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Detection of broad bean stain virus in lentil seed groups

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Abstract

Broad bean stain virus (BBSV) was easily detected in lentil seed groups each of 25 seeds by the enzyme-linked immuno-sorbent assay (ELISA). When seeds were dissected into axes, cotyledons, and seed coats BBSV detection was highest in cotyledons followed by the germinating axes and least in seed coats. Detection in ground intact seeds was less than in germinated seedlings. Testing lentil seed groups provided a practical mean in monitoring seed-borne BBSV in lentil seed lots.

Introduction

The seed-borne broad bean stain virus (BBSV) infects a number of leguminous crops including lentils (*Lens culinaris*) (Bos *et al.* 1986; Boswell and Gibbs 1983). No loss estimates have been reported for lentils infected with BBSV. Nevertheless, symptoms induced in response to infection are very mild and virus incidence is usually low, an indication that losses incurred by BBSV on lentils are most likely minimal. However, since BBSV is beetle-transmissible, infection can spread to susceptible crops such as pea (*Pisum sativum*) and faba bean (*Vicia faba*) in places where the insect vector is prevalent.

Testing for BBSV is, therefore, important in areas where susceptible economically important crops are grown. Because of the low seed infection rate, testing for BBSV in single seeds would be very time consuming and not economical. In this study we investigated the possibility of testing groups of lentil seeds for the presence of BBSV.

Materials and Methods

A lentil seed lot (cv Syrian Local), which contained seed-borne BBSV, was used as the seed source for this study. Seeds for testing was randomly picked and each 25 were grouped together as one sample. Seeds were tested as (i) ground, (ii) intact seedlings, and (iii) dissected seedlings to axes, cotyledon, and seed coat. Seeds were sown in moistened sterile sand in germination boxes and incubated at 22-24°C for one week.

Testing for BBSV was carried out using the enzyme-linked immuno-sorbent assay (ELISA) following the procedure of Clark and Adams (1977) with one exception. The Standard extraction buffer was replaced by 0.2M phosphate buffer, pH 6.0. The antiserum used was produced in the virology laboratory of the Faculty of Agricultural and Food Sciences, American University of Beirut, Beirut against a purified BBSV isolate from faba bean (SV 173-85). ELISA values were taken by a Dynatech micro ELISA minireader (MR 590). In each ELISA plate, eight healthy samples were used to determine the negative threshold value. ELISA sample values higher than the healthy mean plus three standard deviations were considered positive.

Table 1. Detection of broad bean stain virus (BBSV) in ground intact seeds, intact seedlings, and dissected seedlings (developing shoot and root, cotyledons, and seed coat) of lentil seed groups when tested by ELISA.

Sample	Number of groups* tested	Number of groups infected	Seed infection rate estimate
Dissected seedlings			
shoot and root	39	36	9.8
cotyledon	39	37	11.2
seed coat	39	17	2.3
Intact seedlings	40	39	13.8
Ground intact seeds	40	31	5.8

* Each group was a mixture of 25 seeds.

Results

The seed infection rate obtained for ground intact seeds was lower (5.8%) than that of intact germinated seedlings (13.8%) (Table 1). In dissected seedlings, both germinated axes (shoot + root) and cotyledons gave a higher infection rate than the seed coat. The reason for this is mainly due to the nature of the different tissues extracted. Samples were homogenized for 30 seconds in a Waring blendor using a microcontainer. This extraction time permitted more virus to be extracted from the germinating embryo than from the seed coat. Using a similar procedure for the detection of BBSV in faba bean seeds, virus was detected in the germinating embryo, but not at all from the seed coat (unpublished).

The seed infection rates in Table 1 were estimates calculated by the formula $p = [1-(\frac{Y}{N})^{\frac{1}{n}}] \times 100$ where p = % of seed infection rate, Y = number of groups found healthy, N = total number of groups tested, and n = the number of seeds which constituted a group (Maury *et al.* 1985). In the experiments presented in Table 1, n = 25 and N was 39 or 40. The precision of the seed infection rate estimate is higher with low infection rates than with high infection rates. Precision could have been increased if preliminary tests were carried out to determine the optimal values of n and N that one should use (Maury *et al.* 1985). However, in this study our aim was not the precise comparison between seed infection rates of different samples, but rather to identify seed lots which contain the virus for the purpose of discarding them for use in seed multiplication.

Conclusion

Groups of 25 lentil seeds could be easily tested for the detection of BBSV. Sensitivity of group testing was higher with germinated seedlings as compared to ground intact seeds. It is recommended then to use groups of germinated seedlings for the determination of BBSV infection rate in lentil seeds.

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