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SUMMARY

The present work on group testing describes the reliable detection by ELISA of pea seed-borne mosaic virus (PSbMV) in pea seed lots. In the cultivars studied, the percentage of embryos that were ELISA-positive was correlated with the percentage of seed transmission. But unlike the seed-borne lettuce mosaic virus/lettuce and soybean mosaic virus/soybean systems, when whole pea seed was blended, the assays for PSbMV-infected seed in the experimental lot greatly overestimated the known percentage of seed transmission, due to the extraction of non-seed-transmissible virus from the seed coats. The removal of seed coats thus becomes imperative and a promising mechanical procedure is proposed for routine application. Before grouping embryos, the maximum size of a group can be determined on the basis of the variation in PSbMV titer among about 20 infected embryos of a given cultivar. In case of pea cv. Amino, the embryo with the lowest virus titer could be detected when mixed with a maximum of 100 healthy embryos. In case of pea cv. Arpad the group size limit proposed is 60 embryos. This limit may thus vary among cultivars and antisera ; a workable limit should therefore be determined for routine use.

Additional key words : *Seed transmission rate, epidemiology, routine testing, seed certification, detection.*

RÉSUMÉ

Analyse de lots de graines de Pois par la technique ELISA : taux de transmission du pea seed-borne mosaic virus.

L'objectif de ce travail est de définir les modalités de traitement de l'échantillon de graines qui permettent d'utiliser la technique ELISA pour la détermination du taux de transmission du pea seed-borne mosaic virus (PSbMV) sur différents lots de graines de Pois.
Pour les cultivars étudiés, une bonne corrélation existe entre le pourcentage de transmission et le pourcentage d'embryons qui réagissent positivement au test ELISA. Mais à la différence des observations faites sur graines de Laitue lorsqu'on dose le virus de la Mosaïque de la Laitue ou sur graines de Soja lorsqu'on évalue la transmission du virus de la Mosaïque du Soja, si l'on pratique le test ELISA sur des suspensions résultant du broyage de l'ensemble de la graine avec un homogénéiseur, le pourcentage de graines infectées dépasse très largement le pourcentage de transmission du lot, car, dans ces conditions de broyage, l'antigène présent dans les téguments est lui aussi extrait. Il est donc nécessaire d'éliminer ces téguments avant le test et un procédé mécanique est proposé pour le traitement d'un nombre important de graines dans les conditions de routine.
Avant de procéder au test sur les embryons ainsi obtenus, il faut déterminer le nombre maximum d'embryons sains qui, ajoutés à un embryon infecté, forment un groupe positif en ELISA. Cette taille limite a été évaluée à 60 embryons pour le cv. « Arpad », et à 100 embryons pour le cv. « Amino » sur la base d'une étude de variabilité de la concentration en virus sur une vingtaine d'embryons infectés. Avec un antiserum donné, cette limite peut être déterminée de façon simple pour un cultivar considéré.
Les problèmes de l'élimination des téguments et de la détermination de la taille du groupe d'embryons étant résolus, la technique ELISA se prête avec précision et reproductibilité à l'analyse de routine des lots de graines de Pois pour le taux de transmission du PSbMV.

Mots clés additionnels : *Taux de transmission par la graine, épidémiologie, test de routine, détection, certification des semences.*

I. INTRODUCTION

Pea (*Pisum sativum* L.) is grown all over the world for the production of proteins and its area under cultivation is increasing every year. Among the thirty five viruses of pea, pea seed-borne mosaic virus (PSbMV), is one of the four which are seed-transmitted. It is also efficiently transmitted by aphids. It has commonly been found in germplasm collections and had a noticeable incidence on field production as was demonstrated in North American pea-growing regions. It was also shown that short-term control of the disease could be achieved by eradication of the virus from germplasm lines and commercial seed lots (HAMPTON, 1983 ; HAMILTON, 1983).

To be able to make accurate surveys, as well as to help growers to produce healthy seed, an attempt has been made, in the present paper, to define the conditions for routine and accurate estimation of the transmission rate of PSbMV in different seed lots.

