IN VITRO EXPLANT DEVELOPMENT IN THE PRESENCE OF SOME EXTRATERRESTRIAL FACTORS

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Abstract. These experiments were performed in calli of *Krainzia longiflora* (*Cactaceae*) maintained for 816 hours at different values of geomagnetic (natural or double) and geoelectrical field (natural or screened). Were analysed the growth rhythm, as well as the enzymatic activity at the calli level (isoperoxidase, catalase, esterase and total peroxidase) and the assimilatory pigments amount. The calli development is dependent on calli type (normal or anthocyanic) and on the value of geomagnetic and geoelectrical field.

INTRODUCTION

In the last years, more researches of spatial biology were performed regarding to the effect of some component from the extraterrestrial environment on living organisms. The experiments on plant were effected at *in vivo* and *in vitro* culture. From the component of the extraterrestrial environment, the effect of different values of geomagnetic and geoelectrical field, were performed in different species, at *in vivo* (Körösföy S. et al. 1997; Morariu 1992, 1997; Neamțu et al. 1997, a/o.) and at *in vitro* culture (Corneanu et al., 1995 - 2002; Lupa et al., 1997, a/o). At *in vitro* culture, the geomagnetic and geoelectrical field affects the explant development, their enzymatic activity, the ultrastructural features, the production of secondary metabolites a/o (Corneanu et al., 1995-2002, a/o.). The effect is dependent on genotype, the medium culture composition, the analysed factors from environment and different interaction between them, analysed feature. In this paper, was analysed the geomagnetic and geoelectrical field effect on *in vitro* development in *Krainzia longiflora*, as well as on the metabolic activity of the calli.

MATERIAL AND METHODS

The *Cactaceae* species are used in different experimental conditions because they present a compact shape, with an orientation of the division spindle, and a high vitality in anabiosis conditions (Corneanu, 2002).

Krainizia longiflora (Br. & R.) Backbg. [sin. *Mammillaria longiflora* (Br. & R.) Berger] is native from Mexico (Durango). Is a globular specie, with a cluster shape. Every stem present about 8-9 cm in diameter, dark-green, with long trabecula and axila slightly distinctive. The radiate thorns, white or yellowish, of 1-1.3 cm in length; four central thorns, yellowish or brown, of the same length, one having hooked. The flowers are pink, until 4-5 cm in length and 4 cm in diameter.

The calli fragments of about 3/5 mm sizes, originating from an in vitro subculture, were inoculated on a MS basal medium supplied with 1.8 mg/l IAA and 0.011 mg/l KIN. 48 hours after inoculation the explants were introduced in the experimental conditions. Were used four experimental variants with different values for geomagnetic and geoelectrical field:

1. Natural geomagnetic and geoelectrical field (Control);
2. Natural geomagnetic field, screened geoelectrical field;
3. Double geomagnetic field, natural geoelectrical field;
4. Double geomagnetic field, screened geoelectrical field.

In these conditions the vessels with explants were maintained time of 816 hours (34 days), at dark and a temperature of $22^0 \pm 2^0$ C. After this period, the explants from the all experimental variants, were maintained in a growth room, time of 76 days, at a temperature of $24^0 \pm 2^0$ C, and a light regime of 16 light per day and 2600 lx light intensity. The time period of the experiment was 120 days long.

The biometrical observations were effected steadily, time of 120 days. Were analysed the growth rhythm, the organogenesis and callusogenesis processes. At the end of the experiment, were performed biochemical investigations on the calli and in vitro neoformed plantlets. The assimilatory pigments amount (the

A- and B-chlorophyll and carotenoids pigments) was establish in the green, normal calli, as well as in an anthocyanic calli, after the classical methods. The peroxidase isozyme activity was establish through electrophoresis in a thin agarose layer and densitometration of the electrophoregrams at 460 nm. The enzymatic activity was establish recording of the total amount of peroxidase, catalase and esterase from the calli of *K. longiflora*.

The recorded values were statistically interpreted with CSS Statistica program. The analysis of significance variance was made with the ANOVA/MANOVA test.

The duoble geomagnetic field was obtained in a Helmholtz coil. The vertical component of this geomagnetic field presents a value of about 76,000 nT, which represent about double towards the natural geomagnetic field (with a value of about 47,000 nT). The screening of the geoelectrical field, was obtained through the covering of the experimental vessels with an aluminium folio.

RESULTS AND DISCUSSIONS

***In vitro* development of the *Krainzia longiflora* calli.** *K. longiflora* developed two calli types: (a) one normal, green, with a high development rhythm, which after 90 days of *in vitro* culture, presented organogenesis process (clone α), and (b) another, with a high content in anthocyan, with a low development rhythm (clone β). At this type, the growth takes place especially in diameter, under shape of some layers of cells at the medium surface. Moreover, at this calli type, are not present the organogenesis processes. The development of the green, normal calli, was dramatically affected by the geomagnetic field modification and screening of the geoelectrical field, while in anthocyanic calli was slightly stimulated in a double geomagnetic field, and inhibited at the screened of the geoelectrical field (Table 1).

