂ families from crosses between homozygous fertile plants of T241 and heterozygous fertile plants of T258.

***Segregating F₂ families from crosses between homozygous fertile plants of T242 and heterozygous fertile plants of T258.

Table 4

Observed ratios in segregating F_2 families from crosses between heterozygous fertile plants of T241 and heterozygous fertile plants of T258

Families that were considered to be samples from a 3:1 population of $\underline{St}_2 \underline{St}_2 \underline{St}_4 \underline{st}_4$ or $\underline{St}_2 \underline{st}_2 \underline{St}_4 \underline{St}_4$ F_2 plants

F_2 number	Fertile	Sterile	Chi-square and probability for expected ratios			
			$\chi^2(3:1)$	P	$\chi^2(9:7)$	P
PR 19-2	69	23	0.0	0	13.14	<.005
PR 25-2	112	42	0.42	.75-.50	16.99	<.005
PR 28-2	122	44	0.20	.75-.50	20.06	<.005
PR 29-1	88	35	0.78	.50-.25	11.69	<.005
PR 29-4	186	74	1.66	.25-.10	24.69	<.005

Families that were considered to be samples from a 9:7 population of $\underline{St}_2 \underline{st}_2 \underline{St}_4 \underline{st}_4$ F_2 plants

F_2 number	Fertile	Sterile	Chi-square and probability for expected ratios			
			$\chi^2(3:1)$	P	$\chi^2(9:7)$	P
PR 19-1	106	82	34.75	<.005	0.001	.975-.95
PR 25-1	67	57	29.08	<.005	0.25	.75-.50
PR 26-1	61	47	19.75	<.005	0.002	.975-.95
PR 28-1	46	42	24.24	<.005	0.57	.50-.25
PR 29-5	57	39	12.50	<.005	0.38	.75-.50

Table 5

Observed ratios in segregating F_2 families from crosses between heterozygous fertile plants of T242 and heterozygous fertile plants of T258

Families that were considered to be samples from a 3:1 population of $\underline{St}_3 \underline{St}_3 \underline{St}_4 \underline{st}_4$ or $\underline{St}_3 \underline{st}_3 \underline{St}_4 \underline{st}_4$ F_2 plants

F_2 number	Fertile	Sterile	Chi-square and probability for expected ratios			
			$\chi^2(3:1)$	P	$\chi^2(9:7)$	P
PR 2-1	71	21	0.23	.75-.50	16.37	<.005
PR 15-2	146	58	1.28	.50-.25	19.45	<.005
PR 15-3	127	42	0.002	.975-.95	24.53	<.005
A 1258-1	190	76	1.81	.25-.10	24.90	<.005
A 1258-2	294	108	0.75	.50-.25	46.57	<.005
A 1260-1	238	77	0.05	.90-.75	47.71	<.005
A 1260-2	268	94	0.18	.75-.50	46.52	<.005

Families that were considered to be samples from a 9:7 population of $\underline{St}_3 \underline{st}_3 \underline{St}_4 \underline{st}_4$ F_2 plants

F_2 number	Fertile	Sterile	Chi-square and probability for expected ratios			
			$\chi^2(3:1)$	P	$\chi^2(9:7)$	P
PR 10-1	47	31	9.04	<.005	0.51	.50-.25
PR 13-1	42	26	6.35	.025-.01	0.84	.50-.25
PR 13-2	87	63	23.12	<.005	0.19	.75-.50
PR 13-3	50	30	6.67	.01-.005	1.27	.50-.25
PR 13-4	27	20	7.72	.01-.005	0.03	.90-.75
PR 13-5	32	18	3.23	.01-.005	1.22	.50-.25
A 1258-3	229	180	78.83	<.005	0.01	.95-.90
A 1261-1	222	153	49.93	<.005	1.33	.25-.10
A 1261-2	156	143	83.09	<.005	2.02	.25-.10

References

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2. Male transmission of an extra chromosome.*

Three primary trisomes were studied in 1973. Two trisomes originated from an asynaptic T241 (st₂ st₂) plant; the other trisome came from a T258 (st₄ st₄) plant. We had previously observed 42-chromosome plants among the selfed progeny of 41-chromosome plants and we suspected simultaneous female and male transmission of the extra chromosome. We were also interested in testing to determine if the extra chromosomes present in the three trisomes were identical or different. Therefore, the trisomes were reciprocally intercrossed, crossed both as male and female parents with the cultivar 'Hark' and also selfed. Chromosome numbers of the parental trisomes and F₁ plants were determined from root tip squash preparations.

Observations on male transmission of the extra chromosome are presented in Table 1. The values for male transmission of each trisome, A73-T23**, A73-T25**, and A73-T33***, are very high (approximately 25%), when compared with published reports describing primary trisomes in other plant species (generally 0 to 15%). Additional F₁ seedlings will be classified for chromosome number to determine if the values obtained are representative of male transmission in soybeans. The identity of the three trisomes will be established in the summer of 1974.

