DYNAMICS OF ALKALOID BIOSYNTHESIS IN CASE OF INTRASPECIFIC SOMATIC HYBRIDIZATION IN SOME *CLAVICEPS PURPUREA* STRAINS

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In this paper, the dynamics of T:S:P ratio and of total alkaloid content was registered, for *Claviceps purpurea* sclerotia of ergotamine (T), ergocristine (S) and ergocryptine (P) type and their descendance, both for parental strains and hybridization products (also for those of backcrosses), and their descendance, in successive generations. The results differed depending on origin-sclerotia alkaloid type, hybrid or non-hybrid nature of ascendants, number of analysed generations. Thus, in *ergotamine strain hybridizations*, possibly a manifestation of so-called "variability of generation average", for the case of multiple selection generations was observed. Also, a hybrid vigour effect was noted for F1 hybrid descendances, as a result of heterokaryosis degree and gene heterozygosity increase after somatic hybridization, phenomenon not evidenced in F1 *ergocristine hybridizations*. In *ergocryptine strain hybridizations*, frequently a turning from ergocryptine to the ergocristine or ergotamine type takes place. The nuclear reassortment and the new rates between genetically different nucleuses determined the significant modification of the alkaloid spectrum.

Key words: *Claviceps purpurea*, ergopeptide alkaloids, somatic hybridization, successive generations.

INTRODUCTION

The genetic studies on the *Claviceps purpurea* ergot fungus and their application in the alkaloid biosynthesis potential amelioration were difficult, because of the complex life cycle of the species belonging to the *Claviceps* genus (2). The insufficient knowledge about the genetic determinism of alkaloid synthesis and the great phenotype variability at the *Claviceps purpurea* phytopathogen fungus reflects in the amelioration activity of the character. Even the sclerotia formed on the same ear have a variable alkaloid content, because the infection could be due to some ascospores or some conidia of different origins. Likewise, using spores from a pure culture will not lead to obtaining uniform sclerotia under the biosynthesis potential aspect (20). Spalla, 1973 and Spalla and Marnati, 1978 consider that the great variability of the level and the quality of the ergot alkaloids is given by the complex genetic nature of the *Claviceps purpurea* fungus, but also by the strong interaction with the environmental factors (16, 17). At the filamentous fungi, the haplophase has the greatest temporal extent, all the genes being freely expressed, without the intervention of the dominance that would mask the phenotype expression of the recessive determinants. Plasmogamy is important, because not only the nucleuses are gathered in same place but the cytoplasms too.

Dikaryophase and the diploidy are reduced as time. Karyogamy and the meiosis take place in the developing asci, assuring the recombination and segregation of the genetic material. This phase succession is under the control of a complex gene system (3, 6). Beneath the sexual cycle, a parasexual cycle is described, that includes an event sequence, that results in the somatic hybridization and which is considered as a characteristic way of fungi recombination (13). There is a correlation between the nucleuses number and the biosynthesis capacity, expressed in the total level and the quality of synthesized alkaloids. In this way, there are at least two nucleuses in sclerotia cells, between 2–22 nucleuses in the structures forming and accumulating alkaloids in submerged conditions (1, 11, 13) and in the cells of the alkaloid non-producing cultures only one nucleus is found (21). This fact could prove that the biosynthesis of clavine alkaloids and simple amides of lysergic acid is dependent on the presence of 1–2 nucleuses/cell, while the peptide alkaloids are synthesized under the conditions of a more complex gene interaction, that has needed the existence of more nucleuses. The viability and virulence of the conidia formed in the submerged culture are much better expressed in multinuclear state (15, 22).

The filamentous fungi somatic hybridization, as well as of other eukaryotes, is a way of genetic variability improvement, not only through sexual compatibility overrun but also through the genomes combination (nuclear, mitochondrial) into a new profile (10). There are at least two pairs of alleles that influence the forming, growth, vigour and maintaining of the heterokaryosis state, defined as the "coexistence state of genetically different nucleuses in two in continuity lying cytoplasm" (3, 5). The mono- and multinucleate cells of active mycelium show continuity through the septal pores. For this reason, it can be considered as an organism but as well as a population of nucleuses in a "cytoplasmatic medium". As a result, it is possible that the phenotype of one colony is uniform, but the presence of some sectors, as a result of the phenotype expression by the gene determinants of several nucleuses, is not excluded. This determinism mechanism, present at *Claviceps* too, can be a proof for the heterokaryosis state (1, 17).

