SOME BIOCHEMICAL, PHYSIOLOGICAL AND CYTOGENETICAL ASPECTS IN *VICIA FABA* L. SEEDS, DURING GERMINATION

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Abstract
Four seed samples of different ages, belonging to one faba bean (*Vicia faba* L.) landrace, represented the biological material studied along 168 hours of germination to see if the storage period length and/or storage conditions or viability influence the respiration, the peroxydase and mitosis activity of the seeds. The peroxydase activity was more reduced, and the respiration was more intense (after 48 hours of germination), in lower viability seed samples, kept a longer period in inadequate storage conditions. In the same seed samples, lower cells division number and mitosis index value, but a greater aberrant cells, beside blank, have been registered.

Resumé
Quatre échantillons de semences, appartenant à une variété de fève (*Vicia faba* L.), avec différentes capacités germinatives et périodes de dépôt (garde) ont été etudiés pendant la germination (en cours de 168 heures), pour établir s'il existe une liaison entre la longueur de la période, les conditions de dépôt (garde) ou la viabilité et la respiration, l'activité de peroxidase et l'activité mitotique des semences. L'activité de peroxidase a été plus réduite et la respiration plus intense (après 48 heures de germination) à les échantillons de semences avec moins viabilité et gardé une periode plus longue en conditions neclimatique. Aux mème échantillons de semences ont été enregistrés valeurs plus réduites des cellules en division et du index mitotique moins un nombre plus grand de cellules aberrantes, comparatif au (avec) blanc (témoin).

Rezumat
Patru eşantioane de seminţe, cu germinaţii şi perioade de depozitare diferite, aparţinând unei populaţii locale de bob (*Vicia faba* L.), au fost studiate pe parcursul a 168 ore de germinaţie, pentru a constata în ce măsură lungimea perioadei şi/sau condiţiile de depozitare ori viabilitatea influenţează respiraţia, activitatea peroxidazei şi activitatea mitotică a seminţelor. Activitatea peroxidazei a fost mai redusă, iar respiraţia mai intensă (după 48 ore de germinaţie) la probele de seminţe cu viabilitate mai mică şi păstrate o perioadă mai îndelungată în condiţii neclimatizate. La aceleaşi eşantioane de seminţe s-au înregistrat valori mai mici ale celulelor în diviziune şi ale indicelui mitotic, dar un număr mai mare de celule aberante, comparativ cu martorul.

Introduction

The seeds storage can lead, in time, to the loss of viability because of some external and internal factors action. Regarding the inner factors, the genetically integrity, determining an efficient enzymatic and respiratory activity, has a great importance. It seems that it is one direct relationship between loss of the seeds viability and reduction of some enzymatic activities. On the other hand, the respiration proportionally increases to seed moisture content and to temperature rise, leading to senescence (Harrington, 1963).

Trying to find a relationship between mitosis, respiration and enzymatic activity in some seeds, with different storage periods and viabilities, this work analyses the mitosis as well as peroxydase and respiration evolution during germination of some seeds, belonging to one faba bean (*Vicia faba* L.) landrace.

Materials and methods

Four seed samples, belonging to one faba bean landrace were used as biological material. Some features of the samples, at the tests start date, are reproduced in the table 1.

Table 1. Characteristics of the used biological material

Samples	Storage period (years)	G (%)	WTG (g)	SMC (%)
MB	1	97	1040	11.304
B1	10	82	1044	11.312
B2	16	75	1038	11.286
B3	14	70	1042	11.293

G= germination percentage; WTG = weight thousand grain; SMC = seed moisture content

MB, having the greatest germination percentage (G%) and stored in proper conditions (+4°C, and 30% relative humidity), was used as blank. Concerning germination, except the blank, it was 70-82% for seeds whose storage period varied between 10 and 16 years. Thousand grain weight and seed moisture content registered small differences between samples. In order to estimate the germination percentage, 4 replicates of 50 seeds for each sample were achieved (Ellis et al., 1985). Petri dishes, with special filter paper, have been used and medium of germination was distilled water. The maximum term of the test evaluation was 10 days.

The peroxydase activity was investigated, according to a colorimetric method (Artenie and Tănase, 1981), at different times of germination, within 0-168 hours interval, and was expressed as IU/g DM.

The respiratory activity was assessed, accoding to Boysen-Jensen proceeding (Boldor, Raianu and Trifu, 1983), by measuring CO_2 released at different times of germination, within 0-168 hours interval, and was expressed as cm^3 CO_2/g fresh material/h.

The mitosis activity was determined using Feulgen method (Raicu et al., 1983), which allows the cytological examination of the first meristem division in the root tips. The mitosis activity assesment consisted in examination of 1000 cells in two replicates (for each sample). The result was expressed at 1000 examined cells. There was also estimated the number of cells in different mitosis division phases. Besides, it has been reported the percentage of aberrant cells (AC%), as well as the mitosis index value (MI%) as number of cells in mitosis / total number of examined cells x 100.

