INFLUENCE OF ASCORBIC ACID ON SCLEROTIA PRODUCTION OF DESCENDANCE OF SOME *CLAVICEPS PURPUREA* HYBRID AND NON-HYBRID PARENTAL STRAINS

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Key words: ascorbic acid, *Claviceps purpurea*, sclerotia production

Abstract: This paper includes the experimental results regarding the effects of ascorbic acid, present in culture media, on the phenotypisation of “sclerotia/ha production” character. The behaviour of descendance of some monoparental and hybrid strains of *Claviceps purpurea* is analysed; the obtained data evidenced variations of strain bioproductivity, under the influence of ascorbic acid, variations which can be correlated with strain origin, alkaloid type etc.

INTRODUCTION

The source for the obtainment of ergopeptine alkaloids, which confer the particular importance of *Claviceps purpurea*, is represented by sclerotia. A greater sclerotia production signifies greater quantities of ergot alkaloids. To optimize these characters - sclerotia production and alkaloid level – methods to amplify the genotype and phenotype variability (experimental mutagenesis, protoplast isolation and fusion, intraspecific somatic hybridization by hyphal anastomosis, quantitative and qualitative variation of growth factors in culture media) were used (OLTEANU et al., 2005; SPALLA and, MARNATI, 1982; SURDU et al. 2005a, b; TRUŢĂ et al., 2005)., followed by selection and perpetuation of wanted genotypes. One of tested organic acids is ascorbic acid, the most important soluble antioxidant in plant organism. Vitamin C is the L-enantiomer of ascorbic acid. Together with flavonoids, polyphenols and some water insoluble compounds (α-tocopherol, for example), ascorbic acid diminishes the negative effects of free radicals and reactive oxygen species, of some chemical and toxic polluters.

For certain micro organisms, the ascorbic acid is an important growth factor (NEAMŢU, 1996). For this reason, we proposed to investigate the influence manifested by this compound on *Claviceps purpurea* strains with different bioproductive traits. The ascorbic acid present in plants (especially in chloroplasts, meristems, regions with an intense growth activity) is a factor that influences the parasite micro organism still in earlier growth stages of this.

MATERIAL AND METHOD

Claviceps purpurea strains, with different biosynthetic features, were used as biological material. To obtain the sclerotial descendance, several steps are necessary to be performed. The selected sclerotium, which constitutes the strain origin, is asepsized, and obtained fragments are placed on agar surface (STRNADOVA and KYBAL, 1974). The cultures are incubated at 28^{O}C, for 18-21 days. To obtain submerged cultures, the colonies formed on agar media are inoculated in liquid medium (WACK et al., 1983). The flasks are 3-5 days incubated, at 24^{O}C, under stirring. Ascorbic acid was introduced in culture media before colonia inoculation. The added quantities are integrated to the 10^{-4} - 10^{-8} domain. The obtained cultures are kept to refrigerator. Before rye infection, they are suspended in glucose solution. Sclerotia used to obtain in vitro cultures were coded conforming to predominant alkaloid (T, for ergotamine; S, for ergocristine; P, for ergocryptine). This symbol is followed by a number. The first digit of this number is an indicative for generation succession. The sclerotium and its descendance have the same code.

The ascorbic acid ((2-oxo-L-threo-hexono-1,4-lactone-2,3-enediol) is an organic acid with antioxidant properties, with molecular weight=172.12 g/mol and melting point=190-192 °C. The chemical formula is $C_6H_8O_6$, and its chemical structure is presented in Fig. 1.

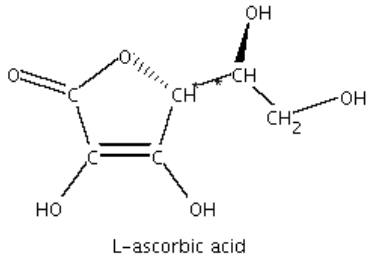

Figure 1. Chemical structure of ascorbic acid

RESULTS AND DISCUSSIONS

Determined quantities of ascorbic acid were introduced in *Claviceps purpurea* submerged culture and the behaviour of this material was analysed in parasitic cultures on rye, from „sclerotia production" point of view. The values of this parameter are presented in Table 1 and Fig. 2.

