PRELIMINARY INVESTIGATIONS ON THE VIABILITY OF THE USE OF ABSCISIC ACID (ABA) IN CRUCIFER SEED HEALTH TEST FOR DETECTING *PHOMA LINGAM*[1]

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ABSTRACT - *Phoma lingam*, a seedborne fungal organism causes blackleg disease in crucifer (Brassicaceae). The recommended International Seed Testing Association (ISTA) procedure for determining the incidence of *P.lingam* in crucifer seed calls for examination of seed and blotters for typical pycnidia and conidial ooze, after 10-11 days of incubation at 20°C on blotters wetted with 0.2%, 2,4-D solution. The use of ABA at 100 mg/l is proposed as a new method for testing crucifer seed for *P.lingam*. The advantages of this seed assay are: a) ABA is totally soluble in water and using low concentration eliminates the risk of laboratory contamination; b) Seed pretreatment with sodium hypoclorite to reduce saprophytic contaminating fungi is not necessary; c) ABA is not toxic to *P. lingam*, thus it does not affect seed health testing, and d) ABA is available world wide and there is no difference in formulations. Suggestions have been made to the working group on seed-borne diseases of crucifer of the ISTA Plant Disease Commitee to compare this method with the presently recommended 2,4-D test.

Index terms:seed assay, germination.

INVESTIGAÇÃO PRELIMINAR DO USO DO ÁCIDO ABSCÍSICO (ABA) NO TESTE DE SANIDADE DE SEMENTES DE CRUCIFERA PARA DETECTAR PHOMA LINGAM

RESUMO - Phoma lingam, um fungo transmissível pela semente, é o agente causal da doença "canela-preta" em crucíferas (Brassicaceae). A técnica recomendada pela International Seed Testing Association (ISTA) para determinar a incidência de P.lingam na semente consiste no exame de picnídios no papel de filtro e nas sementes, e exudatos conidiais do fungo, após dez a onze dias de incubação a 20°C em placas embebidas em uma solução de 0,2% de 2,4-D. Neste trabalho, o uso de ácido abscísico (ABA) a 100 mg/l é proposto como um novo método para detectar *P.lingam* em sementes de crucíferas. As vantagens desse novo teste de sanidade são: a) ABA é totalmente solúvel em água, e usando-se baixa concentração elimina-se o risco de contaminação ao laboratório; b) O pré-tratamento das sementes com hipoclorito de sódio para reduzir a contaminação de fungos saprofíticos não é necessário; c) ABA não é tóxico a *P.lingam*; e d) ABA não apresenta diferença em formulação e é facilmente disponível. Tem-se sugerido, ao grupo da ISTA que trabalha com doenças das crucíferas, provindas de sementes, que compare este método com o teste "2, 4D", atualmente recomendado.

Termos para indexação: teste de sanidade, germinação.

INTRODUCTION

Blackleg of cruciferous is incited by P. lingam (Tode ex Schw.) Desm. (teliomorphic stage: *Leptosphaeria maculans* (Desm.) Ces and de Not. Its importance, epidemiology and control have been discussed by Gabrielson & Maguire (1977) and more recently by Gabrielson (1983).

The International Seed Testing Association (ISTA) published a laboratory method for detecting *P. lingam* on seeds. (Cabbage... 1965). The seed health test recommended by ISTA(International... 1966), evaluated by Maguire et al. (1978) and reviewed by Maguire & Gabrielson (1983), consisted of a 2,4-D assay where crucifer seeds are seeded on

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a blotter wetted with a 0,2% 2,4-D dichlorophenoxyacetate solution). The 2,4-D inhibits the growth of the seedling and allows the blackleg organism, which seed is internally infected with it, to grow and sporulate. After 10-11 days the seeds and blotters are examined for pycnidia, conidial ooze and spores of *P. lingam*.

This test was developed by European scientists who have had a serious continuous concern for seedborne *P. lingam*. Maguire et al. (1978); however, noted considerable variation in the materials and methods employed by European laboratories that conduct testing programmes for seedborne *P. lingam*. They evaluated the effects of these differences on the sensivity of the assay and found that solutions of 2,4-D derived from the acid formulation were toxic to *P. lingam*, reducing pathogen counts, but that some commercial sodium salt formulations were not toxic at the recommended rates.

Laboratory contamination with 2,4-D salt is a serious problem that has concerned most seed analysts. To avoid this contamination, freezing the seed and other techniques have been employed to inhibit germination and allow *P. lingam* evaluation. Gabrielson (1978) in a report to the plant disease committee working group on crucifers described the comparison among laboratories evaluating freezing as an alternative germination inhibitor to 2,4-D. In general, lower *P .lingam* counts were found with freezing, although four to eight-hour freezing periods were more comparable to 2,4-D counts than a 24-hour-freezing period. Inadequate inhibition of germination was reported with a four-hour freezing schedule by some laboratories.

Abscisic acid is a well known natural germination inhibitor present in different tissues of many species. The inhibitory effects of ABA on seed germination have been fully described by Walton (1980/1981); King (1982) and Black (1983). In view of all these, we envisaged that ABA, a potent inhibitor could be used as an alternative method for assaying the presence of *P. lingam* in crucifer seed.

The main goal of this study, therefore, was to compare the ABA with the recommended 2,4-D as a germination inhibitor in the assay of *P lingam* in crucifer seed.