Results and discussion

Table 2 includes the peroxydase activity in four faba bean samples during germination. Along the whole analysed interval, one can see an increasing of the enzyme activity once with the germination time increasing, as well as significant differences between blank and the other samples. The lowest enzyme values were found at 0 and 24 hours of germination, and the highest ones at 96 and 168 hours. Except 24 hours, at the other germination times the greatest peroxydase values were held by the blank (MB). Between samples with less viability (and older) there were differences too (B_1 beside B_2 and B_3).

Table 2. The mean values of the peroxidase activity in the faba bean seed samples, at different periods of germination

	Peroxydase activity (IU / g DM)					
Time	0 h	24 h	48 h	72 h	96 h	168 h
SAMPLES						
MB	7.41	6.97	23.67	18.54	31.04	30.60
B1	4.64	7.98	20.59	18.17	29.15	27.27
B2	5.43	4.67	17.07	15.53	23.02	22.03
B3	2.82	5.40	16.74	16.46	16.80	15.54

IU = international units; DM = dry matter

Working on caryopses of barley, having different storage periods, Mac Leod (1952) found an important reducing of the peroxydase activity mostly in caryopses older than 10 years.

The respiratory activity has registered differences along the assessed interval - very low value at 0 and 24 hours and much higher ones, beginning with 48 hours of germination (table 3). For the four tested samples, 96 hours of germination represented the greatest respiratory mean value in the whole analysed cycle.

Table 3. The mean value of the respiration in the faba bean seed samples, at different periods of germination

Respiration values (cm^3 CO_2 / g / h)						
Time / Samples	0 h	24 h	48 h	72 h	96 h	168 h
MB	0.017	0.054	0.150	0.130	0.190	0.147
B1	0.019	0.066	0.144	0.130	0.190	0.160
B2	0.009	0.045	0.125	0.120	0.200	0.216
B3	0.016	0.049	0.126	0.135	0.192	0.200

During the first stages of germination, as a result of seeds imbibition and of the embryo cells hydration, the respiration, having as substratum the simple sugars in the reserve tissue increases very much. After 2-7 days, depending on species, the starch hydrolisation begins, and the respiration intensity, in this stage, depends on the rate of its hydrolisation (Peterfi and Sălăgeanu, 1972). It seems the differences between seeds, as to the water uptake or mitochondria activity (the latter ones representing the respiratory enzymatic center) or the both, determine great differences, between samples, regarding the respiration, because the ageing process would not reduce so much the respiration substratum (i. e. the sugars reserve), but especially its using efficiency (Heydecker, 1969).

Analysing and comparing the data of the both tables, one can better see the relationship between the peroxydase activity and respiration. Thus, at MB and B1, on the second part of the germination time (96-168 h), the peroxidase value increase, while of the respiration ones decrease. At B_2 and B_3 the same report is inverted.

The table 4 reproduces the value of mitosis index, the number of cells in the four cell division phases, as well as the aberrant cell percentage.

Table 4 The cell division dynamics in broad bean samples

Index / Samples	Dividing cells (%)	Prophase (cells no.)	Metaphase (cells no.)	Anaphase (cells no.)	Telophase (cells no.)	Mitosis index (%)	Aberrant cells (%)
MB	112	52	19	14	27	13.2	0.60
B1	94	36	22	10	25	9.4	2.90
B2	75	31	18	9	15	7.8	5.70
B3	82	27	23	11	19	5.4	3.00

As seen, the most intense mitosis activity (dividing cells) was carried out by blank (MB) - the lowest value being în B2. Compared to other division phases, the prophase prevailed in the four samples.

The aberrant cells percentage, except the blank where this index registered a very low value, seems to be correlated especially to seeds age than to seeds viability.

Working on 3 broad bean landraces, Fărtăiş and Avramiuc (1991) found the aberrant cell percentages close to those in the table 4, and a direct correlation between abnormal cells and seeds age.

Conclusions

- In the four seed samples of different ages, belonging to one faba bean landrace, the peroxidase activity was more reduced in older seed samples than in blank, especially between 96 and 168 hours of germination.
- The respiration process, based on volume of CO_2 released, evidenced much greater value at 48 hours beside 0 and 24 hours of germination. The decrease of peroxydase activity and the increase of respiration can cause the accumulation of a great amount of toxic produce during seed metabolic processes.
- As to mitosis activity, the samples with more reduced viability has registered lower values of dividing cells and mitosis index as well. The aberrant cells percentage seems to be into a direct correlation more with storage period length and storage conditions than with viability (G).

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