The Development of Diagnostics for Evaluating *Beta* Germplasm

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ABSTRACT

The development of diagnostics for evaluating *Beta* germplasm should lead to the more informative cataloguing and rapid exploitation of *Beta* gene banks. At Broom's Barn Experimental Station, resistance or tolerance to aphid transmitted yellowing viruses (BYV and BMYV) and to *Polymyxa betae*, the fungal vector of the Rhizomania virus (BNYVV) is being examined using ELISA techniques and molecular probes. *Beta* material is also being evaluated for drought tolerance by estimating the damage incurred during stress through chlorophyll fluorescence measurements. Characteristics which confer tolerance are also being identified for subsequent development of specific markers.

Additional Key Words: *Beta vulgaris* L., sugarbeet, pathogen resistance, molecular probes

During the past 10 years diagnostic methods have assumed an increasingly important role in the sugarbeet industry. In particular, the ability to diagnose the presence of sugarbeet pests and pathogens has provided new insights into their epidemiology. This knowledge has enabled more accurate forecasting of potential pest and disease problems and improved advice on crop protection measures. A further important role for diagnostics, particularly those based on molecular biology, is to identify useful characteristics in sugarbeet and its related species which can be incorporated into new varieties, either through genetic engineering or conventional breeding. At Broom's Barn, diagnostics are being developed and utilized mainly in crop protection programs. Increased emphasis in sugarbeet physiology and biochemistry programs is also demanding such approaches.

DNA PROBE TO DETECT *POLYMYXA BETAE* IN SUGARBEET ROOTS

Currently, Rhizomania is partly controlled by growing varieties possessing some resistance to the causal virus BNYVV. However, control should also be possible by introducing resistance to *Polymyxa betae,* the fungal vector. Quantitative resistance to the fungus has been detected in specific lines of *Beta maritima* (Asher and Barr, 1990). The availability of a molecular probe for the fungus would be of considerable help in any breeding program.

Since *Polymyxa betae* cannot be cultured axenically, infected sugarbeet roots have been used to develop this probe (Mutasa et al., in press). It has been produced from a repetitive genomic DNA fragment cloned from the DNA library from a *P. betae* infected sugarbeet. Homology of this DNA fragment to sequences on at least four different *P. betae* chromosomes was confirmed by pulse field gel (CHEF) analysis. Southern hybridization experiments demonstrated that the probe is highly specific to the fungus in infected plant roots. When tested against a wide range of fungi commonly found in soil and/or associated with plant roots, including the fungi most closely related to *P. betae (Polymyxa graminis, Plasmodiophora brassicae),* the probe did not cross-hybridize. This probe, pPbKES-1, hybridizes to all *P. betae* isolates tested. The probe is highly sensitive. Tests carried out so far show detection of *P. betae* from as little as 10 ng of total genomic DNA from infected roots.

The DNA fragment has been partially sequenced and PCR primers have been designed which are now being used for screening sugarbeet plants naturally infected with low levels of *P. betae* in the

field. To date, we have used the probe on southern blots of PCR gels to successfully amplify and confirm products of *P. betae* from 30-day-old sugarbeet seedlings. In addition to searching for additional probes in our library, we are developing a quantitative PCR assay that uses primers engineered for ELISA-type color reactions, which can be rapidly evaluated on standard microtitre plate readers. The development of this sensitive and specific DNA probe and PCR protocol will allow rapid screening of sugarbeet plants for *P. betae* infection, with a goal of selecting plants with resistance to *P. betae* for incorporation into breeding programs.

Sequence data analysis suggests that the DNA fragment may originate from a part of the *Polymyxa* genome involved in the BNYVV/*Polymyxa* interaction. A highly representative cDNA library of *P. betae* has also been constructed. These developments should provide opportunities to study the mechanisms of the BNYVV/*P. betae* interaction and to isolate and study the genes expressed by sugarbeet in response to fungal invasion, thus providing new insight into defense mechanisms expressed by the plant.