MATERIALS AND METHODS

Procedures outlined by Maguire et al. (1978) and Maguire & Gabrielson (1983) were slightly modified in this study. Using a perforated vacuum counting plate, 100 seeds were placed 8-10 mm apart in a 15 cm Lab-Tek plastic or Pyrex glass petri dish on two Whatman No.1 filter papers saturated with 10 ml of seed germination inhibitor solution. The standard inhibitor was 0,2% (w/v) aqueous solution of the Dow sodium salt of 2,4-D, 95% active ingredient. ABA mixed isomers, white crystalline, 90% pure was purchased from Sigma Chemical Co., St.Louis, MO.

In testing the effect of germination inhibitors, treatments with 2,4-D, 2,4-D + Thiram (0,2%), ABA (100 mg/l) and ABA + Thiram (0,2%) aqueous suspensions were used in the first experiment. A naturally infected cabbage cv. Asgrow X (*Brassica oleracea* var. *capitata* L.) seed lot previously 5% *P. lingam* was used for most trials. Five petri dishes were incubated on laboratory benches under ambient conditions of approximately 20°C and 12h day/12h night of near ultraviolet light. Seeds and blotters were examined for typical pycnidia and conidial ooze at 6 and 11 days after inhibition.

In order to determining the optimum concentrations of ABA; 50 mg/l, 100 mg/l and 200 mg/l of the abscisic acid solution were tested on *P. lingam* incidence and percentage of seedling sprouts. The incubation procedure was similar as outlined above, except the percentage of seedling emergence was counted on 11 days after inhibition.

The experimental design was a completely randomized block with five replicates. The analysis of variance and the means pairwise comparisons by Duncan's Multiple Range Test were calculated.

RESULTS AND DISCUSSION

The development of *P. lingam* in seed assays in a 2,4-D standard procedures and ABA are presented in (Table 1). ABA at 100 mg/l resulted in higher *P. lingam* incidence than the sodium salt at 6 days after imbibition and continued increasing until the final counting, while the reading of 2,4-D inhibitor remained constant. Thiram applied together either with ABA or 2,4-D substantially reduced the percentage of *P.lingam* but did not erradicate it, indicating that there is no antagonism between fungicide and ABA. Thus, even in the presence of chemical seed treatment ABA was effective in

determining the seed infection. Again, the ABA + Thiram treatment resulted in higher readings of *P. lingam* than the standard method, but they are not statistically different.

The higher concentration of ABA up to 200 mg/l did not reduce the incidence of *P. lingam* and at any ABA concentration. The percentage of *P. lingam* was higher than 2,4-D treatment at 6 and 11 days after imbibition (Table 2). However, a dramatical difference was observed for seedling inhibition; no germination was obtained with ABA at 200 mg/l and only 10% was recorded at 100 mg/l. The 2,4-D treatment was not effective as ABA in inhibiting seedling emergence. The data indicate, therefore, that the most feasible concentration of ABA is 100 mg/l. If seeds of a particular genotype germinate during the treatment the concentration of the inhibitor should be increased (Table 2).

TABLE 1. Percentage of cabbage seed infected with *P. lingam* using different seed assay treatments.

Seed assay	Inhibitor dose	Percentage of *P. lingam*	
treatment	mg/l	at 6 days	at 11 days
ABA	100	6.8 b	7.2 b
2,4-D	2000	5.4 b	5.4 ab
ABA+0.2% Thiram	100	2.6 a	3.1 a
2,4-D+0.2% Thiram	2000	2.2 a	2.6 a

1. Means within column followed by the same letter are not statistically different at 5% level by the Duncan's Multiple Range Test.

TABLE 2. Percentage of cabbage seed infected with *P.lingam* and percentage of sprouting as compared with different concentration of ABA and the standart 2,4-D assay.

Seed assay	Inhibitor	Percentage of *P. lingam*		Percentage of
treatment	concentr. mg/l	at 6 days	at 11 days	sprouting
2,4-D	2000	4.2 a	5.0 a	98 a
ABA	50	5.0 a	6.2 a	24 b
ABA	100	4.6 a	6.0 a	10 c
ABA	200	4.8 a	6.2 a	0 d

1. Means within column followed by the same letter are not statistically different at 5% level by the Duncan's Multiple Range Test.

Saprophytic contaminating fungi are commonly encountered in seed assays and vary significantly among seed lots. Sometimes these rapidly growing contaminants, e.g. *Rhyzopus* and *Alternaria* spp. make readings more difficult. In case of 2,4-D method, Hewett (1977) and Maguire et al. (1978) have effectively controlled them with dilute sodium hypoclorite. However, these saprophytic fungi grew very slowly on seed treated with ABA, thus surface sterilization is not needed.

Seed companies, seed analysts, and seed quarantine officials need an accurate, repeatable, reproducible, rapid and economical test for monitoring *P. lingam* in seed production, international seed trade and germplasm exchange.

CONCLUSIONS

1. ABA assay at 100 mg/l is proposed as a new alternative, since it inhibits the germination process, it reduces substantially the rapidly growing saprophytes, making readings more accurate and therefore, there is no need of seed pretreatment. Another important feature of ABA with regard to 2,4-D is to eliminate the risk of laboratory contamination and the use of several formulations with varying disease evaluations.

2. A major goal of the working group on seedborne crucifer diseases of the ISTA Plant Disease Committee is to develop standard methods for *P. lingam* detection in crucifer seed. The proposed ABA assay should aid in ameliorating such a test. The laboratory evaluation of crucifer seed for *P. lingam* is assential in the production, processing, and marketing of high quality seed and must be standardized for use by laboratories worldwide.

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