RESEARCH ON SEED EXUDATE INDICES AND PEROXIDASE ACTIVITY IN SOME MAIZE AND BROAD BEAN SEEDS

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Rezumat

Analizând exudatul seminal şi activitatea peroxidazei unor semințe **de porumb şi de bob** cu viabilități diferite, această lucrare a evidențiat unele modificări ale indicilor exudatului seminal şi ale **activității acestei enzime.** Interpretarea **testelor de laborator şi calculul diferitelor corelații au arătat** o legătură directă între scăderea viabilității (facultății germinative) semințelor şi creşterea valorilor **conductivității electrice şi a efluxului transmembranar de zaharuri** totale şi **aminoacizi liberi. Valorile** foarte scăzute ale activității peroxidazei unor semințe în timpul germinației au putut fi direct asociate **viabilității reduse (sau lipsei de viabilitate) a acestora, având drept cauză anumite deteriorări structurale** şi funcționale care au determinat şi modificările indicilor exudatului seminal.

Abriss

In dem Werk wurden das seminäle Exsudat und die Tätigkeit der Peroxydasse einigen Mais- und Pferdebohnenkörner analisiert. Es wurden einige Änderungen dem Exsudatindex sowie auch der **Peroxydasentätigkeit herausgegeben. Die Auslegung den Laboruntersuchungen sowie auch die** Rechnung verschiedenen Korrelationen zeigte eine direkte Verbindung zwischen der Verringerung der **Samenlebensfähigkeit (Keimungsfähigkeit) und die Zunahme der elektrischen Leitfähigkeit und den** transmembranären Abfluss von totalem Zucker und freie Aminosäure. Die sehr geringe Werte der **Peroxydasentätigkeit einigen Samen während der Keimung konnten mit einer kleinen Lebensfähigkeit** bzw. der ihre Abwesenheit. Die Ursache dafür ist eine strukturelle- und Funktionsbeschädigung die **auch die Änderungen dem seminäle Exsudat stellten fest.**

Résumé

Cette recherche sur l' exudate et l' activité de peroxidase des quelques semences de mais et de fève (avec differente viabilité) a mis en évidence des modifications des indices d' exudate et de l' **activité de cette enzyme. L' évaluation des analyses de laboratoire et le calcul des differentes** correlations ont relevé une relation directe entre la diminution de la viabilité des semences et la **croissance de la conductivité électrique et d' eflux transmembranaire des sucres totals et des** aminoacides libres. Les valeurs tres reduites de l' activité de la peroxidase, des quelques semences **pendant la germination, ont été directement correlées avec la viabilité reduite (ou l' absence de la** viabilité) des celles-ci, ayant comme cause certaines détériorations de la structure et de la fonction qui **ont déterminé aussi les modifications des indices d'exudate de la semence.**

Introduction

Although tackled within last four decades scientific works, the damage of living organism membrane structures still remains a research field both for physiologists and biochemists.

It seems that one of the major cause of the membrane damages would represent the free radicals metabolically formed which, by certain reasons, it can no more be neutralized by the detoxifiation systems, and they accumulate into the living tissues. This free radicals accumulation favours some irreversible transformations leading to the tissues ageing and death.

Whatever woud be the cause, the main membrane damage indices are: the increase of electrical conductivity and the transmembranal loss of organical and anorganical substances from cell.

This work analyses three basic indices of the seed exudate (electrical conductivity, sugars and aminoacids leakage) as well as the peroxidase activity, in order to indirectly evidence some membranal modifications in maize and broad bean seeds having different viabilities.

Materials and methods

The biological material in this work was represented by eight seed samples : four of maize and four of broad bean, belonging to some local cultivars (within parenthesis is reproduced the germinative capacitY).

EP-95 (95%); **EP-80** (80%); **EP-50** (50%); **EP-0** (0%) – maize samples

EB-92 (92%); **EB-85** (85%); **EB-68** (68%); **EB-0** (0%) – broad bean samples

Except **EP-95** and **EB-92**, stored some years under special conditions (with controlled humidity and 4°C constant temperature), the other samples have been stored in diverse

unclimatized places. For obtaining no viability samples (O%), their seeds were subjected to some repeated hidrous-thermic shocks

For viability assessment there were used 50 seeds in 4 replicates for each sample (Anghel et al., 1959; Ellis et al., 1985). The distilled water, which imbued a special filter paper put into Petri boxes, represented the germination medium. The germination temperature was 25°C (for maize) and 20°C (for broad bean), and the maximum test assessment duration was 7 days (for maize) and 10 days (for broad bean). There were considered germinated the seeds whose rootlets were at least 1 mm length.