The effect of a double geomagnetic field. The geomagnetic field value, didn't present a significant influence in the first phases of development after treatment, the significant effects being evidently after the explants transfer, 60 days after the experiment began, on a fresh culture medium. The variance analysis, reveal that the calli growth in height was affected ($F = 76.627$; $p = 0.00001$), and finally the growth in diameter at 120 days ($F = 46.39$; $p = 0.0001$) and calli mass weight ($F = 41.49$; $p = 0.0001$). The recorded values are slightly reduced at the calli development in the variants from a double geomagnetic field, but the variance analysis didn't record significant differences. Also, was not recorded significant correlation between the calli growth and the value of the geomagnetic field.

The screened geoelectrical field effect. The screened geoelectrical field manifests a significant effect on the calli development, as well as for the differentiation and morphogenesis processes. The variance analysis, emphasised the very significant effect of the geoelectrical field on the initial development, and past over the organogenesis processes. The screened of the geoelectrical field, affected especially the growth in diameter of the calli, probably through affecting of the mitotic division spindle. The analysis of the differences between the recorded values for this parameter (the value of the geoelectrical field, screened or not), demonstrate the very significant influence in the first development phases ($F= 49.52$; $p = 0.001$). This effect is converted in a significant effect, when the explants are developed in normal conditions for the geoelectrical field ($F= 25.67$; $p = 0.005$)). These findings are in concordance with the

negative correlation recorded between these development parameters ($r = -0.3321$; $p = 0.04$).

The effect of the interaction geomagnetic field - geoelectrical field. The interaction between geomagnetic and geoelectrical field, influenced the cell multiplication in the first phases of the *in vitro* development, especially regarding the in height growth, but the variance values are not significant. Was observed a different response at the two stress factors in the two calli types, the anthocyanic calli being the most severely affected.

The photosynthetic activity in the *Krainzia longiflora* calli. The amount of assimilatory pigments was established in the green normal calli and in the anthocyanic calli, 120 days after the experiment began (Table 2).

Table 2. The assimilatory pigments amount (in mg% fresh substance) in *Krainzia longiflora* calli

Variant	A-Chl	B-Chl	Caroten. pigm.	A+B-Chlor.	A-Chlor./ B-Chlor.	A+B-Chl/ Carot.pig
Normal calli						
gm.+ge.	0.066	0.027	0.018	0.093	2.444	5.167
gm.-ge.	0.079	0.050	0.060	0.129	1.580	2.150
2gm.+ge.	0.095	0.003	0.003	0.098	31.667	32.677
2gm.-ge.	0.002	0.046	0.027	0.048	0.043	1.777
Anthocyanic calli						
gm.+ge.	0.022	0.161	0.072	0.183	0.137	2.542
gm.-ge.	0.024	0.333	0.023	0.357	0.072	15.520
2gm.+ge.	0.036	0.099	0.037	0.135	0.360	3.649
2gm.-ge.	0.063	0.069	0.020	0.134	0.940	6.700

Legend: gm. = natural geomagnetic field; 2gm. = double geomagnetic field;
ge. = geoelectrical field; +/- presence of absence of the geoelectrical field.

The total amount of assimilatory pigments presented higher values in the anthocyanic calli, in comparison with the green, normal calli (Table 2). The recorded values for the assimilatory pigments, presented a variability, depending on the experimental variant and the calli type. In the green, normal calli, the A-chlorophyll presented higher values in the natural geomagnetic field (indifferent of the screening or not of the geoelectrical component), as well as in the variant from double geomagnetic field, in the presence of the geoelectrical component. The carotenoids pigment amount, recorded different values, depending on the calli type and the geomagnetic and geoelectrical field value. In the presence of the geoelectrical field (indifferent of the geomagnetic field values: natural or double), the carotenoid pigments amount was higher in the anthocyanic calli. In the absence of the geoelectrical field, the values are opposite (Table 2). Some interesting values were recorded in the ratio between different assimilatory pigments, suggesting their different implication in the photosynthesis process, depending on the experimental conditions.

The peroxidase isozymes activity in the *Krainzia longiflora* calli. The peroxidase isozymes were established through electrophoresis in agarose thin layer. The isoperoxidases activity was established through electrophoregrames densitometration, the recorded values being expressed through arbitrary units (a.u.; Table 3). The recorded values for the isoperoxidasic activity, are depending on the calli type, the value of geomagnetic and geoelectrical field. In a natural geomagnetic field, the isoperoxidasic activity was intensely in the anthocyan calli, being slightly reduced at the screened of the geoelectrical field. In a double geomagnetic field, the isoperoxidasic activity was intensely in the green calli, having higher values at the screening of the geoelectrical field (Table 3).