ELISA has already been used to detect PSbMV in individual seeds (HAMILTON & NICHOLS, 1978). However, for determining low levels of virus transmission, a large number of seeds for testing are required and group testing is a more economical alternative. In order to determine the rate of virus transmission using groups of seeds, two questions need to be answered.

1 — What is the maximum number of healthy embryos which, when added to the embryo having the lowest virus titer, will still give a positive ELISA reaction ?

2 — Can ELISA tests of homogenized whole seeds detect seed-transmitted virus, or is it necessary to remove testas (frequently containing virus that is not seed-transmitted), for detection of virus in embryos ?

In tests of soybean seed for the presence of soybean mosaic virus (SMV), the feasibility of estimating the incidence of seed-transmitted SMV by testing groups of seeds by ELISA was established and the mathematical basis revised (MAURY *et al.*, 1985). In the SMV/soybean system, assays of homogenized whole seeds provided accurate detection of SMV-infected soybean seeds, i.e. infected embryos from which infected seedlings arise. In the course of applying these principles to tests for PSbMV in pea seeds, however, assays of whole pea seeds greatly exaggerated estimates of seed-transmitted PSbMV. The current study focused on the resolution of this obstacle to pea seed assays for PSbMV and on the feasibility of using ELISA for detection of the virus in seed homogenates representing a range of seed infection.

II. MATERIALS AND METHODS

Experimental PSbMV-infected seedlots of pea cvs. Amino and Arpad were produced by mechanically inoculating healthy plants, growing plants to maturity and harvesting the seeds produced. The rate of PSbMV seed transmission was determined to be 17 % (± 7.5 %, P = .95) for cv. Arpad by planting seed and individually testing 100 resulting plants by ELISA ; and 2 % (± 0.7 %, P = .95) for cv. Amino by planting 5 000 seeds, sampling 3 000 leaves from the resulting plants and testing homogenates from 60 leaves in each of 50 groups of leaves (37 groups ELISA positive).

Antiserum to PSbMV was provided by R. I. HAMILTON (Agriculture Canada Research Station, Vancouver, Canada V6TIX2). Immunoglobulin from this antiserum was conjugated to alkaline phosphatase, as per AVRAMEAS (1969).

Seed samples to be tested by ELISA were randomly assigned to seed groups, and soaked in water overnight. Testas were then manually removed and embryos were blender-homogenized for 30 s in 1/10 w/v (w = weight before soaking) phosphate-buffered saline containing 0.05 % Tween (PBST) and 1 % polyvinyl pyrrolidone 6 000 (PVP). An aliquot of homogenate was centrifuged at 10 000 g for 15 min. The supernatant fluid, as antigen in the double antibody sandwich type of ELISA, was added to duplicate wells of an ELISA microplate. Mean absorbance values (A_{405}) for duplicate wells represented the serological reactions for seed groups. The healthy limit value, above which a positive serological reaction was assumed, was defined as before (MAURY *et al.*, 1985) by a 0.005 probability for a healthy group to exceed this threshold ; it consisted of the mean A_{405} value for healthy controls (10 × 2 wells per microplate) plus 3.25 s (standard deviation per plate). Homogenized, presoaked healthy cv. Amino seeds were used as virus-free control and as diluent to produce experimental blends containing 3.3 %, 1.0 %, 0.5 % and 0.2 % proportions of PSbMV-infected/healthy seeds. Seed-preparation methodology and ELISA sensitivity were in turn tested against these standardized homogenate mixes.

One wrinkled-seeded pea seedlot, provided by R. STEGMARK from Sweden and included because of its high concentration of PSbMV in/on testas but low rate (1 % approx.) of PSbMV seed-transmission, was mechanically decorticated (dry seed) by use of a barley-pearling mill (Seedburo Barley Pearler, Model 109B, Seedburo Equipment Co., 1022 W. JACKSON Blvd, Chicago, Illinois 60607-2990). Decorticated seeds (i.e. embryos with varying amounts of residual, bound testas) were prepared and ELISA-tested, in comparison with whole seeds and with manually prepared embryos, by the above methods. Results provided a feasibility assessment for mechanical/automated removal of testas.