MONOCLONAL ANTIBODIES TO DETECT YELLOWING VIRUSES AND MOLECULAR PROBES FOR VIRUS RESISTANCE IN SUGARBEET

A method has been devised for screening sugarbeet plants for resistance to BYV and BMYV, based on the assumption that plants which resist virus multiplication during the critical early stages of growth are likely to give higher yields than their susceptible counterparts. The stability and mode of inheritance of this resistance and the use of molecular markers to identify resistant material also are being studied.

Seed lots, derived from crosses between *Beta maritima* and *B. vulgaris,* were inoculated at the cotyledon stage by placing five to 10 *Myzus persicae* carrying BYV or BMYV on each plant. The aphids were killed 48 h later by nicotine fumigation and the plants grown in the glasshouse. We used ELISA to assess the virus concentration of each plant 28 days later; those plants that gave lower absorbance readings, indicating low virus concentration, were retained. Monoclonal antibodies were used to improve the sensitivity of virus detection in the triple-antibody-sandwich ELISA method (D'Arcy, Torrance and Martin, 1989).

Three of the nine selected seed lots gave small numbers (<1%) of plants with low virus concentration when inoculated with BYV. The remaining seed lots (six) gave up to 9% of plants with low concentration

of BMYV. These plants with low concentrations of BMYV were vernalized and used in a commercial breeding program. Their progeny were evaluated in artificially-infected field trials. A number of crosses derived from the original selections exhibited a degree of resistance to infection and remained green through the season.

The stability and mode of inheritance of this resistance is now being investigated by use of clones of resistant and susceptible beet in inoculation studies and in a breeding program. The clonal material was produced by tissue culture of inflorescence tips (Zhong et al., 1993), from 35 BYV-resistant and 36 BMYV-resistant plants selected with the ELISA technique. The use of clones will facilitate the determination of environmental effects on resistance, and should overcome the problems of genetic variability. Subsequent ELISA testing of inoculated cloned plants showed that they reacted uniformly to infection.

The possibility of using molecular markers to identify resistant material is now being examined. DNA has been extracted from leaf tissue of each of the virus-resistant plants and compared with that from susceptible plants. RAPD analysis with four 10-base pair primers has indicated three polymorphic bands present only in resistant individuals. Of the 71 resistant types selected, 10 BMYV-resistant and 8 BYV-resistant lines had one or two of the polymorphisms present. It is hoped that this technique can be used to monitor the resistance through successive generations in a breeding program and to construct a more accurate linkage map.

SELECTION FOR TOLERANCE TO DROUGHT STRESS IN *BETA* SPP.

Sugarbeet is a relatively drought tolerant crop as would be expected from its maritime ancestry. However, wilting occurs frequently due to both high evaporative demand and large soil moisture deficits, resulting in poor quality and reduced yields. *Beta* material is being evaluated for drought tolerance by estimating the damage incurred during stress and identifying characteristics which confer tolerance for subsequent development of markers (Clarke et al., 1994). The extent of damage to a leaf can readily be determined from its chlorophyll fluorescence induction curve. The ratio of variable (Fv) to peak (Fp) fluorescence has been shown to be a sensitive indicator of photochemical efficiency and thus leaf health.

During water deficiency stress, the leaf relative water content falls and wilting occurs, beginning with the oldest leaves. However, in the absence of other stresses, e.g. heat or low temperatures, leaf damage

does not occur until much later (Clarke et al., 1994). The drought tolerance of different lines can be measured by imposing water deficiency stress and quantifying any damage after a fixed period of time.

Drought tolerance is due to several diverse plant characteristics, including the content of compatible solutes, sugar, stress proteins, plant hormones as well as stomatal number and control, cuticular resistance and fibrous root growth. Some of the biochemical components being measured by chromatographic techniques (HPLC) are particularly important since they also influence the internal quality of beet roots.

CONCLUSIONS

Cultivated and wild forms of the genus *Beta* have been collected by genebanks to safeguard the genetic diversity of the sugarbeet crop. Although several useful genes have been transferred from wild beet into sugarbeet, there is still much unknown about the breeding potential of these germplasm collections. An important factor in the failure to utilize what is undoubtedly a considerable reservoir of genetic diversity is the lack of rapid reliable screening methods. The development of such methods, as indicated here, should lead to the more informative cataloguing and rapid exploitation of *Beta* genebanks.

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