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BACKGROUND

Many viruses are known to infect faba bean and chickpea crops worldwide (Kumari and Makkouk, 2007; Makkouk *et al.*, 2003, 2012; Kumar *et al.*, 2008), even though only a few are reported to occur in Tunisia (Kumari and Makkouk, 2007; Najjar *et al.*, 2000). More than 10 viruses are known to be seed-borne in faba bean and chickpea (Makkouk *et al.*, 2012), some of them were reported in Tunisia, such as Broad bean mottle virus (BBMV), Broad bean stain virus (BBSV), Pea seed-borne mosaic virus (PSbMV), Bean yellow mosaic virus (BYMV), Alfalfa mosaic virus (AMV) and Cucumber mosaic virus (CMV) (Najar *et al.*, 2000; Kumari and Makkouk, 2007).

Seed-borne viruses of faba bean and chickpea crops in Tunisia have not been extensively identified and no information is available on their incidence. Due to the potential economic importance of legume viruses, a study was conducted to identify seed-borne viruses affecting faba bean and chickpea seed lots in the major production areas of Tunisia, and to determine their incidence and relative importance.

SEED COLLECTION AND LABORATORY TESTING

Seed collections were conducted during November/December 2022 for faba bean and during January/February 2023 for chickpea. The survey covered 20 locations (10 faba bean and 10 chickpea) in northeastern (Bizerte, Cap Bon) and northwestern regions (Bousselem, Kef, Beja) (See Tables 1 and 2). From each farmer, around 2 kg of seeds were randomly collected for each crop variety. Seed samples were placed in labeled plastic bags and brought to the virology laboratory of INRAT. Around 500 to 700 collected seeds were planted in sterilized sand in germinations boxes/trays and incubated at 20-25 °C under greenhouse conditions for 1-2 weeks (Figure 1). The germinated seedlings were blotted on nitrocellulose membranes in six replicates and tested by tissue-blot immunoassay (TBIA) (Makkouk and Kumari, 1996) at the Virology Laboratory of INRAT-Tunisia using six polyclonal antisera for BYMV, BBMV, BBSV, PSbMV, AMV and CMV provided by ICARDA Virology Laboratory.



Figure 1. (A) Planting chickpea seeds in trays at greenhouse of INRAT station; (B) faba bean seeds after emergence are ready for testing; (C) Blotting faba bean plants on nitrocellulose membranes (NCMs).

RESULTS

When 4,587 faba bean seedlings from 10 seed lots and 4,000 chickpea seedlings from 10 seed lots were tested by TBIA, only BYMV was detected in 2 faba bean seed lots, whereas AMV and PSbMV were detected in 3 and 9 chickpea seed lots out of 10 tested (Tables 3 and 4).

Results obtained showed that the average seed transmission rates of AMV and PSbMV were 1.0% and 3.2% in chickpea, respectively. Whereas the average seed transmission rate with BYMV was 1.7% in faba bean seed lots. The highest PSbMV seed transmission rate (6.8-8.3%) was found in two chickpea (cv. Bouchra) seed lots (Table 2).

Table 1. Serological results (TBIA) of faba bean seeds lots collected from 10 Tunisian farmers and tested against six polyclonal antibodies of seed-borne viruses during 2023.

| No. of faba bean seed lots | Regions | Faba bean variety | No. of seeds tested | No. of seeds infected with BYMV (% virus incidence) |
|----------------------------|---------------|-------------------|---------------------|---|
| 1 | Jendouba | Najeh | 555 | 0 |
| 2 | Kef | Chourouk | 503 | 0 |
| 3 | Bousselem | Najeh | 473 | 5 |
| 4 | Beja | Najeh | 408 | 35 (8.5%) |
| 5 | Nabeul | Najeh | 458 | 0 |
| 6 | Beja | Najeh | 419 | 0 |
| 7 | Bizerte | Najeh | 450 | 0 |
| 8 | Jendouba | Chourouk | 500 | 42 (8%) |
| 9 | Cap-Bon -Mida | Chahbi | 335 | 0 |
| 10 | Cap-Bon -Mida | Chahbi | 486 | 0 |
| Total | | | 4,587 | 77 (1.7%) |

All tested seeds were negative to BBMV, CMV, AMV, BBSV and PSbMV

Table 2. Serological results (TBIA) of chickpea seeds lots collected from 10 Tunisian farmers and tested against six polyclonal antibodies of seed-borne viruses during 2023.

| No. of chickpea seed lots | Regions | Chickpea Variety | No. of seeds tested | No. of seeds infected with (% virus incidence) | |
|---------------------------|-----------------|------------------|---------------------|--|-------------------|
| | | | | AMV | PSbMV |
| 1 | Beja | Nour | 400 | 11 (2.8%) | 16 (4%) |
| 2 | Beja | Joud | 400 | 0 | 15 (3.8%) |
| 3 | Beja | Bouchra | 400 | 16 (4%) | 27 (6.8%) |
| 4 | Beja | Rebha | 400 | 0 | 9 (2.3%) |
| 5 | Cap-Bon | Bouchra | 400 | 14 (3.5%) | 33 (8.3%) |
| 6 | Mateur | Nour | 400 | 0 | 9 (2.3%) |
| 7 | Menzel Bourgiba | Beja 1 | 400 | 0 | 14 (3.5%) |
| 8 | Bousselem | Beja 2 | 400 | 0 | 1 (0.3%) |
| 9 | Cap-Bon | Bouchra | 400 | 0 | 0 |
| 10 | Cap-Bon | Béja 1 | 400 | 0 | 2 (0.5%) |
| Total | | | 4,000 | 41 (1%) | 126 (3.2%) |

All tested seeds were negative to BYMV, BBMV, CMV and BBSV.

CONCLUSIONS AND RECOMMENDATIONS

- Surveys need to be repeated regularly to determine the extent of seed transmitted viruses in Tunisian faba bean and chickpea seed stocks.
- There is a need to conduct seed tests to confirm the presence of the virus, and to inform Tunisian farmers through extension services precise information on the importance of seed quality and health in improving the productivity of their crops.

- Research centers and farmers should be advised to plant healthy seeds to combat emerging virus epidemics in the country.
- It was evident that some of chickpea and faba bean seeds used by farmers in Tunisia, contained viral infections which may be transmitted by other means of spread (such as aphids) to healthy plants.
- Conduct further experiments to study the relationship between the infection rate in planted seeds and production loss under local conditions.
- To reduce the rate of viral infection in faba bean and chickpea fields, and thus increase productivity of these crops in Tunisia, it is necessary to produce virus-free seeds. This is a necessary first step, and can be implemented easily if the efforts of all concerned parties are combined.

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