III. RESULTS

A. Maximum number of seeds per group

1. *Variation in virus titer among infected embryos from a seed lot with a high transmission rate*

Each of one hundred seeds from a batch of pea cv. Arpad (percentage of PSbMV transmission : 17 ± 7.5) was manually dissected with a scalpel into three parts : testas (average weight 11 mg), axis (1 mg) and cotyledons (75 mg). Each part was ground using a mortar and pestle at 1/300 (w/v) dilution. For each axis, 0.1 ml of this dilution was further diluted 75 times

(1/300 × 1/75 = 1/22 500) to obtain the contribution of this axis to the ELISA value of the related whole embryo ground at a dilution of 1/300 (w/v).

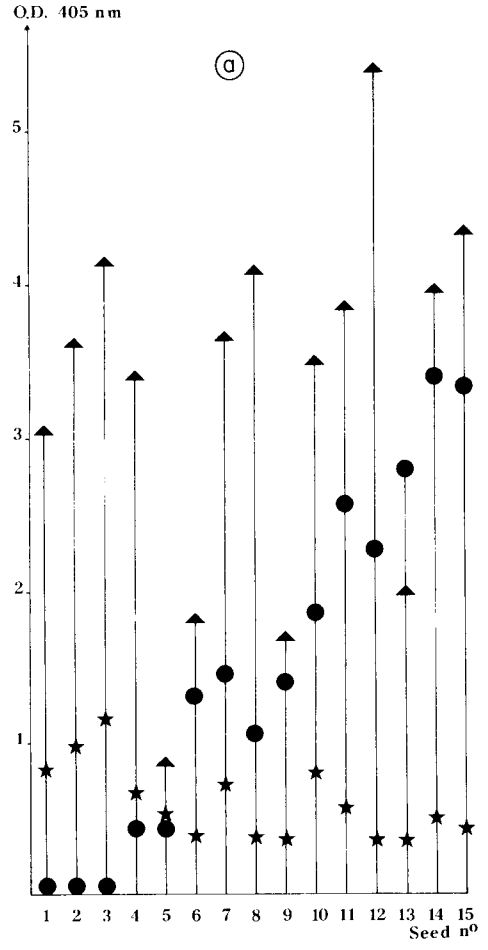
From this sample of 100 seeds, 15 embryos gave a positive ELISA reaction (fig. 1a). PSbMV concentration was higher in the axis than in the cotyledons. Particularly for 3 among these 15 infected embryos, the ELISA value obtained for the cotyledons was in the healthy background. The contribution of each axis in the embryo reaction is shown in figure 1b. The lowest reaction would be positive even when diluted twice, thus giving a dilution around 1/600 (w/v) for the embryo with the lowest titer. In the corresponding volume of buffer, 60 embryos could be ground to reach the group dilution 1/10 w/v.

2. *Determination of the group size limit using a seed lot with a low transmission rate*

The batch of infected pea seed used (cv. Amino) was earlier found to have a 2 ± 0.7 % transmission rate : under these conditions, the number of seeds to be individually tested to estimate the virus titer in 20 to 30 infected embryos would be too high. So we took 900 seeds, containing approximately 20 embryo-infected seeds and divided them into 30 groups of 30 seeds each. Thus, the probability of having more than one infected embryo per group was not nil though it was low. After an overnight soaking period, the embryos were separated manually. Each group of 30 embryos was then ground in 60 ml buffer (w/v = 1/10) using a Waring blendor.

Healthy embryos of cv. Amino were ground in the same conditions. The resulting suspension was used as a healthy control, and for serial dilutions of the 30 suspensions obtained from infected seed : each group was thus diluted with the suspension from healthy seed up to ratios of 100/30, 200/30 and 500/30 corresponding to the mixing of one infected embryo with 100, 200 and 500 healthy embryos.

