

TSWV development, transmission, and escape in *Taraxacum kok-saghyz*

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ABSTRACT

Taraxacum kok-saghyz (TK) is highly researched for its natural rubber properties and as a possible supplement to rubber from the rubber tree, *Hevea brasiliensis* (Figures 1 and 2). The TK plant can be infected by the Tomato Spotted Wilt Virus (TSWV) while remaining reasonably healthy. However, TSWV-positive TK plants may be a source of infection to tomatoes, petunias and other susceptible species. Following a pervasive TSWV multi-species infection in an OARDC greenhouse complex, leaf samples of 52 TK plants relocated to a quarantine greenhouse were tested using a TSWV-specific ELISA (enzyme-linked immunosorbent assay). Of these, 19 TK plants tested positive, while 33 were negative. The roots of positive TK plants were then retested using the TSWV ImmunoStrip to evaluate the distribution of TSWV throughout the plant. All 15 TK plants with positive leaf infections also had TSWV in their roots, although the roots appeared completely normal. The level and rate of infection spread in seedlings compared to adult plants was investigated by inoculating two leaves of TSWV-negative plants with TSWV positive serum. A total of four adult TK where tested using one as control. Two out of the three adult TK inoculated with the virus became infected and the other stayed negative. After one week visible symptoms appear on the two positive TK plants. The last test was to expose seeds to infected leaf tissue and determine if the germination rate was affected and if TSWV persisted after storage at ambient temperature or at 4°C, and when treated with a 1% bleach solution. All treated seeds germinated and TSWV was not present in the seedlings. The virus-free plants cannot be generated by cloning roots of infected plants but, if they can be taken through seed production, the progeny are freed of the virus.



Figure 1: *Taraxacum kok-saghyz*



Figure 2: *Hevea brasiliensis*

INTRODUCTION

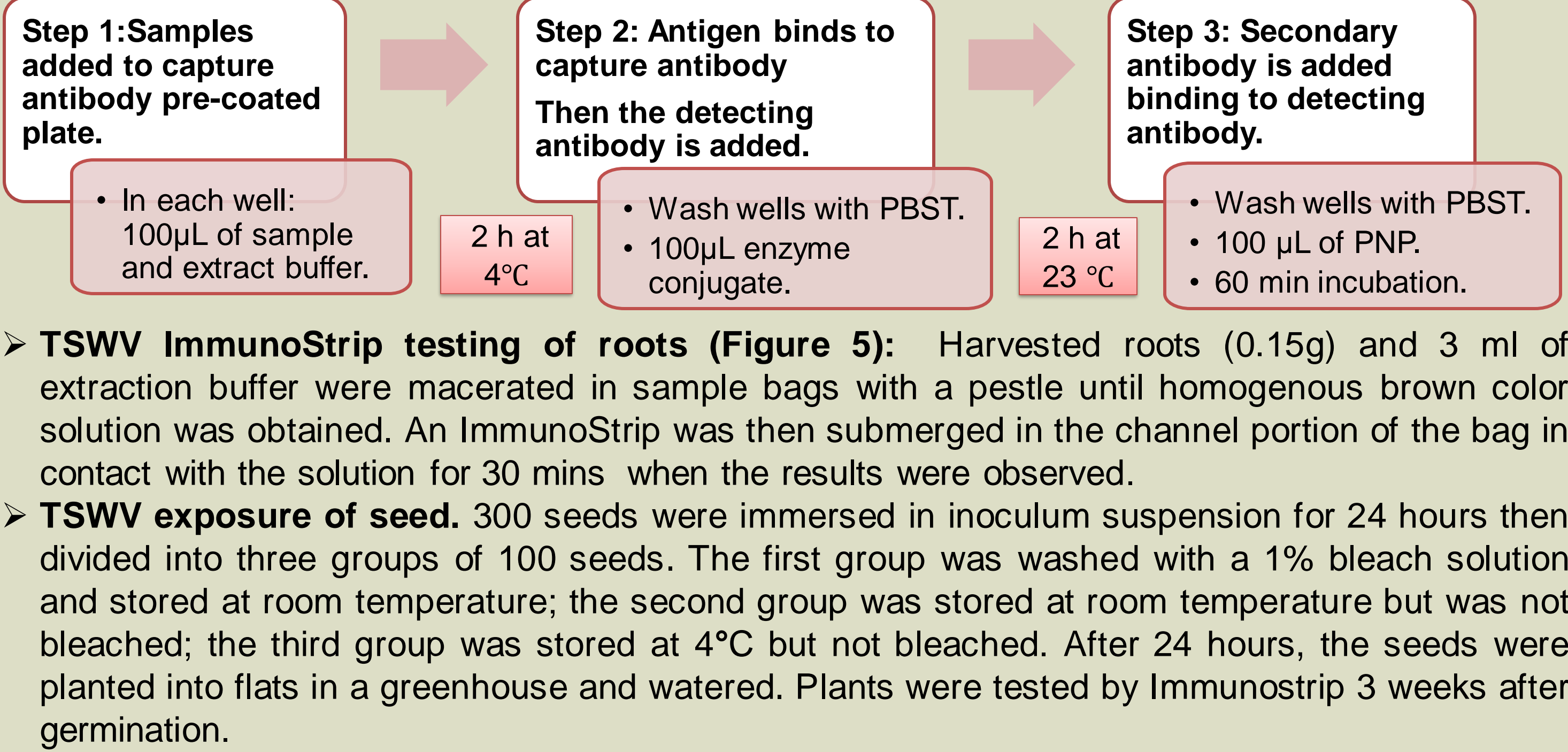
- *Taraxacum* is a large genus of flowering plants in the family Asteraceae, which includes the dandelions. *Taraxacum kok-saghyz* (TK) is a rubber-producing dandelion under development in North American and Europe.
- A shortfall of natural rubber is predicted because of increasing global demand for rubber which cannot be met by *H. brasiliensis* rubber trees. *H. brasiliensis* grows in plantations in tropical Southeast Asia. TK plants produce natural rubber with similar mechanical properties to *H. brasiliensis* and is a suitable supplement to the *H. brasiliensis* natural rubber supply.
- As TK develops as a new crop, it is important to understand the types of pathogens that can affect TK, perhaps leading to a decreased productivity or restriction of growing areas. TK can be infected by Tomato Spotted Wilt Virus (TSWV) but is often asymptomatic leading to concerns that it might be a source of infection of more susceptible species. TSWV causes serious disease in many economically important plants, including ornamentals, vegetables, and field crops, and appears in field and greenhouse production.
- TSWV is one of a few viruses transmitted by thrips, a common insect found in many crop production systems. The virus can be acquired at the thrip's larval stage but is transmitted only by adult thrips.