MATERIAL AND METHODS

The analysed biologic materials are the *Claviceps purpurea* (Fr.) Tul. sclerotia harvested from the cultivated graminaceous, especially autumn rye (*Secale cereale*), *Ergo* and *Dankovskoe zlote* cultivars. The sclerotia were characterized from the point of view of the total quantity of synthesized alkaloid (CAT) and of the alkaloid spectrum (ergo*T*amine:ergocri*S*tine:ergocry*P*tine – T:S:P). These determinations were made on the material resulted from one half of a sclerotium, the other half being destined for obtaining sclerotial descendance.

The asepsis of vegetal material was made through successive sclerotia treatment with isopropyl alcohol solution and formol, each of them being preceded

and followed by rinses with sterile distilled water. The sclerotium is cut up, and the fragments are placed on the agar medium surface. The cultures are incubated at 28 °C, for 18–21 days. To obtain submerged cultures, the colonies formed on agar media are inoculated in liquid medium. The flasks are 3–5 days incubated, at 24 °C, under stirring. The nutritive agar T_2 medium contains: sucrose; α-asparagine; $Ca(NO_3)_2.4H_2O$; $MgSO_4.7H_2O$; levulose extract; KCl; $FeSO_4.7H_2O$; $ZnSO_4.7H_2O$, pH 5.2 (STRNADOVA, 1984). The medium is sterilized at 0.8 atm., for 20 minutes. For the submerged cultures, two medium variants were used: the T_2 liquid medium, that has the same ingredients, but does not contain agar, and the I_2 medium, that contains corn extract; sugar; ammonium sulfate; $CaCO_3$, pH 6–6.2. For the total alkaloid content determination, the Rumpel method is used (14), consisting in the alkaloid extracting with a tartric acid methanol solution at 55–60 °C and the extract purification with a zinc acetate solution. The extract reacts with the stain reactive (a para-dimethyl-aminobenzoic-aldehyde sulfuric solution), the result being a photocolorimetrable blue compound. The alkaloid spectrum was determined through thin layer chromatography, on formaldehyde impregnated silica gel plates. The mobile phase is the ethyl ether. The spots identification is made in UV light, at $\lambda = 254$ nm.

For easier understanding there are some specifications to be made. The sclerotia are encoded after the predominant alkaloid (T, for ergotamine; S, for ergocristine, P, for ergocryptine). The symbol is followed by a number, of which first digit indicates the generation succession. The sclerotia and its descendance do not have the same code. The total alkaloid content values (CAT) and the alkaloids spectrum represent the average of the determinations made on 10 sclerotia, randomly chosen from each variant. Conform to the CARLILE and WATKINSON, 1994 concept, which defines the strain as "a genetic variety of a fungus, which is isolated from nature, either produced in a lab, through mutation or recombination", in this work the sclerotium is considered to be the origin of a strain. The generation linkage constitutes a "normal line", when the culture belongs to one sclerotium, or "hybrid", when the culture results from two different sclerotia. In the case of hybrid cultures, by analogy with diploid organisms, we are talking about the F1 hybrid generation, back-cross, or consanguinity.

RESULTS AND DISCUSSIONS

Spontaneous hyphal anastomosis, evidenced in species of *Claviceps* genus, is, together with mutations, a way to amplify heterokaryosis. In somatic hybridization experiments, realized by hyphal anastomosis, the dynamics of T:S:P ratio and of total alkaloid content (CAT) was registered. The values were analysed for sclerotia and

their descendance, both for parental strains and hybridization products, and their descendance. The intraspecific hybridizations between strains of same biosynthetic type (Figs. 1–3) are the object of this paper, but we also effectuated hybridizations between strains of different alkaloid type. The *Claviceps purpurea* strains are grouped in predominantly ergocryptine producing strains (1%), exclusively ergotamine producing strains (22%), and strains with all peptide alkaloids, in various proportions (77%). Ergocristine is the solely alkaloid that does not overtake 80% of total alkaloid content (18).

In ergotamine strain hybridizations, the selection criterion for parental strain origin-sclerotia was the average of total alkaloid level and ergotamine percentage predominance in total alkaloid level.

CAT average value expresses the character phenotypisation tendency in descendance of respective sclerotia. As shown in Fig. 1, in T-605 and T-607 parental lines perpetuation during three generations, a variability of average of this character from generation to generation was observed, an alternating dynamics of decrease – increase – decrease type, comparatively with the reaction norm of descendance and of origin-sclerotium. This behaviour is, possibly, a manifestation of the so-called "variability of generation average" (Falconer, 1969), extrapolated to the situation of multiple selection generations.

According to this author, the modification of selection derived populations average is a consequence of gene frequency change for the genes controlling the respective metric character.