The catalase, esterase and peroxidase activity. The enzymatic activity of the catalase, esterase and peroxidase was establish only in the green, normal calli, at 120 days after the beginning of the experiment (Table 4). The peroxidas's activity presents in generally higher values in the presence of the geoelectrical field, the values recorded in a natural geomagnetic field, being higher in comparison with the those recorded in a double geomagnetic field. The catalase and esterase activity in the green, normal calli, presented the highest values in a natural geomagnetic field, with screened geoelectrical component (Table 4).

Table 4. The enzymatic activity of the catalase, esterase and peroxidase in *Krainzia longiflora* green calli (clone α)

Variant	Catalase[1]	Esterase[2]	Peroxidase[3]
gm + ge	69.75	1.555	8.517
gm – ge	96.00	4.222	5.162
2gm + ge	63.00	1.777	5.678
2gm – ge	58.80	0.888	6.552

[1]expressed in ml oxygen delivered in 3 min, from a 3% H_2O_2 solution, by the catalase from a fresh calli, at 25^0 C

[2]expressed in ml acetic acid formed after enzyme action, per minute and gram fresh enzymatic preparative at 30^0 C

[3]expressed in ascorbic acid micromols oxidative per minute by the peroxidase from a gram fresh calli at the room temperature.

CONCLUSIONS

The *in vitro* development of the *Krainzia longiflora* calli (the organogenesis processes, the metabolic activity a/o) is dependent on the calli type and on the value of geomagnetic and geoelectrical field.

The analysis of the calli development in *Krainzia longiflora* at different values of the geomagnetic (natural or double) and geoelectrical field (present or screened) suggest that in the undifferentiated tissue of the calli, the mitotic division spindle is affected (modified orientation) by the values of the geomagnetic and geoelectrical field. In a double geomagnetic field, screened geoelectrical, there are alterations of the development being inhibited the organogenesis processes. Also is affected the anthocyanic calli development, in the variant with a double geomagnetic field, screened electrical.

The total amount of the assimilatory pigments from the normal (clone α) and anthocyanic calli (clone β), presented higher values in a natural geomagnetic field, in comparison with the values recorded in a double geomagnetic field.

The results recorded for the isoperoxidasic activity present the highest values in the anthocyanic calli (β) in Control (natural geomagnetic and geoelectrical field). In a double geomagnetic field the recorded values are opposite, being higher in normal calli (α) at the screening of the geoelectrical field.

The catalase and esterase activity recorded the highest values in natural geomagnetic field with the screened electrical component. The peroxidasic activity, recorded higher values in the presence of the geoelectrical field, in the normal calli (α), the values recorded in a natural geomagnetic field, being higher in comparison with the values recorded in a double geomagnetic field.

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Table 1.The calli mass development and organogenesis processes in *Krainzia longiflora*, at different values of geomagnetic and geoelectrical field, 120 days

Geomagnetic field	Natural				Double			
Geoelectrical field	Natural		Screened		Natural		Screened	
Feature	clone α	clone β	clone α	clone β	clone α	clone β	clone α	clone β
Diameter (mm)	38.5	15.3	34.8	9.0	42.7	19.4	45.3	11.2
Height(mm)	30.0	8.6	33.3	5.8	30.3	12.2	31.5	6.6
Shoots/explant	3.8	0.0	0.7	0.0	4.0	0.0	0.0	0.0
Fresh weight (g)	10.2	1.1	12.2	0.2	14.5	2.1	14.1	0.4
% development	28.6	35.7	23.1	38.5	40.0	40.0	10.0	50.0
% necrosis	0.0	35.7	0.0	38.4	0.0	20.0	0.0	40.0

Table 3. The isoperoxidase activity in *Krainzia longiflora* calli

Var	a.u. total	total fract	Anodic fractions					Cathodic fractions				
			No.	$a.u._1$	$a.u._2$	$\%_1$	$\%_2$	No.	$a.u._1$	$a.u._2$	$\%_1$	$\%_2$
Normal calli (α)												
1	0.92	2	2	0.64	0.28	69.7	30.1	0	-	-	-	-
2	0.59	3	2	0.28	0.16	47.5	27.1	1	0.15	-	25.4	-
3	2.84	3	2	2.20	0.40	77.5	14.1	1	0.24	-	8.5	-
4	4.54	3	2	3.85	0.44	84.8	9.7	1	0.25	-	5.5	-
Anthocyanic calli (β)												
1	2.77	4	2	1.97	0.42	67.5	15.2	2	0.30	0.18	10.8	6.5
2	0.83	2	2	0.66	0.17	80.0	20.0	0	-	-	-	-
3	2.03	3	2	1.67	0.24	81.3	11.9	1	0.14	-	6.9	-
4	4.48	3	2	3.10	0.78	69.2	17.4	1	0.60	-	13.4	-