OBJECTIVES

- Determine the presence of the TSWV in different organs of *Taraxacum kok-saghyz* using the enzyme-linked immunosorbent assay (ELISA) and the ImmunoStrip test.
- Observe phenotypic symptoms and virus spread through the plants over time, in both seedlings and adult plants.
- Determine the viability of TSWV on contaminated TK seed.

MATERIALS AND METHODS

- **Weekly photos documenting TSWV progression in TK plants (Figure 3):**
- **TSWV inoculation of healthy TK plants:** Leaf tissue from TSWV positive plants was extracted with a 50 mM sodium phosphate buffer (PB) and 2g of leaf tissue. The tissue was macerated until a homogeneous green solution was obtained. A small amount (about 0.1g) of a mild abrasive carborundum was then added. This suspension was applied to leaves using a pestle to gently rub each leaf eight times. The presence of TSWV was determined by ELISA with a double antibody sandwich (DAS) format, with yellow color indicating the presence of TSWV (Figure 6).



RESULTS



Figure 3. Timeline of TK plants with established TSWV infections. Weekly photos of symptomatic *Taraxacum kok-saghyz* plants, A1) Week 1: a few leaves with pale green to yellow spots; A2) Week 3: all leaves with pale green to yellow discoloration; A3) Week 5: stunted growth and necrotic tissue showing a progression growth of the virus in the plant.



Figure 4. Phenotypic symptoms of TSWV, present in *Taraxacum kok-saghyz* plants. A1) TK plant shows leaves with pale yellow spots; A2) Chlorotic tissue on tips of the leaves and curly phenotype of most leaves; A3) Necrotic leaves with brown spots and dark streaking in the plant's terminal stems.

- The root test indicated that all 15 adult TK plants were positive with TSWV, confirming that the virus spread through all the plants' organs.
- The ImmunoStrip test used in the TSWV exposure test, demonstrated that the virus was not present in the plants after germination and no visible symptoms were observed.