Table 1. Ascorbic acid (C) induced effects on sclerotia production/ha in *C. purpurea* strains of different alkaloid type, in comparison with parental strains

Origin	**Sclerotia production of parental strain (Kg/ha)**	**Strain**	**T:S:P (%)**	**Sclerotia production in 2005 (Kg/ha)**
TS 808	21.93	TS 911	70/30	12.72
		CTS 911		*7.61*
		T 941	80/20	24.92
		CT 941		*30.30*
TS 808 x ST 814	2.95	T 946	90/10	41.73
		CT 946		*36.96*
ST 814	1.71	T 953	90/10	35.84
		CT 953		*16.65*
T 810 x ST 814	5.31	TS 914	50/50	11.21
		CTS 914		*8.59*
T 810	11.11	TS 913	60/40	13.25
		CTS 913		*11.42*
ST 809 x T 810	145.96	S 919	20/60/20	43.53
		CS 919		*47.88*
		ST 916	50/50	49.59
		CST 916		*46.54*
TS 805 x ST 814	14.81	TS 939	60/40	40.26
		CTS 939		*46.23*

Origin	Sclerotia production of parental strain (Kg/ha)	Strain	T:S:P (%)	Sclerotia production in 2005 (Kg/ha)
TS 803	50.57	ST 920	30/70	38.73
		CST 920		*52.72*
ST 809 x TS 803	70.41	ST 917	30/60/10	34.17
		CST 917		*64.67*
S 803 x S 811	10.24	S 908	20/60/20	10.92
		CS 908		*14.86*
		S 909	20/60/20	7.41
		CS 909		*12.14*
TS 805 x S 811	29.30	T 919	60/40	103.23
		CT 919		*28.89*

The analysis of results evidences several interesting aspects. Thus, for predominantly ergotamine descendance, the ascorbic acid adding in submerged cultures induced a diminution of sclerotia production/ha, comparatively with control. Exceptions are the variants T 941 and TS 939, in which sclerotia production of treated variants is 22 %, respectively 15%, greater than respective controls. Without doubt, the parental hybrid or non-hybrid origin, as well as alkaloid type of strains, have an influence on phenotypic expression of analysed bioproductive character. Thus, the TS 911 and T 941, although of common origin (TS 808), are phenotypically different, both between them and comparatively with parental strain, from the point of view sclerotia production, even contrastive manifestations being evidenced. Also, differences exist regarding T:S:P ratio (70:30:0, respectively 80:20:0). The intraspecific hybridization TS 808 x ST 814 leads to rearrangement of genic determinants for alkaloid synthesis, fact evidenced by the marked predominance of ergotamine (90:10:0) in alkaloid total, and to important growth of ascendance production, comparatively with parental (2.95 Kg/ha), both for control and variant with acid ascorbic adding. Differences exist between T 946 (control) and CT 946 (that has o smaller, but not significant, production).

The positive genic potential regarding sclerotia production, repressed in ST 814 strain, but derepressed in descendance, follows from the analysis of the decendance of following parental combinations: TS 808 x ST 814 (formerly analysed), ST 814, T 810 x ST 814, TS 805 x ST 814. It’s obvious the bioproductive character phenotypisation at values superior to parental, in all cases. The growth is reduced for T 810 x ST 814 descendance, fact explicable if we analyse the T 810 strain behaviour. Both T 810 (non-hybrid parental) and TS 913, respectively CTS 913 descendance, have small and similar sclerotia production. Therefore, the presence of ST 814 strain in hybrid combinations, beside strains of same type, with phenotypisation both for T and for S, has positive repercussions on sclerotia production, while association with ergotamine predominant strains is reflected in a diminished sclerotia production.

Besides the two exceptions, with the control being inferior values to ascorbic acid treated variant, it can be analysed the T 919 strain. The control produced with 72.02% more sclerotia than the treated variant (103.23 Kg/ha, comparatively to 28.89%). Relative to the TS 805 x S 811 hybrid parental strain, the treated variant maintained at almost same level, while the control registered a significant increase. These aspects confirm the inhibitory effect of acid ascorbic adding in culture media of respective variant.

