

PRELIMINARY STUDIES REGARDING THE INFLUENCE OF UV RADIATIONS WITH DIFFERENT WAVELENGTHS ON TISSUE SAMPLES FROM *ZEA MAYS*

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Abstract

The main objective of our research was to determine the influence of the UVB radiations on the organogenesis and callogenesis of the monocotyledonous species, taking as a study subject *Zea Mays* (corn, the ZP471 and Helga hybrids).

The paper presents the results of the *in vitro* tests that have been conducted on tissue samples from the leaves and the vine of the biological material that was used regarding the influence of external factors on the organogenesis "via" callus (water, temperature, UV-B type radiations), on the capacity of regeneration of certain types of explants of a different nature.

The results lead to the conclusion that the enzyme activity and the morphogenesis were affected, depending on the wavelengths and the environments used for the cultures, by comparison to the studied biological material.

Rezumat

Lucrarea prezintă rezultatele testelor *in vitro* efectuate pe secțiuni tisulare din frunze și tulpini provenite de la specia *Zea mays*. S-a cercetat influența factorilor externi asupra organogenezei „via” calus (apă, temperatura, radiații de tip UV-B), capacității de regenerare a unor tipuri de explant (mugur nod, apex) de natură diferită.

Rezultatele au demonstrat faptul că a fost afectată activitatea enzimatică și morfogeneza, în funcție de lungimile de undă și de mediile de cultură utilizate.

Keywords: UV-B type radiation, enzymatic activity, apex, nod and bud

Introduction

The development of the plants requires knowledge on the exact optimization of water and chemicals, as well as the irradiation in a timely manner, so as not to be wasteful.

The corn (*Zea mays*) is a crop originally from Central America, which is cultivated nowadays in many parts of the world. It contains many hydrocarbons, starch, albumins, large amounts of B vitamins, vitamin E,

iron, phosphorus, magnesium, zinc and potassium. Corn is effective against stress, and the magnesium, which can be found in large quantities in the fruit of the plant, supplements in an excellent manner the lack of this element, due to diseases linked to the aging process of the organism. It is rich in B vitamins, especially vitamin B₁, which influence the functioning of the nervous system, muscles, the heart and the production of red blood cells [7]. Being a caryopsis, its fruit also contains starch, proteinic substances and oils, substances which are equally used in the preparation of pharmaceutical products [6].

The effects of the increase in the amount of UV radiation, but also the wavelength variation, include biomass reduction, the inhibition of photosynthesis, photo-morphogenetic and phenological changes, with important implications in maintaining the balance of the ecological system, of preserving certain plant genotypes and/or of the socioeconomic life. A series of studies have shown that plants respond with high variability when subjected to UV-B light. [4]

The effect of the UV-B radiation on the plant systems is ambivalent: - on the one hand, it is harmful, producing specific reactions of cellular, molecular, anatomic, mutagenic and photosynthetic stress[8], having on the other hand, through the UV-B receiver, a role in the acclimatization process, for example the induction of the protection pigments synthesis, which also involves the protection of the vitamins and minerals content found in the *Zea Mays*.

Materials And Methods

In preparing the culture environments, from our experience with different corn genotypes (*Zea mays* L.), taking into account the study of the ZP471 and *Helga* hybrids, we utilized auxin, α - naphthaleneacetic acid (NAA) at low concentrations, 0.5 – 1 mg/L, which, according to the studies in the plants biotechnology field [1], can be successfully used for callus induction and plant regeneration through organogenesis [3], and the cytokines as well, represented by 6-benzylaminopurine (BAP), of 1 – 1.5 mg/L concentration.

In order to select the response related to the hormones and other biostimulating substances, antibiotics, of the 6 days seedlings cultivated in a photoperiod of 12 hours light/ 12 hours darkness, the corn genotypes were placed in microtiter plates and preincubated for 24 hours.

On day 7, just before the measurements, 100 μ M of abscisic acid (ABA) (Duchefa) solution had been added for the hormonal treatment, 250

mM of NaCl solution in order to study the saline stress, and 100 mM of mannitol solution for the evaluation of the osmotic stress.