A similar dynamics appears for T 605 × T-607 and T-706 × T-707 F1 hybrid descendances, but with some particular aspects. The T 605 × T-607 and T-706 × T-707 hybrid descendances, different from parental non-hybrid descendance, display CAT levels superior to those of parental origin-sclerotia (0.92 mg%, for the first case, compared to 0.85 mg%; 0.81 mg%, and 0.96 mg%, for the second hybrid descendance, comparing to 0.78 mg%, respectively 0.77 mg%). These values should be manifestation of a hybrid vigour (heterosis) effect, as a result of heterokaryosis degree and gene heterozygosity increase after somatic hybridization. In backcrosses of "hybrid descendant x parental" type (T-706 × T-708, respectively T-708 × T-707), CAT is smaller in the first generation descendance, more evident for the first backcross, while in the following generation was confirmed the fluctuation previously discussed, by similar or superior CAT values, comparatively to origin-sclerotia values. Distinct from total alkaloid content, ergotamine biosynthesis and T:S:P ratio a relative stability was registered. Ergotamine maintains at higher than 90% levels, indifferently of CAT level. The ergocristine and ergocryptine presence in F1 and b descendance suggests that, although resembling the biochemical phenotype, being exclusively ergotamine producers, the parental strains are genotypically different. The ergocryptine and ergocristine genetic determinants are phenotypically expressed only after hybridization and backcross induced reassortment.

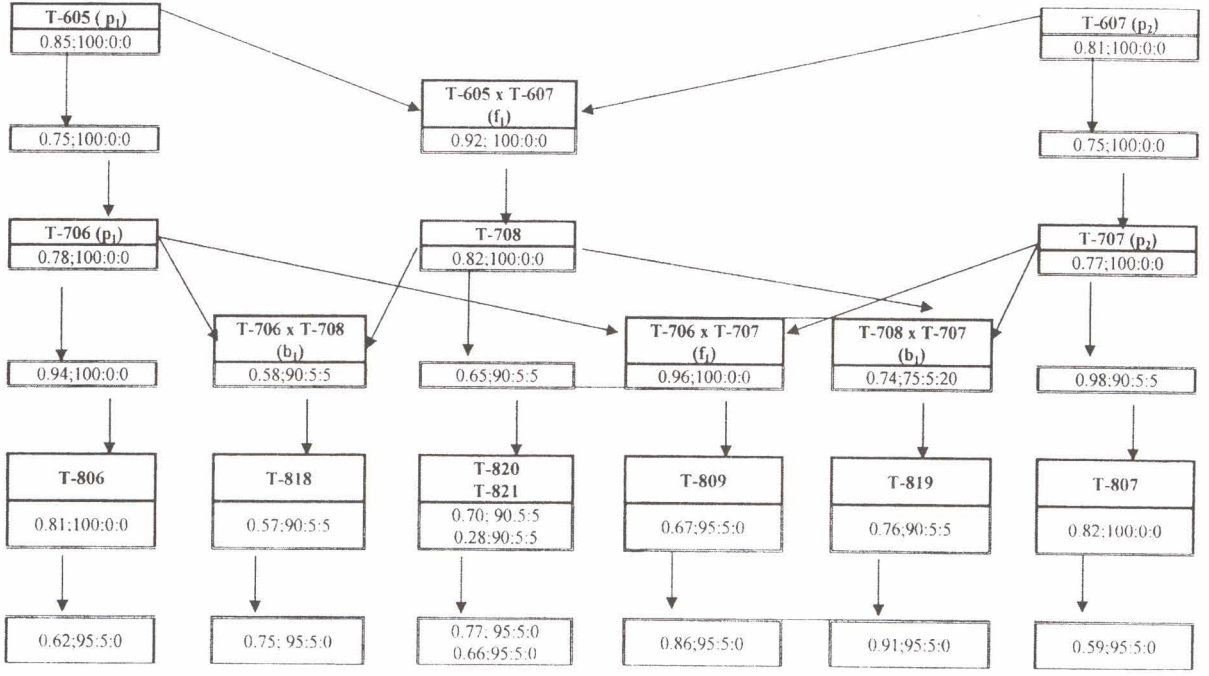

Fig. 1. Hybridization scheme in *Claviceps purpurea* ergotamine strains, and phenotypisation of some quantitative characters in origin-sclerotia, and their parental and hybrid descendances, in successive generations.