The seed exudate electrical conductivity was evaluated by measuring this index on a Radelkis type conductmeter. Thus the samples, containing each one 20 seeds (in 2 replicates), have been introduced into Berzelius tumblers with distilled water (30 ml for maize and 40 ml for broad bean) and incubated at 25°C (at darkness). After 5 hours, the distilled water, containing the seed exudate, was collected for measuring its conductivity. The results have been reported as μ S/g (μ mho/g), seeds being previously weighed.

The total sugars dosing in exudate has been accomplished în 2 replicates of each 3 g for maize, and 5 g for broad bean, which were incubated in 7 ml, and respective 8 ml distilled water at 25°C (darkness conditions). After 5 hours there were dosed the total sugars, using a method based on reducing of picric to picramic acid by monoglucides (Schell, 1980). The results were expressed as μg sugars at 1g of seed.

For aminoacids exudate dosing there was worked in 2 replicates, each one of 3,5 g seed + 5 ml distilled water (maize), and 5 g seed + 6 ml distilled water (broad bean), which were introduced în thermostat at 25°C (darkness conditions). After 5 hours, it has dosed the free aminoacids in extract, using a micromethod relied on colour reaction between the aminoacids from exudate and ninhidrine reagent (Villegas and Mertz, 1975). The results were expressed as μg aminoacids at 1g of seed.

The peroxidase assessment has been made by means of a colorimetric method (Artenie and Tănase, 1981). The enzyme extract was obtained through breaking of some 0.1-0.5 g seeds together with natrium phosphate buffer M/15 (pH=6,7), followed by centrifugation. The activity dosing was made colorimetric (at 430 nm), using pyrogalol (189 mg in 25 ml distilled water). The peroxidase activity was estimated, according to the reaction speed, as conventional units, expressed at 1 g seeds (flour).

Results and discussion

The table no. 1 reproduces the seed exudate indices values in maize and broad bean samples.

As to electrical conductivity, one can observe that in the both analysed species the lowest values were in samples with maximum viabilitie (EP-95 and EB-92), and the highest ones in samples having zero viability (EP-0 and EB-0). Except one sample, it can see that the germination (G) decrease led to the electrical conductivity (C) increase. This fact is also confirmed by simple correlation coefficients between these parameters (G and C), whose negative values were distinct significant (– 0.937°° for maize, and – 0.915°° for broad bean).

Table 1

Maize and broad bean seed exudate indices

Indices	Electrical conductivity (μS/g)	Sugars (μg/g)	Aminoacids (μg/g)
Samples		Maize	
EP-95	16,08	170,25	17,54
EP-80	19,70	253,14	82,45
EP-50	29,75	425,05	56,60
EP-0	128,30	613,24	259,00
		Broad bean	
EB-92	41,86	159,15	43,24
EB-85	121,40	482,85	211,08
EB-68	89,17	371,50	253,90
EB-0	220,25	1103,10	505,35

Like conductivity, the total sugars and free aminoacids content of seed exudate had the geatest values in zero viability samples too. Also in this case the correlation coefficients estimation, between exudate sugars, aminoacids and germination capacity, has shown negative values significant for samples of the both species: – 0.994^{ooo} for maize and –0.952^{ooo} for broad bean (as for total sugars) and –0.915^{oo} for maize, respective –0.950^{ooo} for broad bean (as for free aminoacids).

In order to establish the relatioships between the three seed exudate indices, there were calculated the coefficient correlations between these ones. Thus, between conductivity (C) and sugars (S), conductivity (C) and aminoacids (A), sugars (S) and aminoacids (A) there were found positive correlations with values between 0.872* (for the relationship S – A, in maize) and 0.993*** (for the relatioship C – S, in broad bean), which shows that, in the analysed samples, the electrical conductivity increase was into a direct relationship with exudate sugars and aminoacids enhance.

According to some authors, the presence of electrolits within the seed exudate, which is caused by membrane damages, can reach very high values. In this respect, Ching and Schoolcraft (1968), working on *Trifolium incarnatum* seeds, found conductivity values between 44 and 89 μS/g and an aminoacids leakage between 299 şi 650 μg/g. According to Abdul-Baki and Anderson (1970), in barley damaged embryos the glucose transmembranar lost can be about 60-70%, and sucrose 20-30% from total reserve.

In the table no. 2 are rendered the peroxidase activity mean values at 0, 48 and 96 hours of germination.

In the both species samples during pregermination (zero hours), the enzime activity values have registered small differences between the 4 samples having different viabilities. After 48 and 96 hours of germination the differences between sample seeds increased, the peroxidase activitiy values being situated on a ascending way (fig. 1) into direct correlation with germination capacity (G) values of the analysed samples. The table no. 3 indicates significant (**) and very significant (***) distinct direct correlations between G and peroxidase activity only at 48 and 96 hours of germination, resulting that, during germination, the enzimes activity (this case peroxidase) reaches maximum values according to very intense metabolic changes in this process, and the differences between samples are related to certain structural or functional changes of seeds, indirectly expressed through G and seed exudate index values.