The *in vitro* organogenesis process was assessed by measuring the size of the seedlings (mm) developed from meristems[3]. The observations noted at 20, 40 and 60 days of culture have emphasized the fact that on the MS culture medium, but also on the LS one, supplemented with NAA as a source for auxin, under the effect of the UV-B type radiation, on different wavelengths, meristems were formed. (*Table III*)

Two basic environments have been utilized, Murashige-Skoog [5], abbreviated MS, and Lindsmaier-Skoog (LS), and also complex fertilizer LB (Sambrock, 1989) and MStop-Agar (0,5% agar), for the drawing of meristems and their inoculation on the culture environment, in order to initiate proper *in vitro* cultures.

A certain range of concentrations was used for each type of hormone, depending on the stage that was covered in the *in vitro* multiplication process. The types of vegetal hormones and the concentrations used are presented in Table I.

Table I.
Biostimulating substances used for the *in vitro* corn culture (mg/L)

Type of hormone	The <i>in vitro</i> culture initiation	The callogenesis introduction	The callus regeneration
NAA (mg/L) α - naphthaleneacetic acid	0.5	1	0.5
BAP (mg/L) 6-benzylaminopurine	1	0.5-1.5	-
Sucrose (g/L)	20	20	-
Thiamine	0.35	1.2	-
Pyridoxine	0.5	5	-

The observations were made using meristems from the stem and crown, which were previously disinfected by immersion in 75% ethanol and 1% sodium hypochlorite with a drop of Tween 20/100 mL. Philips UV lamps were alternatively used for disinfection as well, in order to avoid fungal infestations of various types or other microorganisms that might have influenced the results, but also a complex of antibiotics. (*Table II*).

Table II.
Antibiotic substances used in the experiment

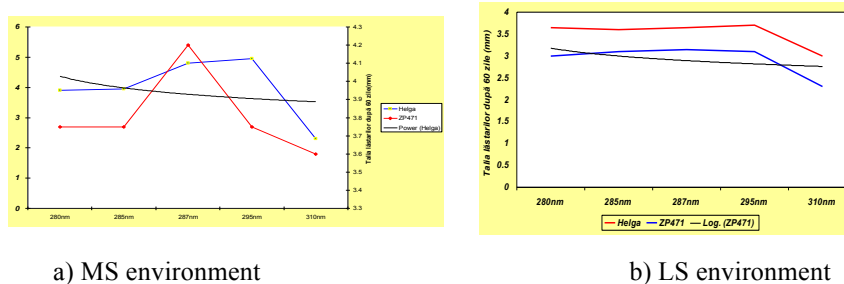
Antibiotic	Concentration %
Ampicillin (Amp)	100 ng/mL
Streptomycin (Sm)	100 ng/mL
Tetracycline (Tet)	25 µg/mL
Carbenicillin (Cb)	100 pg/mL
Rifampicin (Rfp)	100 pg/mL
Kanamycin (Km)	25 µg/mL
Hygromycin (Hyg)	15 µg/mL
Cefotaxime (Ctx)	250 µg/mL

Results and Discussion

The results obtained in the irradiation experiments of the explants, subjected to different wavelengths during the *in vitro* organogenesis for the corn, have shown the fact that the organogenesis processes for corn are different, depending on the source of the explant, on the culture environments and on the combination of the growth regulators. This fact is reflected by the difference in size between the seedlings developed from the Helga and ZP471 hybrids (Table III).

Table III
The size of the seedlings (mm) developed from the meristems grown in the MS and LS environments and the irradiation with different wavelengths, after 20, 40, 60 days of culture