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** From a T241 (st₂ st₂) plant.

*** From a T258 (st₄ st₄) plant.

Table 1
Male transmission of an extra chromosome in three
primary trisomes in soybeans

Female parent		Male parent	Frequency of F ₁ seedlings with	
(40 chromosomes)		(41 chromosomes)	40	41
Cultivar		Plant No.	chromosomes	chromosomes
Hark	x	A73-T23-5	9	1
Hark	x	A73-T23-18	6	4
Hark	x	A73-T25-2	6	4
Hark	x	A73-T25-13	8	2
Hark	x	A73-T33-6	4	1
Hark	x	A73-T33-9	4	1
Hark	x	A73-T33-11	3	2
Hark	x	A73-T33-13	3	2
Hark	x	A73-T33-16	5	0

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3. A new mutation for sterility*

In our genetic studies of st₄ (T258) we routinely harvest seeds from the near-sterile plants (st₄ st₄) and study the progeny. Near-sterile plant number 3008-1, found in 1970, had one very small seed, which was subsequently planted in summer 1971. A chromosome count was not obtained from this plant (A71-T48), but it was highly sterile as judged by I₂KI pollen staining; however, it produced four seeds. The pollen from A71-T48 was not typical of the st₄ sterile. It had large, dark-staining pollen grains instead of the smaller, collapsed, non-starch-filled pollen found in st₄ steriles. The pollen grains of A71-T48 seemed to have general characteristics of pollen produced by ms₁ plants (Brim and Young, 1971). The line carrying the ms₁ gene, however, was not grown at Ames, Iowa prior to 1971.

In 1972, all four seeds from A71-T48 were each determined by root tip squash preparations to have 40 chromosomes and were transplanted to the field.

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Of the three survivors, one was completely fertile and produced many seeds (A72-T28); the other two plants (A72-T30 and A72-T31) were highly sterile with sterility phenotypically identical to the A71-T48 plant.

Two crossed seeds (male parent cultivar 'Clark 63') and two outcrossed seeds were obtained from plant A72-T30. One F_1 seed of the Clark 63 cross and one outcrossed seed were grown in the greenhouse in winter 1972-73 and both plants were highly fertile. The results from the F_1 and F_2 generation in summer 1973 are presented in Table 1.

Table 1
Pollen fertility of F_1 and F_2 progeny from a mutant
found in the st_4 sterile

1972 family number	fertile plants	1973 - number of		
		sterile plants		χ^2 test
		st_4	non- st_4	
A72-T28 fertile	51	16	24	0.14(9:3:4)
A72-T29 died				
A72-T30 sterile (non- st_4)				
F_2 (Clark 63 male)	19	0	7	0.05(3:1)
F_1 (Clark 63 male)	1			
F_2 (outcross origin)	22	0	7	0.01 (3:1)
F_1 (outcross origin)	1			
A72-T31 sterile (non- st_4)	No progeny			

Meiotic studies indicated first division was normal in the non- st_4 plants, A72-T30 and A72-T31; second division was not studied. Thus, the mechanism responsible for the non- st_4 sterility could either be operative in second division of meiosis or it could be post-meiotic.

We hypothesized that pollen from 6 F_2 plants (1/16 of 91) from A72-T28 were phenotypically classified as being from non- st_4 steriles even though genetically the plants were recessive for both genes. Meiosis will be studied from non- st_4 plants from A72-T28. It should be possible to classify

those plants that are double recessive, both on the basis of asynapsis (desynapsis), and on the appearance of pollen grains.

References

Brim, C. A. and M. F. Young. 1971. Inheritance of a male sterile character in soybeans. *Crop Sci.* 11:564-566.

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4. Distribution of canavanine in the genus *Glycine* and related genera.

Relatives of the soybean in the genus *Glycine* have been studied by many workers. Immediate kindred in other genera have not been examined for characters of agronomic interest or possible close relationship to the soybean itself. This report is one facet of our study of the soybean and related genera.

The free amino acid canavanine is known primarily from seeds of advanced legume tribes. Absence of canavanine in some species is thought to be due to the loss of ability to produce the compound, and is considered advanced. Since previous surveys have shown canavanine to be a useful marker of other legume groups, we felt it might help evaluate relationships in the *Glycine* subtribe, the Glycininae, and its parent tribe, the Phaseoleae.

Procedures for detection of canavanine were adapted from Bell (1958).

Canavanine presence-absence, compiled in Table 1, is constant within most subtribes: all sampled Galactiinae contain canavanine; all Cajaninae, Erythrinae, and Phaseolinae lack it. But Glycininae are inconsistent, and the homogeneity of the subtribe may be questioned. Group 3, conventionally including *Glycine*, is primarily negative, but there are three exceptions: *Shuteria*, which perhaps better belongs with group 2; *Ophrestia* (*Paraglycine*) *hedysaroides*, anomalous in that genus on morphological bases; and *Glycine wightii*, exceptional in *Glycine*, both on cytological and morphological grounds.