Table 1 shows that 16 groups reacted positively when the number of embryos was less than or equal to 100. This result was compatible with the percentage of transmission previously determined for this batch. Groups n° 2 and n° 23 gave the lowest positive ELISA values ; most probably these groups contained only one infected embryo, which was no longer detected when mixed with 200 or more healthy embryos. In the case of pea cv. Amino, there is therefore a good probability that group testing using a maximum of 100 embryos per group would take into account all the infected embryos irrespective of the virus titer.



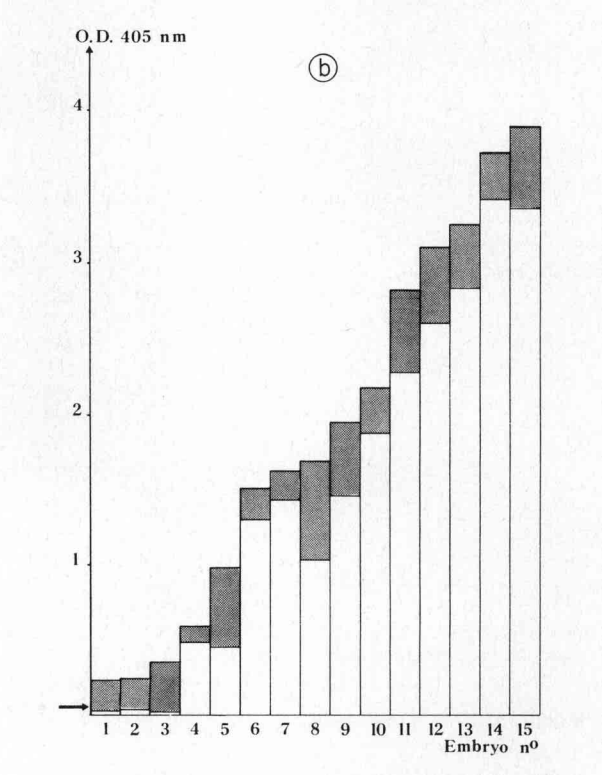


Figure 1

Variation of PSbMV titer among infected seeds.
1a - ELISA values (A_{405}) for extracts of parts from 15 embryo-infected seeds, the testas (★), axis (▲) and cotyledons (●). Tissues were ground at the same dilution (1/300 w/v) using pestle and mortar.

1b - Relative contribution of the axis (dilution 1/22500 ▨) and of the cotyledons (dilution 1/300 □) to the resultant A_{405} values of the same embryos as in fig. 1a had they been ground at a dilution 1/300 w/v. The arrow shows the limit of the healthy background.

Variation de la concentration en PSbMV dans les graines infectées.
1a - D.O. 405 nm correspondant à 15 graines à embryon infecté après broyage au mortier des téguments (★), axe (▲) et cotylédons (●) à la dilution 1/300 w/v.

1b - Importance relative de l'axe (dilution 1/22500 ▨) et des cotylédons (dilution 1/300 □) dans les valeurs qui auraient été obtenues en ELISA pour les embryons précédents s'ils avaient été broyés à la dilution 1/300 w/v.

TABLE 1

Group size limit. ELISA values ($A_{405} \times 10^3$) for infected embryos when mixed with 30, 100, 200 and 500 healthy embryos. Each column corresponds to one microplate, in which were incubated in duplicate wells 30 groups + 10 replications for the healthy extract.

Taille limite du groupe. D.O. 405 nm ($\times 10^3$) pour des extraits d'embryon infecté mélangé à 30, 100, 200 et 500 embryons sains. Chaque colonne correspond à une plaque ELISA, dans laquelle ont été déposés les extraits correspondant à 30 groupes ($\times$ 2 puits par extrait) et 10 ($\times$ 2) répétitions pour l'extrait sain.