In the case of predominantly ergocristine descendance, the profile of graph 2 evidences a positive effect of acid ascorbic adding. The only exception is CST 916, but the difference is not significant. The S 919 and ST 916 strains, with common origin in ST 809 x T 810 hybrid (145.96 Kg/ha), have 3 fold smaller productions, both for controls and CS 919 and CST 916. As we already seen, from the point of view of ascorbic acid effect, for CST 916 a little and insignificant diminution exists (approximately 4%), comparing to control. The strain ST 809 is also present in ST 809 x TS 803 hybrid, which is the ST 917 (T:S:P 30:60:10) origin. In this case, different from the previous case, the hybrid parental has a reduced production (70.41 Kg/ha), with a tendency to lowering in 2005 generation. For CST 917, the ascorbic acid has the strongest positive influence on sclerotia production. Therefore, the descendance of ST 809 x TS 803 (70.41 Kg/ha) and ST 809 x T 810 (145.96 Kg/ha) hybrids registered an important decrease, but it must be noted that, in both situations, the treated variant is superior to respective control.

It is visible the uniform bioproductive behaviour of S 803 x S 811 hybrid parental and of 2005 descendance controls, respectively S 908 and S 909, not only regarding sclerotia production, but also the phenotypisation amplitude of genetic determinants coding for synthesis of the principal ergoline alkaloids (20:60:20). The hyphal anastomosis by which was obtained the hybrid parental probably produced a homozygosity of respective determinants, fact that could explain the phenotypic identity of descendance. The CS 908 and CS 909 variants have values superior to controls, the ascorbic acid adding being a benefit for sclerotia production.

The TS 803 strain in this non-hybrid state displayed in 2004 a production (50.57 Kg/ha) smaller than in combination ST 809 x TS 803, although the tendency at the descendance (ST 920) is the same with the previous situation, namely of decrease. The CST 920 strain produced 1.3 fold more sclerotia than control.

The result analysis evidences certain situations due, probably, not to ascorbic acid treatment, but rather to rearrangements and transmission of hereditary factors to descendance and, also, to the intrinsic characteristics of sclerotia used to start the cultures for the conidia obtention, conidia used to rye infection. Also, another factor may be the climatic factors. The observations and the metric determinations established that the ergotamine strains produce greater sclerotia, while those of ergocristine type have smaller sclerotia (SURDU et al., 2005). This aspect can explain some exceptions previously discussed, issued especially in non favourable conditions (rains, strong winds) during sclerotia maturation. The great sclerotia easier detach from rye, fact that determines diminutions of real sclerotia production in these strains.

CAGAŠ and MACHÁČ (2002) argued that the virulence of ergot fungus, expressed by sclerotia production, depends on more factors: the age of infected plant, climatic conditions, the variety (cultivar) of host plant, cultivation method, the post-culture management. A very possible cause of the results in 2005 could be the extreme conditions of temperature and hydric regime, as well as the strong winds manifested on long time periods during sclerotia maturation.

CONCLUSIONS

For predominantly ergotamine descendance, the ascorbic acid adding generally induced a diminution of sclerotia production/ha, comparatively with control.

In the case of predominantly ergocristine descendance, the ascorbic acid adding generally had a positive effect on sclerotia production.

The hybrid or non-hybrid parental origin, as well as alkaloid type of strains, have influence on phenotypic expression of analysed bioproductive character.

The climatic conditions (the strong winds manifested on long time periods during sclerotia maturation) influenced the sclerotia production of strains (for example, the greater sclerotia of ergotamine strains easier detached from rye, fact that determined diminutions of real sclerotia production in these strains).