These preliminary findings give rise to several considerations:

1) The correlation between canavanine presence or absence, and the morphological characters upon which several subtribes are based, tends to

Table 1
Species of Phaseoleae examined for canavanine

Genera-Species examined ^a	Reaction ^b	Genera-Species examined ^a	Reaction ^b
Cajaninae		Glycininae ^c	
Cajanus (1)	-	<u>Group 1</u>	
Cantharospermum (1)	-	Centrosema (5)	+
Eminia (1)	-	Periandra (1)	-
Eriosema (1)	-	Clitoria (7)	-
Rhynchosia (9)	-	<u>Group 2</u>	
Diocleinae		Cologania (4)	-
Canavalia (5)	+	Amphicarpaea (1)	-
Pueraria (1)	-	Dumasia (1)	-
Galactiinae		<u>Group 3</u>	
Calopogonium (1)	+	Shuteria (1)	+
Galactia (3)	+	Glycine clandestina	-
Erythrinae		Glycine falcata	-
Apios (2)	-	Glycine canescens ^d	-
Erythrina (3)	-	Glycine tabacina	-
Mucuna (4)	-	Glycine tomentella	-
Phaseolinae		Glycine wightii	+
Dolichos (6)	-	Glycine soja	-
Phaseolus (9)	-	Glycine gracilis	-
Strophostyles (1)	-	Glycine max	-
Vigna (4)	-	Teramnus (1)	-
		Ophrestia hedysaroides	+
		Ophrestia radicata	-
		Pseudoeriosema (1)	-
		<u>Group 4</u>	
		Hardenbergia (2)	+
		Kennedya (9)	+

^aNumbers in parentheses are for numbers of species tested.

^b+ = positive; - = negative.

^cGroups as given by Bentham and Hooker (1865).

^dThe determination for Glycine canescens is dubious: seed viability for these samples is in question.

validate both the significance of these characters and the possible usefulness of canavanine determinations as a marker of relationships. Instances in which the canavanine seems to be 'out of line' should be restudied with respect to the reasons for placing a given species within a certain genus or the affiliation of a genus with a certain subtribe. Such circumstances may, of course, be caused by loss of an ability to produce canavanine among closely related species (or genera), but in other instances, it may be a clue that a casually assumed relationship is faulty.

2) Previous morphological and cytological evidence suggests that the Glycininae and several included genera are neither homogeneous nor natural, and these data support such a contention. Within Glycine, the conclusions of Pritchard and Wutoh (1964) are supported: these authors, on the basis of cytological studies, separated G. wightii ($2n = 22,44$) from other species of Glycine ($2n = 40,80$).

3) Species and genera which have traditionally been placed outside the Glycininae may more properly belong within it, and may be related to Glycine. The Galactiinae are particularly suspect for this possibility, based on these findings and others.

References

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5. The effect of temperature on the variegation of Y_{18}^m .

In 1951, a variegated plant was found in the cultivar 'Lincoln' at Ames by C. R. Weber. It has been suggested by Peterson and Weber (1969) that this variegation was due to instability at the Y locus (Y_{18}^m). The purpose of this investigation is to determine the effect of temperature on the Y_{18}^m locus.

Seeds from families known to be segregating for variegation were

inoculated with Rhizobium japonicum (serotype 110-8T), and planted in 4-inch clay pots containing a sterile 2 soil : 1 peat : 1 sand mixture. Inoculation with Rhizobium was necessary to obtain nodule formation and nitrogen fixation by the soybean plants. The pots were placed in two growth chambers which maintained 65 F and 85 F environments. After all the leaves had fully expanded, they were removed from the plants and were photographed. The number, size, type of mutation, and leaf area were determined from these photographs. Mutated areas and leaf areas were measured with a polar planimeter. For analysis, it was necessary to assign leaflet position within trifoliate leaves. The middle leaflet was assigned position 2; the leaflet to the left, position 1; the leaflet to the right, position 3. The two unifoliate leaves in the analyses are considered a single unit, and the values presented are an average of both values. This average is designated by \overline{Un} .

At 65 F, there were more total mutations and more leaf area mutated than at 85 F. The percentage area mutated at 65 F and 85 F differed only slightly, but this may be a result of a difference in growth of plants at 65 F and 85 F. Mutations to yellow were fewer at 85 F than at 65 F, but involved a larger proportion of leaf area. Light green areas constituted a greater proportion of the leaf area at 65 F than at 85 F (Tables 1 and 2).