No	The type used	UV-B Λ (nm)	Stem meristem						Crown meristem					
			MS			LS			MS			LS		
			20 daymm	40 daymm	60 daymm	20 daymm	40 daymm	60 daymm	20 daymm	40 daymm	60 daymm	20 daymm	40 daymm	60 daymm
	Helga	Without irradiation	1.1	2.3	4.3	0.98	2.1	4.1	1.2	2	3.7	1.3	2.2	3.98
1	Helga	280	0.9	1.9	3.9	0.85	1.75	3.75	1.25	1.85	3.55	1.15	1.80	3.65
2		285	0.98	1.95	3.95	0.88	1.95	3.85	1.20	1.90	3.60	1.18	1.86	3.60
3		287	1.1	2.1	4.8	1.12	2.15	4.50	1.26	1.95	3.85	1.25	1.90	3.65
4		295	0.98	1.95	4.95	0.98	1.90	4.55	1.20	1.90	3.70	1.22	1.85	3.70
5		310	0.7	1.3	2.3	0.65	1.45	2.55	1.05	1.55	2.30	1.00	1.50	3.0
	ZP471	Without irradiation	0.8	1.9	4.2	0.75	1.81	4	1	2	3.1	1	1.98	2.95
1	ZP471	280	0.75	1.75	3.75	0.70	1.80	3.65	0.9	1.9	3.20	0.98	1.85	3.0
2		285	0.79	1.75	3.75	0.75	1.86	3.70	0.95	1.95	3.20	0.98	1.95	3.10
3		287	0.8	1.8	4.2	0.82	1.82	4.35	0.10	2.10	3.25	1.10	2.15	3.15
4		295	0.73	1.75	3.75	0.75	1.78	3.85	0.10	2.10	3.15	1.00	2.00	3.10
5		310	0.6	1.6	3.6	0.60	1.55	3.55	0.7	1.3	2.85	0.65	1.50	2.3



a) MS environment

b) LS environment

Figure 1

The size of the seedlings (mm) developed from meristems from the stem and crown, grown in the MS and LS environments, after 60 days of culture, under the influence of UV-B

The development of the neo-plants (new plants grown) for the two hybrids, under the given experimenting conditions, is significantly different at a wavelength of 287 nm. The adaptation capacity of the plants after 60 days of culture under UVB 287 is similar in the two hybrids, but Helga tolerates much better wavelengths of 295 nm (Figure 1). Also, the utilized LS culture environment contributes to the good development of the plants, under the influence of UVB, in both cases. Consequently, the stem meristem is recommended for *in vitro* regeneration experiments due to its high tolerance to UVB. What is notable is the similar reaction of the genotypes to the other wavelengths, indicating the more pronounced effect on the regeneration processes, regardless of the type of explant or the type of environment used.

The organogenesis processes for corn differ depending on the variety which was used, the environment, the combination of used auxins and the wavelength which was used to irradiate the explants. The influence of the various wavelengths which was used to irradiate at regular time intervals is exercised by stimulating the growth and development of the neo-plants of the Helga variety, at wavelengths between 287 nm – 295 nm, compared to the ZP471 hybrid (Figure 1).

The results obtained regarding the evaluation of the *in vitro* regenerative capacity of the varieties of corn, depending on the irradiation conditions, on the nature of the explants and the culture environment which was used, have been pursued in three different environments (abbreviated T₀, T₁, T₂ and T₃). In the T₂ environment, the explants from the node have a regeneration rate of 80%, forming however out of each node 2-4 neo-plants with an early root system (Tables IV-V).

Table IV
Wavelengths and the experimental hormonal balance in the case of the corn explants

Utilized option	Base environment	UV-B □(nm)	Cytokinins	Concentration %	Auxin	Concentration%
To	MS	--	--	--	--	--
T1	MS	280-310	BAP	2	ANA	1
T2	MS	280-310	BAP	1	ANA	0.5
T3	MS	280-310	BAP	1	ANA	1

In comparison, from the apex and floral bud fewer neo-plants are formed, for both types of hybrids (Table IV).

Table V
The *in vitro* morphogenesis of the apex type explants for the HELGA corn, under the influence of UV-B and the culture environment

Utilized option	UV-B □ (nm)	Explant	Regeneration degree %		Number of neo-plants		Height (mm)		Other types of tissues	
			MS	LS	MS	LS	MS	LS	MS	LS
To	control	apex	67%	65%	1	1	11.2	10.2	-	-
T1	280	apex	69%	65%	1	1	11.5	11.00	-	-
	285		69%	67%	1	1	11.8	11.50	-	-
	287		70%	73%	1	2	12.0	12.50	-	-
	295		70%	71%	1	2	12	12.15	-	-
	310		45%	55%	1	1	9.5	9.25	-	-
T2	280	apex	72%	75%	2	2	8.0	8.20	-	-
	285		75%	75%	2	2	8.5	8.35	-	-
	287		75%	78%	2	3	8.5	8.40	-	-
	295		75%	78%	2	3	8.5	8.40	-	-
	310		53%	65%	1	1	7.1	7.05	-	-
T3	280	apex	75%	78%	2	2	11.2	11.50	-	-
	285		78%	78%	2	2	11.5	11.50	-	-
	287		80%	85%	3	3	12.1	12.10	-	-
	295		80%	85%	2	3	12.1	12.10	-	-
	310		55%	65%	1	1	10	0.95	-	-