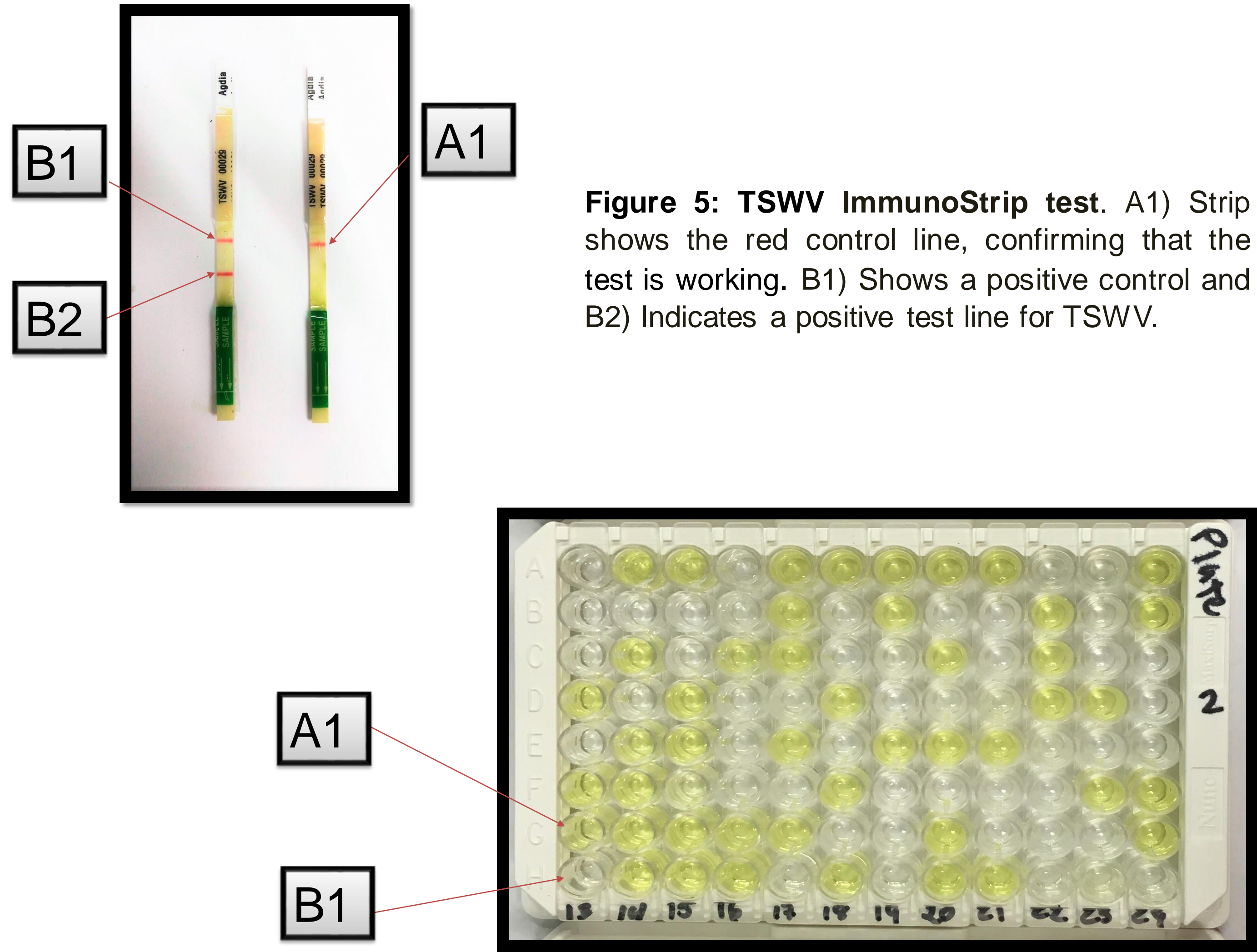


Figure 5: TSWV ImmunoStrip test. A1) Strip shows the red control line, confirming that the test is working. B1) Shows a positive control and B2) Indicates a positive test line for TSWV.

Figure 6. Enzyme-linked immunosorbent assay (ELISA) results. This test shows the positive control well A1) with a yellow coloration and the negative control well B1) with no coloration. This test was used to determine the presence of 19 positive TK plants for TSWV. The 33 wells without color show negative test results.

CONCLUSION

- Inoculating virus free TK plants with TSWV did, indeed, cause infection.
- All positive TSWV TK plants showed a progressive infection period. Although diseased leaves had visible symptoms the roots were asymptomatic. Thus, root cloning to regenerate virus free plants is not a viable option.
- In all three seed treatments, exposure to TSWV leaf inoculum did not negatively affect germination rate and seedlings from these seed were virus-free.
- When the domestication of TK is successful it will be grown in close proximity to TSWV susceptible crops. Diseased plants produced virus-free seed even when bleach treatment was not used. TSWV-free TK seed does not pose a disease risk to neighboring crops.

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