At 85 F (Tables 2 and 3), leaflet position 2 had the largest percentage of area mutated, and the largest number of yellow and light green areas. These results, however, are not found at 65 F, except in the yellow areas. Comparison of the total number of mutations and total area mutated according to leaflet position showed distinct differences between the values at 85 F and 65 F (Table 3). The values for area per mutation differed only slightly with leaf position and temperature. At 85 F the yellow area per mutation was larger than the area per mutation at 65 F. This may indicate that mutations to yellow occur earlier in the leaf ontogeny at 85 F than at 65 F (Table 1).

The data may be summarized as follows: The lower temperature (65 F) caused an increase in the number of mutations and an increase in the total area mutated compared with the results at 85 F. The total percentage area mutated and area per mutation differed only slightly between temperature treatments and leaflet positions. At 65 F the percentage of light green

Table 1

The effect of temperature on the number of mutations,
leaf area mutated, and area per mutation

	65 F	85 F
Number of yellow areas	6,967	2,923
Number of light green areas	<u>8,971</u>	<u>1,838</u>
Total	15,938	4,761
Yellow area (in.) ²	75.7	109.8
Light green area (in.) ²	<u>372.1</u>	<u>57.7</u>
Total (in.) ²	447.8	167.5
Average yellow area	0.010	0.038
Average light green area	<u>0.041</u>	<u>0.031</u>
Total area (in.) ² per mutation	0.028	0.035

Table 2

The effect of temperature on the percent leaf area
mutated with reference to leaflet position

	65 F				85 F			
	Leaflet position				Leaflet position			
	<u>Un</u>	1	2	3	<u>Un</u>	1	2	3
% of total yellow area	4.6	1.7	5.0	4.6	13.8	18.0	23.4	9.9
% of total light green area	<u>15.2</u>	<u>25.5</u>	<u>21.4</u>	<u>24.9</u>	<u>8.5</u>	<u>8.2</u>	<u>9.5</u>	<u>8.4</u>
Total	19.8	27.2	26.4	29.5	22.3	26.2	32.9	18.3

Table 3
The effect of temperature and leaflet position on the number of mutations,
leaf area mutated, and area per mutation

	65 F				85 F			
	Leaflet position				Leaflet position			
	<u>Un</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>Un</u>	<u>1</u>	<u>2</u>	<u>3</u>
Number of yellow areas	1025	1525	1961	2456	275	849	973	826
Number of light green areas	<u>1189</u>	<u>2961</u>	<u>3125</u>	<u>1696</u>	<u>375</u>	<u>483</u>	<u>497</u>	<u>483</u>
Total	2214	4486	5086	4152	650	1332	1470	1309
Yellow area (in.) ²	16.2	13.7	26.9	18.9	12.3	34.8	42.8	19.8
Light green area (in.) ²	<u>52.9</u>	<u>105.9</u>	<u>113.0</u>	<u>100.3</u>	<u>7.4</u>	<u>15.9</u>	<u>17.4</u>	<u>16.9</u>
Total	69.1	119.6	139.9	119.2	19.7	50.7	60.2	36.7
Yellow area per mutation (in.) ²	0.02	0.01	0.01	0.01	0.04	0.04	0.04	0.02
Light green area per mutation (in.) ²	<u>0.04</u>	<u>0.04</u>	<u>0.04</u>	<u>0.04</u>	<u>0.02</u>	<u>0.04</u>	<u>0.04</u>	<u>0.03</u>
Total area (in.) ² per mutation	0.03	0.03	0.03	0.03	0.02	0.04	0.04	0.03

leaf area was greater than the percentage at 85 F. Conversely, the yellow areas constituted a larger percentage of the leaf at 85 F than at 65 F.

References

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1. Seed protein percentage and sulfur-containing amino acid contents in wild soybean (*Glycine soja* Sieb. and Zucc.) strains native in Japan.

Japanese cultivated soybeans (*G. max* (L.) Merrill) have been known as protein-rich. However, as the world protein malnutrition problem has been urged to be solved, the development of much higher protein strains is considered to be an indispensable task for soybean breeders. Since the hybridizations of *G. max* x *G. soja* (= *G. ussuriensis*) by Williams (1948) and Weber (1950), *G. soja* has been regarded as a promising protein gene source in breeding of the *G. max* varieties with high protein. However, little information is available for the qualitative aspect of *G. soja* protein. The authors *et al.* (1972) have already published about the amino acid composition of the species, along with the other *Glycine* species. Subsequently, the present paper aims to elucidate the inter-strain variability of sulfur-containing amino acid contents in *G. soja* seed protein.

Twenty-nine *G. soja* strains were collected from various places of Japan in 1969 and grown in pots at Morioka, Iwate-ken, in 1970, including 8 representative *G. max* varieties as control. Based on morphological differentiation, the 36 strains were separately harvested and exploited for subsequent chemical analyses. From the seed meal of each strain, protein