The apex type morphogenesis of the explants for the Helga hybrid is influenced by the wavelength, not by the composition of the culture environment. Therefore, higher values than the control in the regeneration degree can be noticed at 287-295 nm wavelengths, which are much better tolerated in comparison to the rest of the UVB utilized specter. (Table V)

Table VI

The *in vitro* morphogenesis of the node type, HELGA corn explants, under the influence of the UV-B and the culture environment

Utilized option	UV-B □ (nm)	Explant	Regeneration degree %		Number of neo-plants		Height (mm)		Other types of tissues	
			MS	LS	MS	LS	MS	LS	MS	LS
To	control	node	70%	65%	1	1	10.2	10.00	-	-
T1	280	node	72%	70%	1	1	10.5	10.20	-	-
	285		75%	75%	1	1	10.8	10.25	-	-
	287		75%	75%	1	2	11.50	10.40	-	-
	295		75%	75%	1	2	12	10.10	-	-
	310		55%	50%	1	1	9.5	9.00	-	-
T2	280	node	75%	75%	3	2	6.0	6.60	-	-
	285		80%	80%	3	3	6.5	6.75	-	-
	287		80%	80%	4	3	6.5	6.75	-	-
	295		80%	80%	4	3	6.5	6.75	-	-
	310		63%	63%	2	1	5.0	5.50	-	-
T3	280	node	75%	75%	3	3	7.2	7.35	-	-
	285		78%	80%	2	3	7.5	7.55	Callus 45%	Callus 45%
	287		78%	75%	3	4	7.1	7.65	-	-
	295		80%	65%	5	3	7.0	7.20	-	-
	310		65%	70%	1	1	6.5	6.25	-	-

The balance between auxins and cytokinins from the culture environment has a significant influence on the regeneration degree of the node type explants (Table VI). We can also notice the stimulation of the regeneration on the T₂ environment with percentages of 80% and the formation of callus on the T₃ environment, with a UV-B irradiation of 285 nm. We can notice again a higher tolerance level to the UV-B 297-295 nm at the Helga type hybrid, in comparison with the rest of the analyzed specter. In this case, compared to the regeneration of the apex, it is recommended the usage of a proper hormonal balance to sustain the regeneration and subsequent development process of the neo-plants.

Table VII
The *in vitro* morphogenesis for the bud type explants for the HELGA corn variety, under the influence of the UV-B and the culture environment

Utilized option	UV-B □ (nm)	Explant	Regeneration degree %		Number of neo-plants		Height (mm)		Other types of tissue	
			MS	LS	MS	LS	MS	LS	MS	LS
To	control	bud	57%	65%	1	1	8.2	7.15	-	-
T1	280	bud	60%	70%	1	1	8.5	7.50	-	-
	285		60%	75%	1	1	8.8	7.80	-	-
	287		60%	75%	1	2	8.0	8.10	-	-
	295		60%	75%	1	2	8.8	8.00	-	-
	310		55%	50%	1	1	7.5	7.00	-	-
T2	280	bud	62%	75%	3	2	12.0	12.10	-	-
	285		65%	80%	3	3	13.5	13.50	-	-
	287		65%	80%	3	3	13.5	13.40	-	-
	295		65%	80%	3	3	13.5	13.50	-	-
	310		50%	63%	1	1	10.1	10.00	-	-
T3	280	bud	55%	75%	4	3	7.2	7.00	-	-
	285		58%	80%	4	3	7.5	7.50	Callus 45%	Callus 35%
	287		58%	75%	4	4	7.1	7.15	-	-
	295		58%	65%	4	3	7.1	7.10	-	-
	310		45%	70%	2	1	6.5	6.00	-	-

Table VIII
The *in vitro* morphogenesis for the apex type explants for the ZP471 corn variety, under the influence of UV-B and the culture environment