Group n°	Group size 30 embryos	100 embryos	200 embryos	500 embryos
1	218	206	228	185
2	343 +	252 +	210	215
3	208	209	216	190
4	182	179	210	197
5	193	170	188	173
6	208	169	197	182
7	1 265 +	621 +	402 +	257 +
8	> 2 000 +	1 605 +	1 052 +	574 +
9	> 2 000 +	1 050 +	655 +	374 +
10	> 2 000 +	1 106 +	701 +	375 +
11	1 106 +	570 +	362 +	247 +
12	1 484 +	890 +	529 +	325 +
13	168	164	198	194
14	> 2 000 +	1 689 +	1 084 +	558 +
15	184	174	215	170
16	> 2 000 +	1 354 +	860 +	469 +
17	164	159	172	185
18	174	166	164	177
19	> 2 000 +	1 425 +	888 +	466 +
20	> 2 000 +	1 601 +	950 +	515 +
21	> 2 000 +	1 326 +	806 +	467 +
22	> 2 000 +	1 508 +	921 +	516 +
23	562 +	295 +	231	200
24	190	172	161	193
25	160	172	193	208
26	166	175	165	190
27	178	184	180	178
28	1 880 +	966 +	558 +	332 +
29	> 2 000 +	1 687 +	1 188 +	642 +
30	175	186	174	192
Healthy control				
1	170	164	190	202
2	197	168	201	190
3	225	168	201	169
4	188	205	138	169
5	154	160	152	192
6	182	177	170	175
7	181	172	170	183
8	179	203	161	169
9	197	189	165	184
10	217	210	194	198
average $\bar{x}$	189	182	174	183
st. dev. s	21.1	18.6	21.5	12.3
$\bar{x}$ + 3.25 s	258	243	244	223
n° positive groups	16	16	14	14

B. Viral antigen from testas

1. *Influence of infected testas on ELISA estimation of PSbMV transmission rate*

A blender was the most convenient apparatus to extract virus from groups of seeds. We checked, therefore, the level of viral antigen extracted from testas in a short homogenization period.

After soaking seeds overnight, 4 groups of 30 healthy testas (h_1, h_2, h_3 and h_4) and 2 samples of 30 infected testas were manually prepared. Each of the 30 infected testas was divided, using a scalpel, into two halves to prepare 2 equivalent groups of 30 half testas : t and t(m) ; the group t(m) was pre-ground using a mortar and pestle. The duplicate with the 30 other infected testas gave the groups t' and t'(m). Besides, 8 groups of 30 healthy cv. Amino embryos (A_1, A_2 ... A_8) were homogenized for 30 s in 60 ml buffer using a Waring blendor ; a volume of 0.5 ml was taken for ELISA. Then, each extract was blended once more for 30 s after addition of one group of testas. The results are reported in table 2. The healthy limit calculated from the values of the eight groups of embryos was $\bar{x}$ + 3.5 s = 245 ($A_{405} \times 10^3$). Blending resulted in a significant extraction of PSbMV from the testas. Seed coats thus have to be removed before determining the transmission rate.

TABLE 2

Extractibility of viral antigen from testas while blending seeds. ELISA values ($A_{405} \times 10^3$) after blending mixtures of healthy embryos (A_5 and A_7) and infected testas (t and t'). Positive controls : similar mixtures [A_6t(m) and A_8t' (m)] in which the infected testas were pre-ground using a pestle and mortar. Negative controls : groups of healthy embryos (A_1 to A_8) or groups containing a mixture of healthy embryos and healthy testas (A_1 h_1 to A_4 h_4).

Extraction de l'antigène viral des téguments lors du broyage des graines avec un homogénéiseur. D.O. 405 nm ($\times 10^3$) après homogénéisation d'un groupe A_it composé d'embryons sains (A_i) et des téguments infectés (t). Des groupes identiques A_it(m), broyés au préalable au mortier, servent de témoins positifs ; les témoins négatifs sont des groupes d'embryons et des groupes d'embryons + téguments sains.