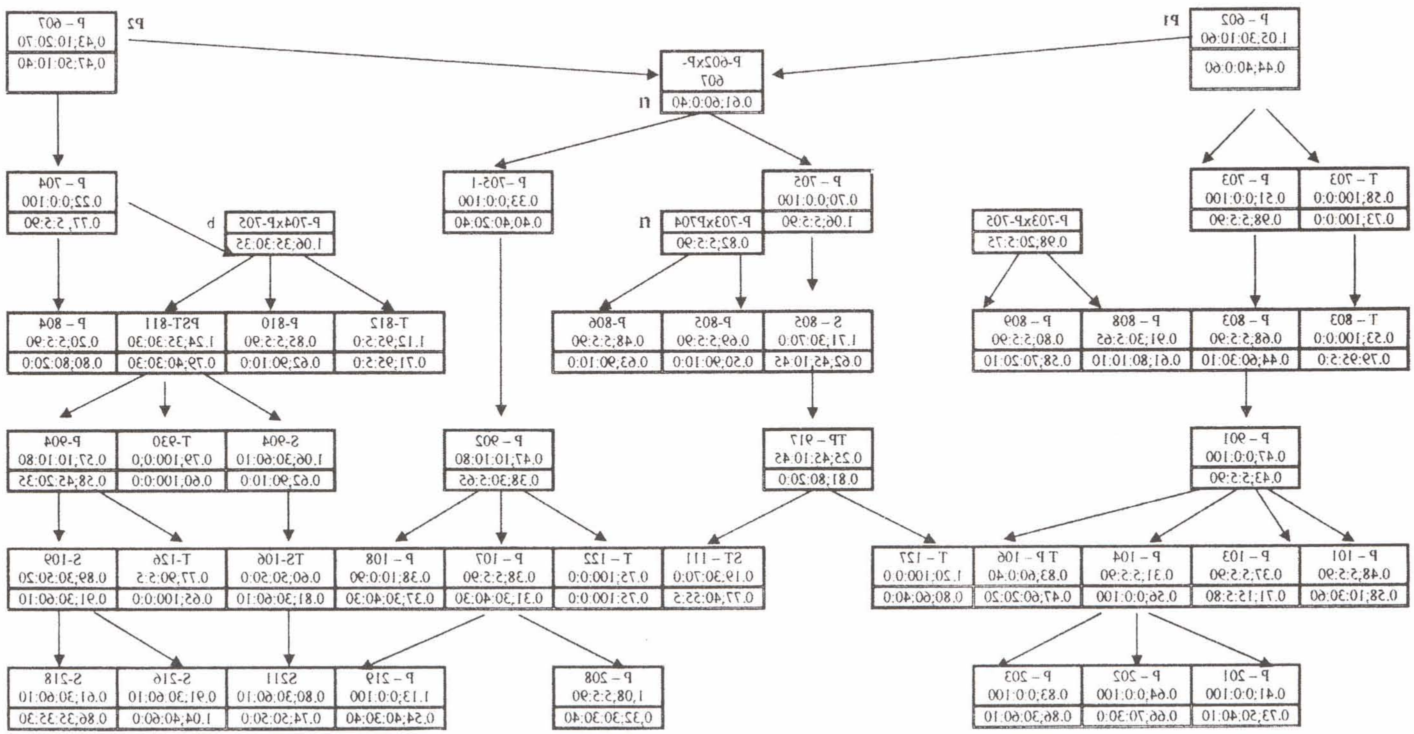
P2
P - 607
0,43;10:20:70
0,47;50:10:40
P-602xP-607
0.61;60:0:40
P1
P - 602
1.05;30:10:60
0.44;40:0:60
P - 704
0.22;0:0:100
0.77;5:5:90
P-704xP-705
1.06;35:30:35
P - 705-1
0.33;0:0:100
0.40;40:20:40
P-703xP704
0.82;5:5:90
P - 705
0.70;0:0:100
1.06;5:5:90
P-703xP-705
0.98;20:5:75
P - 703
0.51;0:0:100
0.98;5:5:90
T - 703
0.58;100:0:0
0.73;100:0:0
P - 804
0.20;5:5:90
0.80;80:20:0
PST-811
1.24;35:30:30
0.79;40:30:30
P-810
0.85;5:5:90
0.62;90:10:0
T-812
1.12;95:5:0
0.71;95:5:0
P-806
0.48;5:5:90
0.63;90:10:0
P-805
0.69;5:5:90
0.50;90:10:0
S - 805
1.71;30:70:0
0.62;45:10:45
P - 809
0.80;5:5:90
0.58;70:20:10
P - 808
0.91;30:5:65
0.61;80:10:10
P - 803
0.68;5:5:90
0.44;60:30:10
T - 803
0.53;100:0:0
0.79;95:5:0
P-904
0.57;10:10:80
0.58;45:20:35
T-930
0.79;100:0:0
0.60;100:0:0
S-904
1.06;30:60:10
0.62;90:10:0
P - 902
0.47;10:10:80
0.38;30:5:65
TP - 917
0.25;45:10:45
0.81;80:20:0
P - 901
0.47;0:0:100
0.43;5:5:90
S-109
0.89;30:50:20
0.91;30:60:10
T-126
0.77;90:5:5
0.65;100:0:0
TS-106
0.60;50:50:0
0.81;30:60:10
P - 108
0.38;10:0:90
0.37;30:40:30
P - 107
0.38;5:5:90
0.31;30:40:30
T - 122
0.75;100:0:0
0.75;100:0:0
ST - 111
0.19;30:70:0
0.77;40:55:5
T - 127
1.20;100:0:0
0.80;60:40:0
T P - 106
0.83;60:0:40
0.47;60:20:20
P - 104
0.31;5:5:90
0.56;0:0:100
P - 103
0.37;5:5:90
0.71;15:5:80
P - 101
0.48;5:5:90
0.58;10:30:60
S-218
0.61;30:60:10
0.86;35:35:30
S-216
0.91;30:60:10
1.04;40:60:0
S211
0.80;30:60:10
0.74;50:50:0
P - 219
1.13;0:0:100
0.54;40:30:40
P - 208
1,08;5:5:90
0.32;30:30:40
P - 203
0.83;0:0:100
0.86;30:60:10
P - 202
0.64;0:0:100
0.66;70:30:0
P - 201
0.41;0:0:100
0.73;50:40:10