REFERENCES

Cagaš B., Macháč R., 2002. *Plant Protection Science*, 38(1), 18 – 22.
Olteanu, Z., Truţă, E., Surdu, Ş., 2005. Lucrările celui de-al X-lea Simp. Naţ. Microbiol., Iaşi, 15 – 16 oct. 2004, Ed. CORSON, 177 - 181.
Spalla, C., Marnati, M. P. ,1982. *Overproduction of Microbial Products* (ed. by V. Krumphanze, B. Sikyta, Z. Vanek, Acad. Press, New York: 563.
Strnadová, K., Kybal, J., 1974. *F. Microbiol.,* 19(4), 272 - 280.
Surdu, S., Truţă, E., Olteanu, Z. & Ioan, I., 2005a. Lucrările celui de-al X-lea Simp. Naţ. Microbiol., Iaşi, 15 – 16 oct. 2004, Ed. CORSON, 165 - 170.
Surdu, S., Truţă, E., Olteanu, Z., Zamfirache, M.M., Rotinberg, P. & Gherghel, D., 2005b. *European Journal of Drug Metabolism and Pharmacokinetics*, 12th Panhellenic Pharmaceutical Congress, Athens, Greece, May 14-16, 2005, 30.
Surdu, S. Ş., Olteanu, Z., Truţă, C. E., 2005. *Genul Claviceps – biologie şi biotenologie*, Ed. CERMI, Iaşi.
Truţă, E., Surdu, Ş., Olteanu, Z. & Zamfirache, M.M., 2004. Lucrările celui de-al X-lea Simp. Naţ. Microbiol., Iaşi, 15 – 16 oct. 2004, Ed. CORSON, 171 – 176.
Wack, G., Kiss, J., Nagy, L., Udvardy-Nagy, E., Zalai, K. & Zsoka, E., 1983. Fermentation Process for the Preparation of Ergot Alkaloids primarly Ergocornine and ß- Ergokryptine, Brevet 4 369 252.

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APPENDIX

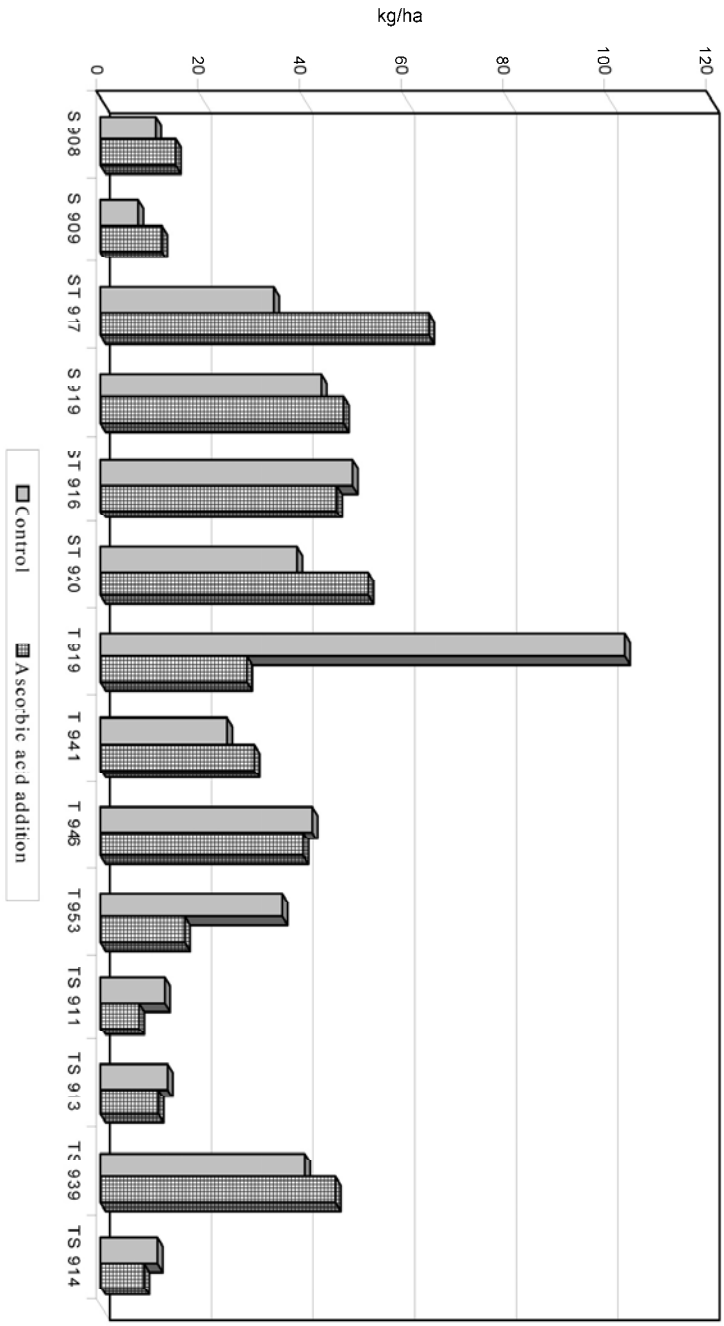

Figure 2. Variation of sclerotia production/ha, in strains of *C. purpurea* of different alkaloid type, in thepresence of ascorbic acid