Utilized option	UV-B □ (nm)	Explant	Regeneration degree %		Number of neo-plants		Height (mm)		Other types of tissues	
			MS	LS	MS	LS	MS	LS	MS	LS
To	control	apex	65%	55%	1	1	10.2	9.5	-	-
T1	280	apex	66%	65%	1	1	10.5	9.75	-	-
	285		66%	65%	1	1	10.8	10.00	-	-
	287		70%	70%	1	1	11.0	10.50	-	-
	295		70%	65%	1	1	11	10.10	-	-
	310		50%	55%	1	1	9.8	9.55	-	-
T2	280	apex	70%	70%	2	1	7.0	7.10	-	-
	285		70%	75%	2	2	7.5	7.55	-	-
	287		75%	75%	2	2	7.5	7.55	-	-
	295		75%	75%	2	2	7.5	7.55	-	-
	310		50%	50%	1	1	6.1	6.55	-	-
T3	280	apex	65%	60%	2	2	11.5	11.0	-	-
	285		68%	65%	2	2	11.7	11.50	-	-
	287		70%	70%	3	2	12.3	12.50	-	-
	295		70%	70%	2	2	12.5	12.50	-	-
	310		65%	60%	1	1	10.5	10.55	-	-

Table IX
The *in vitro* morphogenesis of the node type explants for the ZP471 corn variety, under the influence of UV-B and the culture environment.

Utilized option	UV-B □ (nm)	Explant	Regeneration degree %		Number of neo-plants		Height (mm)		Other types of tissues	
			MS	LS	MS	LS	MS	LS	MS	LS
To	control	node	70%	60%	1	1	9.2	8.50	-	-
T1	280	node	70%	70%	1	1	9.5	9.50	-	-
	285		75%	75%	1	1	9.8	9.65	-	-
	287		75%	75%	1	1	9.8	9.65	-	-
	295		75%	75%	1	1	11	10.50	-	-
	310		50%	55%	1	1	8.5	8.25	-	-
T2	280	node	70%	65%	2	2	6.0	6.20	-	-
	285		75%	70%	3	3	6.2	6.25	-	-
	287		75%	75%	4	4	6.2	6.25	-	-
	295		75%	75%	4	4	6.2	6.25	-	-
	310		60%	65%	3	3	5.0	5.50	-	-
T3	280	node	70%	65%	3	3	7.2	7.25	-	-
	285		70%	60%	1	3	7.6	7.75	Callus 30%	Callus 45%
	287		70%	70%	1	4	7.6	7.70	-	-
	295		75%	75%	1	4	7.8	7.70	-	-
	310		50%	75%	1	1	6.1	6.60	-	-

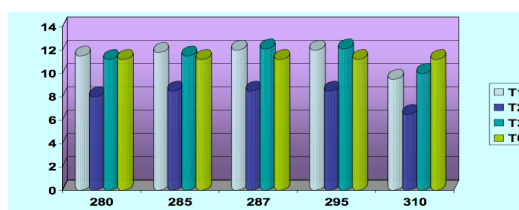
Table X
The *in vitro* morphogenesis for the bud type explants for the ZP471 corn variety, under the influence of UV-B and the culture environment.

Utilized option	UV-B □ (nm)	Explant	Regeneration degree %		Number of neo-plants		Height (mm)		Other types of tissues	
			MS	LS	MS	LS	MS	LS	MS	LS
To	control	bud	65%	60%	1	1	7.4	7.00	-	-
T1	280	bud	65%	65%	1	1	7.5	7.25	-	-
	285		60%	65%	1	1	7.8	7.55	-	-
	287		60%	66%	1	1	7.8	7.70	-	-
	295		60%	66%	1	1	7.8	7.70	-	-
	310		50%	55%	1	1	6.5	6.75	-	-
T2	280	bud	65%	60%	2	2	9.0	9.10	-	-
	285		75%	70%	3	2	9.5	9.45	-	-
	287		75%	75%	3	3	9.5	9.55	-	-
	295		75%	75%	3	3	9.5	9.55	-	-
	310		50%	55%	1	1	9.1	9.25	-	-
T3	280	bud	60%	50%	3	2	9.2	9.10	-	-
	285		62%	65%	3	3	9.5	9.25	Callus 30%	Callus 35%
	287		62%	65%	3	3	9.1	9.75	-	-
	295		62%	60%	3	2	9.1	9.75	-	-
	310		50%	55%	1	1	6.8	7.25	-	-