Fig. 3. Hybridization scheme of *Claviceps purpurea* ergocristine strains, and phenotypisation of some quantitative characters in origin-sclerotia, and their parental and hybrid descendances, in successive generations.

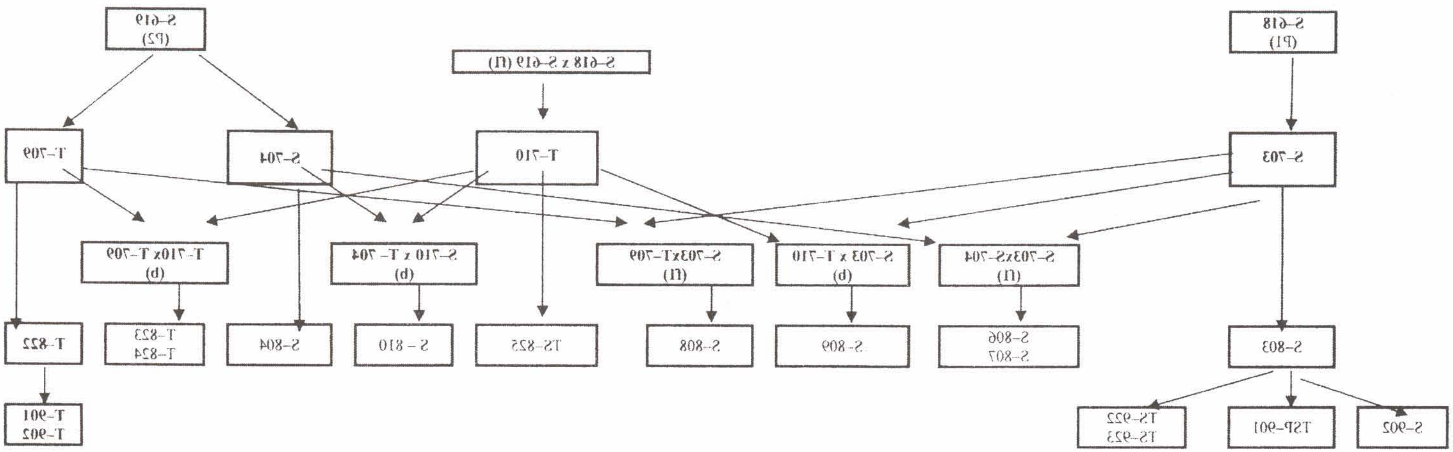

Fig. 2. Hybridization scheme of Claviceps purpurea ergocristine strains.

