ALTERNATIVE METHODS TO CONTROL PLANT VIRUSES

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1 - STRATEGIES TO CONTROL TOSPOVIRUSES ON VEGETABLE CROPS IN BRAZIL.

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Tospoviruses are distributed world wide and are limiting factors in the production of a large number of horticultural crops. In Brazil severe outbreaks on lettuce of 100%, on sweetpepper 69% and up to 84% in processing tomato are reported. According to the ICTV Report from 1995, these viruses are classified within the Bunyaviridae family, in the genus *Tospovirus* and are comprised by the type species Tomato spotted wilt virus (TSWV) and an additional species *Impatiens* necrotic spot virus (INSV). This classification is based on parameters such as broad or narrow host range, thrips species specificity and divergence of the nucleocapsid protein at the serological and molecular levels. Additionally, nine species have already been proposed and named as: Groundnut ring spot virus (GRSV), Tomato Chlorotic spot virus (TCSV), Watermelon silver mottle virus (WSMV), (Groundnut bud necrosis virus GRSV = WSMV), Melon spotted wilt virus (MSWV), Peanut chlorotic fanleaf virus (PCFL), Peanut yellow spot virus (PYSV), Chrysanthemum stem necrosis virus (CSNV), Zucchini lethal chlorotic virus (ZLCV) and Iris yellow spot virus IYSV = BR-10 from onion. From these, TSWV, TCSV, GRSV, CSNV, ZLCV and IYSV occur in Brazil. Worldwide, seven thrips species are competent to transmit tospoviruses in a circulative propagative manner (*Frankliniella fusca*, *F. intosa*, *F. occidentalis*, *F. shultzei*, *Thrips palmi*, *T. setosus* and *T. tabaci*). In Brazil, *F. shultzei* and *T. tabaci* seem to play an important role on the transmission of tospoviruses. The vector seems to acquire the virus only in its first larval stage and becomes infective afterwards for its whole lifespan. The control of tospoviruses has been difficult for several reasons. The virus has one of the broadest host ranges among plant viruses, infecting at least 550 plant species including mono- and dicots out of approximately 70 botanical families. To date more than a hundred species are recorded within the Solanaceae and Compositae. Recently, the emerging ornamental plant industry in Brazil proved to be also an import out source of new, tospovirus species, with CSNV introducing losses in tomato crops. Control measures like roughing and destruction of the thrips vector by insecticides have proved to be very ineffective. Most growers cultivating vegetables within green belts hardly apply preventive measures such as preparation of seedling nurseries protected from viruliferous thrips, elimination of abandoned crops and eradication of weed hosts surrounding the crop fields. Attempts in Brazil by using corn crop as a barrier for tomato culture showed an increase in tospovirus incidence near the windbreak. The use of resistant cultivars is one of the most reliable approaches towards control of diseases caused by tospoviruses on vegetables. Although genetic resistance to tospoviruses on tomatoes has been found in *Lycopersicon peruvianum* (L.) Mill., *L. hirsutum* and in *L. pimpinellifolium*, the majority of the reported sources of resistance are isolate-specific and, therefore, cannot be widely recommended as parental germoplasm. The Sw-5 gene found in the South African *L. esculentum* cultivar 'Stevens' is being used in several tomato breeding programmes in Brazil. This gene was incorporated by backcross breeding into the cultivar IPA-5, the main processing tomato cultivar planted in Brazil. Sw-5 confers resistance to all species of Tospovirus found in Brazil, reflecting an apparent wide-spectrum action. In peppers, sources of resistance to TSWV were found in *Capsicum chilense* and one dominant gene is reported to give resistance. The resistance found in *C. chilense* 'PI 159236' appears to be species-specific rather than isolate-specific, with this genotype being highly resistant against all isolates of TSWV but susceptible to TCSV and GRSV. In lettuce (*Lacttuca spp.*), only a few sources of resistance have been reported. A breeding programme conducted in Hawaii selected PI 342517 as tolerant to the virus. This germoplasm was crossed some years ago with the Brazilian lettuce cultivar 'Regina', although no commercial cultivar has being released so far. Development of transgenic lettuce using the concept of pathogen-derived resistance has been exploited for conferring resistance against tospoviruses. Several genetic engineered resistance strategies have been tested using different parts of the virus genome as transgenes: coat protein (N), non-structural protein (NS_M and NS_S, putative movement proteins), polymerase (L) and glycoproteins (G1 and G2). Use of N and NS_M tospoviral sequences as transgenes proved to be successful to obtain virus resistance. The protein expression level of both, N and NS_M did not affected the resistance level which proved to be species-specific and RNA-mediated. Broad resistance was obtained by simultaneous expression of different nucleoprotein genes. No correlation was observed between steady state RNA expression levels and resistance, but all the resistant plants expressed high levels of transgenic transcripts at nuclear level. The resistance mechanism indicates the induction of an active sequence-specific RNA breakdown similar to co-suppression that confers resistance by degrading viral RNA sequences identical to the transgene. This mechanism is a useful tool to combat tospovirus diseases in crop plants because it minimizes the chances of undesirable genetic exchange between viruses and transgenes, since no protein expression is necessary and RNA is quickly broken down in the transgenic plant cell

2 - OBTAINMENT AND USE OF VIRUS FREE PROPAGATIVE MATERIALS AS PART OF THE INTEGRATED CONTROL OF PLANT VIRUS DISEASES.

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The integrated control of plant diseases is an important component of the sustainable agriculture, in which measures taking into consideration the cost/benefit relation and the less damaging side-effects to the agroecosystem are applied. For virus diseases, a set of methods is usually employed, based on the peculiar forms of surviving, dissemination, infection, and multiplication of the pathogens, as result of the host-pathogen-environment interactions. Though virus free propagative materials (VFPM) are used for virus control, as a general measure of exclusion, its importance is more evident in certain circumstances: maintenance and exchaging of germoplasm, planting vegetatively propagated crops, implementing perenial crops, and cultivating annual plants in presence of a high population of effective vectors. Some major activities with plants are directly related to the obtainment and distribution of VFPM, such as: production of health clones, by selecting virus free materials or cleaning degenerated clones in laboratories of biothecnology; seed and planting material certification programs; and quarantine services. Many different procedures are used for inspecting, sampling, and indexing materials produced "in vitro", under controlled environment, and in field conditions. Depending on the pathosystems, the available materials and facilities, a variety of protocols can be applied: meristem tip and embryo cultures, associated or not with thermo and/or chemotherapy, for virus elimination, and biological, serological, and molecular methods for indexing. In potato, the phenomenon called "degeneration" was observed in Europe in the 18th centure. Though the etiology was not known, plants from botanical seeds were grown by British and Dutch farmers to counteract its effects. Plants generated from nucellar embryos have been used for revitalizing citrus clones, freeing them from virus and viroid diseases. For seedborne viruses, the utilization of the modern methods of indexing has permitted the cleaning of germplasm collections and production of virus free seed for commercial use.