Groups of 30 healthy embryos		+ 30 healthy testas		+ 30 infected half testas	
A_1	139	A_1h_1	158		
A_2	121	A_2h_2	95		
A_3	105	A_3h_3	96		
A_4	100	A_4h_4	124		
A_5	180			A_5t	1 328
A_6	161			A_6t(m)	> 2 000
A_7	87			A_7t'	861
A_8	84			A_8t'(m)	> 2 000

2. *A mechanical procedure for removing testas*

Cv. Amino being a smooth-seeded pea, we preferred to try the efficiency of a mechanical procedure on wrinkle-seeded pea, a more difficult material. Seed was processed in a "Seedburo Barley Pearler" n° 109 B which consists mainly of a carborundum wheel driven by a motor. A 5 s treatment on successive samples (100 seeds per sample approx.) yielded about 50 % decorticated seed with intact embryos which could be visually separated from incompletely decorticated seeds. The time necessary for obtaining 1 000 embryos was about 20 mn.

The quality of the decortication was checked using a seed lot having a low level of transmission (about 1 %) in which extracts from 92 % of the testas were found to contain PSbMV by ELISA. Five groups of 25 decorticated seeds were compared in the same microplate with 8 groups of 25 embryos from the same seed lot ; as positive controls, these 8 groups were blended once more after adding the corresponding testas. In addition, five groups of 25 partially decorticated seeds were also included. Finally 8 groups of healthy cv. Amino

seed were used as negative controls. Each group was extracted with a Waring blendor for 30 s as usual in PBST-1 % PVP, 1/10 w/v. The results are reported in table 3.

TABLE 3

Efficiency of mechanically removing testas. ELISA values ($A_{405} \times 10^3$) after blending in buffer (1/10 w/v) groups of 25 mechanically decorticated seeds in comparison with groups of 25 embryos of the same seed lot, groups of 25 embryos and corresponding testas (ELISA positive control) and groups of healthy cv. Amino seed (negative control).

Efficacité du procédé mécanique d'élimination des téguments. D.O. 405 nm ($\times 10^3$) après broyage dans le tampon (1/10 w/v) de groupes de 25 graines décortiquées par voie mécanique en comparaison avec des groupes de 25 embryons du même lot de graines, des groupes de 25 embryons et téguments correspondants (témoin positif) et des groupes d'embryons « Amino » sains (témoin négatif).

Groups of 'Amino' healthy seed	Infected seed lot			
	Groups of embryos		Groups of decorticated seed	Groups of partially decorticated seed
	(− testas)	+ testas		
68	878	> 2 000	90	> 2 000
64	68	> 2 000	103	> 2 000
42	53	> 2 000	110	> 2 000
64	71	> 2 000	68	1 948
43	41	> 2 000	578	> 2 000
42	32	> 2 000		
42	24	> 2 000		
49	25	> 2 000		

The healthy limit as defined by the 8 healthy groups was $\bar{x} + 3.5\ s = 87$ ($A_{405} \times 10^3$). All the groups of embryos except one were healthy ; when the corresponding testas were added, all the groups reacted very strongly. The same was also true when seed was partially decorticated whereas decorticated seed gave values less than or just above the healthy limit except for the last group (absorbance = .578) which was supposed to contain one infected embryo.

IV. DISCUSSION

The present paper describes how to estimate the percentage of seed transmission of pea seed-borne mosaic virus in pea seed lots by group analysis using ELISA. The maximum number of seeds per group was evaluated by taking into account the variation of virus titer among embryos. For cv. Arpad the transmission percentage (17 ± 7.5) of our seed lot enabled us to analyse single infected embryos as previously described for the soybean mosaic virus/soybean seed (cv. Altona) system (MAURY *et al.*, 1983) and to deduce a size limit of 60 embryos per group. For cv. Amino, from a seed lot with a percentage of transmission of 2 %, a batch of seeds containing approximately 20 infected embryos was divided into small groups, each having mostly 0 or 1 infected embryo (cf. FALK & PURCIFULL (1983) for the system lettuce mosaic virus/lettuce seed). After soaking seed and manually removing the testas, each group of embryos was blended at the dilution 1/10 weight/buffer volume, pre viously shown to be a good compromise for seed testing (MAURY *et al.*, 1983). Each extract was then serially diluted with a similar healthy extract. Most of the infected embryos when individually mixed with extracts from 500 embryos reacted positively in ELISA. But some failed to react when mixed with extracts from more than 100 embryos. It is likely that they corresponded to embryos where only the axis was infected.