In ergocristine hybridizations (Fig. 2, Table 1), the origin-sclerotia of parental strains have identical phenotypes (CAT = 0.90 mg%; T:S:P 20:60:20). Orthoselection on predominantly ergocristine parental strains contributed to the maintenance or even to alkaloid biosynthesis increase. Thus, for S type descendance of S-618 parental, in the first two analyzed generations (G6, and G7), the descendance average (0.94 mg%, respectively 1.27 mg%) is superior to origin-sclerotium CAT value, while in G8 and G9 generations, indifferently of strain alkaloid type (S-803, for G8, respectively S-902, TSP-901, TS-922, T-923, for G9), the general tendency is the diminution of total alkaloid average. The same alkaloid biosynthesis dynamics was registered for S type descendance (S-704, S-804) of the other parental (S-619). The alkaloid spectrum is significantly modified, in the sense of absence of phenotype expression for ergocryptine determinants and of its amplification for ergotamine gene determinants. The G9 generation is originated from sclerotia with different biochemical traits: S-902 (CAT = 0.70 mg%, T:S:P 45:50:5), TSP-901 (CAT = 0.96 mg%, T:S:P 30:40:30), TS-922 (CAT = 1.03 mg%, T:S:P 50:50:0), T-923 (CAT = 0.79 mg%, T:S:P 50:50:0), fact reflected in increased variability of respective descendances, both for CAT, aspect previously commented, and especially for phenotype expression of ergotamine, ergocristine, and ergocryptine coding gene factors. For S-619, it was analyzed the behaviour of descendance of an exclusively ergotamine type sclerotium, in successive generations. In this case, in descendance of 100:0:0 sclerotia type appear not only exclusively ergotamine producing sclerotia, but also predominantly ergotamine sclerotia, which biosynthesize ergotoxines (represented by equal ergocristine and ergocryptine proportions – 5%, or only by ergocristine, up to 20% from CAT level). The F1 hybrid descendance, represented by S-618 × S-619, S-703 × S-704, S-703 × T-709 strains, and their descendants, T-710 (originated in S-618 × S-619), TS-825, S-806 and S-807, respectively S-808, for the last, offers the possibility to discuss on the phenotypisation of the quantitative characters analyzed, in successive generations. The CAT values registered fluctuations in the first generations, less significant, but at an enough high level. A more important CAT decrease is noted for G8 generation. The absence of evident expression of hybrid vigour in F1 generation should be an argument of homozygous state of some factors controlling alkaloid biosynthesis in these hybridizations between predominantly ergocristine strains.

Table 1

Biochemical characteristics of *Claviceps purpurea* origin-sclerotia, of their parental and hybrid descendances,in ergocristine strains, in successive generations

Generations/ P1, P2, f1, b	Sclerotium			Descendance	
	Cod	CAT (mg%)	Report T:S:P (%)	CAT (mg%)	Report T:S:P (%)
G6/P1	**S – 618**	0.90;	20:60:20	0.94;	40:60: 0
/P2	**S – 619**	0.90;	20:60:20	0.84;	60:40: 0
/f1	**S – 618 × S – 619**			0.88;	65:35: 0
G7/P1	**S – 703**	1.09;	40:60: 0	1.27:	30:70: 0
/P2	**S – 704**	1.04;	**40:60: 0**	1 12;	30:70: 0
/P2	**T – 709**	0.96;	**100: 0: 0**	0.66;	90:5: 5
/f1-1	**T – 710**	0.86;	100: 0: 0	0.96;	55:40: 5
/f1	**S – 703 × S – 704**			1.15	30:70: 0
/f1	S – 703 x T – 709			0.91	30:65: 5
/b	S – 703 x T – 710			0.88	30:65: 5
/b	S – 704 x T – 710			0.89	30:65: 5
/b	T – 709 x T – 710			0.73	60:35: 5
G8/P1	**S – 803**	1.03	30:70: 0	0.73;	40:60: 0
/P2	**S – 804**	1.04	30:70: 0	0.66;	80:20: 0
/P2	**T – 822**	0.73	90: 5: 5	0.64;	100:0: 0

Generations/P1, P2, F1, B	Sclerotium			Descendance	
	Cod	CAT (mg%)	Report T:S:P (%)	CAT (mg%)	Report T:S:P (%)
/f1-1	**TS – 825**	0.94	55:40: 5	0.68;	90:10: 0
/f1-2	S – 806	1.10	30:70: 0	0.70	60:40: 0
/f1-2	S – 807	0.79	30:70: 0	0.87	40:50: 0
/f1-2	S – 808	0.92	30:70: 0	0.86	50:50: 0
/b-1	S - 809	0.87	30:70: 0	0.87	50:50: 0
/b-1	S – 810	0.89	30:65: 5	0.70	50:50: 0
/b-1	T – 823	0.39	90:10: 0	0.63	100:0: 0
/b-1	T – 824	0.77	90:10: 0	0.79	100:0: 0
G9/P1	S – 902	0.70	20:80: 0	0.82	45:50: 5
/P1	TSP – 901	0.96	30:40:30	0.69	40:30: 30
/P1	TS – 922	1.03	50:50: 0	0.79	50:50: 0
/P1	T – 923	0.62	80:20: 0	0.73	30:60: 5
/P2	T – 901	0.32	100: 0: 0	0.71	80:20: 0
/P2	T – 902	0.90	100: 0: 0	0.98	80:20: 0