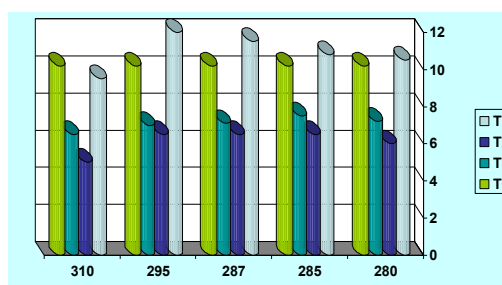
The bud type explants had a superior degree of regeneration on the LS type, T_2 environment regardless of the utilized wavelength (Table VII). Also, the growth rate of the newly formed neo-plants is significantly faster on the T_2 environment. As was expected, in the T_3 environment the callus was obtained. The regeneration capacity of various explants of the ZP471 variety is clearly inferior to that of the HELGA variety, with average differences ranging from 10-20%.

The node and apex type explants reaction is similar compared to the bud type explant which stands out with slightly lower values (Table VIII, IX, X). The graphical representation of the results which were presented so far can be seen in the graphs below (Figures 2-3).

Înălțimea în mm, -apex/Helga



Înălțimea în mm, -nod/Helga



Înălțimea în mm -bococ- Helga

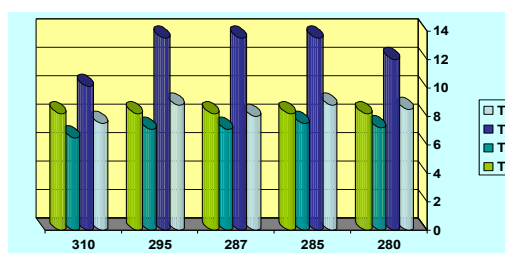
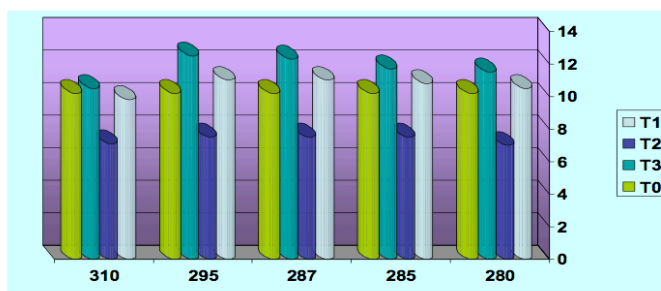
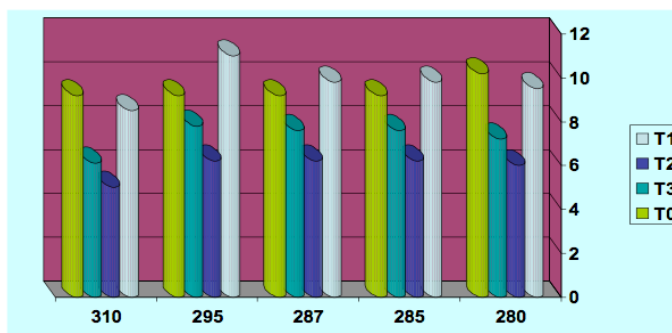


Figure 2. The *in vitro* morphogenesis for the apex, bud and node types, under the UV-B influence, over the three abbreviated environments T_1 , T_2 , T_3 compared with T_0 - Helga

Înălțimea în mm, -apex/ ZP471



Înălțimea în mm, - nod/ZP471



Înălțimea în mm, -boboc/ ZP471

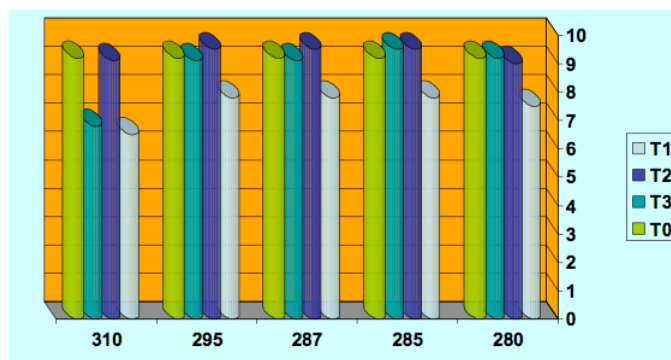


Figure 3.