Maximum group sizes were thus determined for two cultivars. However, the average PSbMV titer can vary among pea cultivars. For example, when comparing PSbMV titer in testas and embryos of ten different USDA accessions (20 seeds of each, collected from infected plants) the average titer in infected embryos was lower in accession 356971 than in cv. Arpad. In contrast, the average titer in infected testas and embryos of accession 356900 was much higher, reflecting presumably its higher susceptibility to PSbMV. However, the group size limit is based on the lowest titer of PSbMV in a single embryo and more data would be necessary to know if this lowest titer, which could be due to infection of only the axis in the embryo, has a range of variation linked in some way to the variation of the average titer. It is therefore suggested that for routine use, a workable group size limit be determined.

This limit might seem low. However, if, for example an embryo-group size of 100 were selected, a seed-sample size of 3000 seeds could be tested in a single ELISA microplate, i.e. 30 groups of 100 embryos. In such a test, for instance, three positive reactions could be interpreted as an infection-frequency range of 0.04-0.30 %, with a 95 % confidence level ; with 0 positive reaction, a range of 0-0.12 %. Hence, even if the tolerance threshold is lower than 1 %, the accuracy with which the transmission level is determined enables good assessment of slightly infected lots.

A complication while testing pea seed comes from the viral antigen in testas which was shown to be extracted by blending and to induce a large overestimation of the percentage of transmission. This situation differs from that of the system soybean mosaic virus/soybean seed (cv. Altona) for which testas having a lower concentration of antigen and a higher resistance to blending were not found to induce false positive reactions ; for lettuce seed, assays of homogenized whole seeds also provided accurate estimation of the transmission rate (FALK & PURCIFULL, 1983). In case of pea, this overestimation was found not only for cv. Amino but also for several cultivars tested. Removing testas before grouping the embryos is therefore an obligatory step in the routine protocol described in this report.

A mechanical procedure for removing testas is shown to be very promising. It however needs some improvements, since the absorbance values corresponding to some groups of decorticated healthy embryos were slightly above the healthy limit : washing the decorticated seeds before blending should eliminate residual pieces of testas and lead to satisfactory results. This procedure would then be of practical use as a quicker alternative to manual processing.

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REFERENCES

Avrameas S., 1969. Coupling of enzymes to proteins with glutaraldehyde. Use of the conjugates for the detection of antigens and antibodies. *Immunochemistry,* **6,** 43-52.

Falk B. W., Purcifull D. E., 1983. Development and application of an ELISA test to index lettuce seeds for lettuce mosaic virus in Florida. *Plant Dis.*, **67,** 413-416.

Hamilton R. I., 1983. Certification schemes against seed-borne viruses in leguminous hosts, present status and further areas for research and development. *Seed Sci. Technol.,* **11,** 1051-1062.

Hamilton R. I., Nichols C., 1978. Serological methods for detection of pea seed borne mosaic virus in leaves and seed of *Pisum sativum. Phytopathology,* **68,** 539-543.

Hampton R. O., 1983. Seed borne viruses in crop germplasm resources : disease dissemination risks and germplasm-reclamation technology. *Seed Sci. Technol.,* **11,** 535-546.

Maury Y., Bossennec J. M., Boudazin G., Duby C., 1983. The potential of ELISA in testing soybean seed for soybean mosaic virus. *Seed Sci. Technol.,* **11,** 491-503.

Maury Y., Duby C., Bossennec J. M., Boudazin G., 1985. Group analysis using ELISA : determination of the level of transmission of soybean mosaic virus in soybean seed. *Agronomie,* **5,** 405-415.