The dynamics of percentage value of the three important alkaloids in total alkaloid level has some particularities, depending on origin-sclerotia characteristics. Thus, in S-618 × S-619 hybrid, although the origin-sclerotia of hybrid culture have a 20:60:20 T:S:P ratio, its descendance produced especially ergotamine and ergocristine producing sclerotia, but predominantly ergotamine (65:35:0). The S-618 × S-619 hybrid behaviour was analysed in descendance by perpetuation of an exclusively ergotamine producing variant. Although this descendance is

characterized by the synthesis of all three principal alkaloids, the perpetuation by TS-825 tends to rebuild the predominantly ergotamine spectrum. In another ergocristine type hybrid (S-703 × S 704), the descendance average remains predominantly of ergocristine type (30:70:0, comparatively to 40:60:0, the value of origin-sclerotia of hybrid). In the next generation (S-806, S-807), a tendency to ergotamine increase was observed, at the same time with ergocristine decrease. For the hybrid resulted by anastomosis of hyphae from different alkaloid type strains (S-703 × T-709), the nucleuses redistribution in heterokaryons was in so manner realized that the factors coding for ergocryptine are also derepressed, the descendance nevertheless remaining predominantly ergocristine producing, similar to S-703 parental. The G8 (S-808) descendance has equal ergotamine and ergocristine levels.

In G7 backcrosses, for S-703 × T-710 and S-704 × T-710 (in which S-703 is P1, and T-710 is F1 hybrid), the CAT average level of descendance tends to that of T-710, tendency that maintains too in G8 generation, although for S-810 (perpetuated from S-704 × T-710) a CAT decrease appears. Under the aspect of alkaloid percentage in T:S:P ratio, the behaviour of variants is identical. The mentioned backcrosses have as a result, in G7 descendance, all the three alkaloid biosynthesis, this remaining prevalently oriented to the ergocristine biosynthesis (30:65:0), such as a non-hybrid partner from these backcrosses (situation different from that registered or CAT), while in G8 descendance the tendency is to an equal ergotamine and ergocristine distribution (50:50:0). In T-710 × T-709 (F1 × P2) backcross, although the origin-sclerotia have an ergotamine phenotype, the descendance allows the expression of genetic determinants for the three principal alkaloids, but in the next generation takes place a coming back to alkaloid phenotype of origin-sclerotia (100:0:0).

In ergocryptine strain hybridizations (Fig. 3), in which the ergocryptine biosynthesis was the selection criterion, for P1 parental (P-602, with 30:10:60 T:S:P ratio), the descendance remains prevalently ergocryptine producing. For P1, the behaviours of one exclusively ergotamine variant and of one obtained from an exclusively ergocryptine sclerotium were analyzed. In first case, the descendance tendency is to biosynthesize a great amount of ergopeptine alkaloids, comparatively with origin-sclerotium level. Regarding T:S:P ratio, the descendances remain predominantly or exclusively ergotamine producers. In the second case, the analysis during five generations, in which the selection was made on the basis of exclusively or prevalently ergocryptine biosynthesis, if in G8 the descendance tendency is to the phenotypisation of determinants for all alkaloid groups, the continuation of directed selective pressure on ergocryptine sclerotia determined, in the next generations, at predominantly ergpcryptine descendances. In the last analyzed generation, the G8 situation repeated, in the sense of ergotamine, ergocristine, and ergocryptine biosynthesis, but with obvious tendency to ergocryptine amount decrease and orientation of alkaloid spectrum to ergocristine or ergotamine.

For P2 parental (P-607), in three analysed generations, on ergocryptine type descendance, the situation is similar to the behaviour of the other parental, for the same generations.

The parental F1 hybridization and the perpetuation in successive generations on the basis of "ergocryptine prevalent biosynthesis" is characterized, in all cases, by the biochemical heterogeneity increase and the alkaloid pattern tendency to turn to ergocristine and ergotamine type. In backcrosses, the same marked phenotype heterogeneity of descendance is visible. To exemplify, we will analyse a PST sclerotium line, which has P-704 × P-705 backcross hybrid as ascendant. On ergocristine type sclerotium descendance, an initial amplification of ergotamine phenotype appears. In the next generations, an ergotamine and ergocristine equalization and a decrease, until absence of ergocryptine, take place. For ergocryptine type sclerotium, in descendances are expressed all hereditary factors for the tree principal alkaloids, they being of ergotamine, ergocristine, or ergocristine-ergotamine type. As a conclusion, the ergocryptine strains have a more reduced biosynthetic stability. Rarely, the ergocryptine sclerotia descendances are biochemically pure. Most often, parental and hybrid descendance becomes heterogeneous, by segregation, and the alkaloid pattern turns to ergocristine type or, the most frequently, to ergotamine type, aspect obvious especially in descendance of strains which are the result of F1 hybridization or of backcross. The nuclear reassortment and the new rates between genetically different nucleuses determine the significant modification of alkaloid spectrum.