Table 2

The peroxidase activity in maize and broad bean seeds during germination

	Peroxidase activity (U.I./g D.M.)		
Time	0 hours	48 hours	96 hours
Samples		Maize	
EP-95	15,60	19,88	32,43
EP-80	12,35	20,50	24,35
EP-50	14,23	16,75	16,03
EP-0	13,38	6,98	10,69
		Broad bean	
EB-92	9,35	31,28	38,10
EB-85	6,44	18,55	31,67
EB-68	5,90	21,03	23,28
EB-0	7,15	2,93	4,07

U.I. = international units; D.M. = dry matter

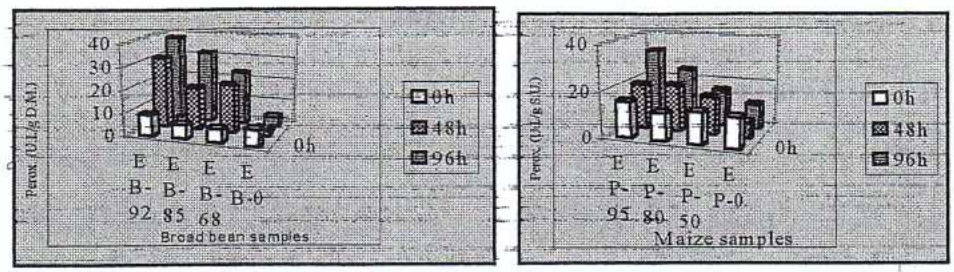
Conclusions

1. The seed exudate analyse in different viabilities maize and broad bean seed samples has indirectly evidenced the presence of some membrane modifications, expressed through electrical conductivity increase as well as through transmembrane leakage of total sugars and free aminoacids.

2. The correlations estimation between conductivity (C), sugars (S) and aminoacids (A) from exudate, on the one hand, and the germination capacities of the analysed samples, on the other hand, has shown that the posibility of membrane changes appearance increases once with seeds

viability decrease. Also, it has resulted that a conductivity enhance has been into a tighter relationship with transmembrane loss of aminoacids (maize samples), and of sugars (broad nean samples).

3. Except no viability seds (EP-0 and EB-0), the peroxidase activity in the other maize and broad bean samples has evdenced, during gemination, an ascending evolution comparatively with the rest period (0 hours). The reduced enzime activity of some samples one could explain by deterioration of some structures or functions, indirectly expressed through germination and seed exudate indices values.



(a) (b)

Fig. 1 The peroxidase activity during germination of maize (a) and broad bean (b) seeds

Table 3

Simple correlation coefficients between germination capacity (G) and peroxidase activity

Correlation indices	Germination capacity	Germination capacity	Germination capacity
Samples	Perox. activ. (0 h)	Perox. activ. (48h)	Perox. activ. (96h)
Maize	0,325	0,967***	0,945**
Broad bean	0,221	0,923**	0,982***

***/ooo = very significant; **/oo = distinct significant; */o = significant

References

1. Abdul-Baki, A.A., Anderson, J.D., 1970 - *Viability and leaching of sugars from germinating barley*. Crop Sci., 10, 31-34.

2. Anghel G., Raianu Maria, Matei C., Bucurescu N., Rădulescu I., Anganu I., Velea C., 1959 - *Determinarea calităţii semînţelor*. Edit. Acad. R.P.R., Bucureşti.

3. Artenie, V., Tănase Elvira, 1981 - *Practicum de biochimie generală*. Centrul de Multiplicare al Univ. "Al. I. Cuza" Iaşi, 138-140.

4. Ellis R.H., Hong T.D., Roberts E.H., 1985 - *Handbook of Seed Technology for Genebanks*. International Board for Plant Genetic Resources, vol. II, Rome.

5. Hosnedl, V., Behal, J., Horcicka, P., 1993 - *Problems of using conductometric tests for pulse seed*. Quatrieme rencontre internationale sur les semences, Angers, France, 20-22 juillet 1992, vol 3, 969-973.

6. Schell, H., 1980 – *Biochimie*. Edit. Did. şi Ped., Bucureşti, 73-80.

7. Villegas, E., Mertz, T.E., 1975 - *Simple chemical and biological methods used at Purdue University to evaluate cereals for protein quality*. St. Bull. No. 70, U.S.A, 17-21.

8. Ching, T.M., Schoolcraft, I., 1968 - *Physiological and chemical differences in aged seeds*. Crop Sci., 8, 407