The *in vitro* morphogenesis for the apex, bud and node types, under the UV-B influence, over the tree abbreviated environments, T1, T2, T3 compared with T₀- ZP471

Table XI

The growth rhythm of the newly formed plants, in the MS environment, under the influence of various wavelengths, regarding the HELGA hybrid.

Utilized option	Wavelength □ (nm)	Length of leaves (cm)	Number of plants	Length of roots (cm)	Degree of subsidy
T ₀	control	0.25	1	2.00	-
T1	280	0.25	2	2.00	-
	285	0.28	4	2.20	-
	287	0.30	5	2.25	-
	295	0.26	3	2.22	-
	310	0.21	2	2.10	-
T ₀	control	0.18	1	1.50	-
T2	280	0.18	1	1.55	-
	285	0.19	2	1.75	-
	287	0.2	3	1.75	-
	295	0.2	2	1.73	-
	310	0.17	1	1.55	-
T ₀	control	0.15	1	1.8	-
T3	280	0.25	2	1.85	-
	285	0.28	3	1.9	-
	287	0.32	6	1.9	-
	295	0.30	4	1.85	-
	310	0.25	2	1.80	-

Table XII

The growth rhythm of the newly formed plants, in the MS environment, under the influence of various wavelengths regarding the ZP471 hybrid.

Utilized option	Wavelength □ (nm)	Length of leaves (cm)	Number of plants	Length of roots (cm)	Degree of subsidy
T°	etalon	0.15	1	1.70	
T1	280	0.15	2	1.80	
	285	0.18	2	1.80	
	287	0.18	4	1.80	
	295	0.17	3	1.80	
	310	0.14	1	1.50	
T°	control	0.20	1	1.90	
T2	280	0.20	1	1.90	
	285	0.25	3	1.95	
	287	0.27	4	1.95	
	295	0.25	3	1.90	
	310	0.10	2	1.75	
T°	control	0.20	1	2.0	
T3	280	0.20	3	2.05	
	285	0.25	5	2.15	
	287	0.30	6	2.20	
	295	0.25	4	1.85	
	310	0.20	2	1.50	

The UVB influence of 287 and 295 nm is obvious in the case of the values regarding the growth rhythm of the different organs of the newly formed plants. We can notice significant statistical differences regarding the control for both the tested hybrids (Table XI-X).

The average regeneration capacity has increased to 80%, the lowest value being 50% (Helga, MS culture environment), and the highest value being 75% (ZP471, LS culture environment).

Conclusions

The morphogenetic potential of the corn root cell activation under the pressure of a certain dose of hormones – auxins and cytokinins combined with wavelengths between 280-310 nm stimulates cellular proliferation, suggesting a dose-dependent effect of UV-B on the corn organogenesis.

The hormonal balance associated with the irradiation at 287 nm is capable of inducing the regeneration of plants with a root system. The phytohormones stimulate the *in vitro* development process; high values of auxin/cytokine support the callogenesis whilst low values induce a proliferation of buds and organogenesis. Auxin and cytokinin favors the organogenesis process.

The size of the regenerated neo-plants vary between 0,35-10 mm (with average values of 0,4-0,5 mm), depending on the culture environment and UV-B type. In the case of the apex for the Helga hybrid and the node and apex for the ZP471 hybrid, we notice a greater influence of the radiation between 285 nm – 287 nm – 295 nm, which actually stimulates the development, growth, regeneration degree, number of developed plants, in comparison to the influence of the wavelength of 310 nm.

As the population grows, so does the food requirement. Studying to obtain as large production of crops as possible is very important, as well as studying the external influences that determine a change in the constituent compounds of the plant. The organism is subjected daily to stressful situations and to the attacks of viruses, bacteria, pollution, and these affect the balance of the cells. Because of its rich mineral and vitamin content, corn can energize and is beneficial to the health of the blood vessels. The high content of flavonoids, polyphenols, phytochemicals and antioxidants recommend it as an aid in treating cancer.

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