The interpretation of the results obtained in the case of hybridization by intraspecific hyphal anastomosis between strains of same alkaloid type is enough difficult, because of the incomplete knowledge of genetic determinism of alkaloid biosynthesis in *Claviceps*, both from total content and T:S:P ratio point of view. The discussions are sometimes speculative, in the absence of complete information regarding the determinism and genetic control of respective characters, and the analysis substantiation is realized exclusively on phenotype bases. The genetics of ergopeptide alkaloid biosynthesis is incompletely deciphered (8). The studies on gene expression evidenced that the genes, cluster organized, are co-regulated, they being activated only in the alkaloid producing conditions. The cluster is constituted by *cpd1* gene, *cpps1-4* genes, and some genes for oxygenases and oxidoreductases. Afterwards, four modular peptid-synthetases (which are non-ribosomal peptide synthases, NRPS), encoded by *cpps1 – cpps4* genes, were identified. This hypothesis regarding the synthesis of peptide alkaloids on the basis of a multienzymatic complex (a peptid-synthetase trimodule) is more and more outlined, the LPS enzymes having large amino acid substratum specificity. These enzymes can naturally synthesize different ergopeptide structures, probably according with the fluctuations of free amino acid reserve. Therefore, the diversity of alkaloids produced by various *Claviceps purpurea* strains is, at least in part, expression of different amino acid

concentrations, in free cell reserves. The *Claviceps purpurea* strains frequently differ by nuclear equipment. The karyotype analysis of some *Claviceps purpurea* isolates evidenced a marked variation of chromosome size and number. The cytofluorometric nuclear DNA estimations showed variable ploidy degrees (9). Additionally, the nucleuses number is important for biosynthesis capacity, as it is mentioned in the introductive part.

The quantitative characters have a polygenic determinism. Their phenol-typisation is strongly enough influenced by environment factors. The quantitative traits can be controlled by additive genes, but it is also possible that the effects of non-allele gene interactions to be an important component in the hereditary variation of these characters. Therefore, it is a polyfactorial determinism (12). Crăciun *et al.*, 1991 sustain the following main characteristics of the polygene heredity: the most quantitative characters are controlled by more genetic loci; the effects of allele substitution at each segregant gene are relatively small and interchangeable (identical phenotypes can be determined by a great variety of genotypes); the phenotypic expression of polygene characters is considerably modified by the environment; the balanced systems of polygene heredity from a population contain a potential of genetic variability, in heterozygous state, partially freed by genetic recombination between polygenes.

Complementary explanations come from the hypothesis of multiple factors (3): the metric characters are determined by more pairs of allelomorph genes; these genes double their effects, which are additive; the dominance is complete or incomplete; the contribution of individual alleles is reduced, being "covered" by environmental variations.

The extrachromosomal genetic elements can be also responsible for the modification of Mendelian segregation ratio for some characters, but the deciphering of the modifications induced by these elements is difficult because of the interaction and/or nuclear genes. These genes modify their multiplication rate and influence the plasmagene balance. In stationary conditions, the hyphae may fuse by anastomosis and form the survival spores, by fusion of hyphal components. As a result, the representative rate of extranuclear genetic elements is modified and can appear different manifestations of the bioproductivity in different age variants of the same strain. It is possible that ergotamine strains increase the density of some *senescent* extrachromosomal elements, as in *Podospora anserina*. Another factor responsible for some apparently contradictory results could be the existence of genetically different nucleuses in a common cytoplasm, because of septal pores which permit their migration between cells.

The modification of Mendelian segregation ratio can be also due to pleiotropy that determines multiple phenotype effects induced by a single gene. It is possible that the phenotypisation of biosynthetic potential and sclerotia length characters to be the result of this type of gene determinism. The researches evidenced a positive relationship in variation of these characters (18, 19). The correlation degree came

from pleiotropy as a measure of influence of two characters by a single gene, although must be considered the effect of all segregant genes that affect each of the two characters. A predominant biochemical phenotype is, from the genetic point of view, a heterokaryon with nuclear rates balanced to one or other of the alkaloids. In gene pool of sclerotia populations all gene types are present, in each genotype the assortment possibilities being infinite. The different nucleuses which assure heterokaryosis state distribute more or less randomly, reason for which it is difficult to maintain a certain heterokaryosis state (a nuclear equilibrium). This can be an explanation for the orientation tendency to the ergotamine type and a certain stability of ergocristine type.

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