GERMPLASM RELEASE: TISSUE CULTURE-DERIVED CURLY TOP-RESISTANT GENETIC STOCK

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Curly top may be caused by three different virus species, ***Beet curly top virus***, ***Beet mild curly top virus***, or ***Beet severe curly top virus***, which are transmitted by the beet leafhopper (***Circulifer tenellus***). Curly top is one of the major diseases of sugarbeet in the semi-arid areas of the western US. The ARS sugarbeet research program at Kimberly is focused on discovering novel genes for resistance to this and other economically important diseases. It is vital in genetics research to develop uniform breeding lines and genetic stocks for inheritance studies, gene transfer (through conventional hybridization), and molecular genetics research. There is almost no information on the inheritance of curly top resistance in sugarbeet. It is a very time consuming process to produce inbred lines in sugarbeet. This process requires several one-year inbreeding cycles because the plant is biennial and the public germplasm has much genetic diversity. We have adopted a well-established tissue culture approach to develop a type of pure breeding lines called 'doubled haploid line' (DHL). Haploid plants have only one-half the normal number of chromosomes, and when doubled, the resulting plants have the normal number of chromosomes (called diploid) and are genetically very uniform. These lines are often produced using unfertilized sugarbeet ovules, pollens, or anthers. This approach has worked well in plant breeding and genetic studies for several decades, but it rarely hasbeen attempted by USDA-ARS sugarbeet researchers.

Fig. 1. DHLs at advanced stages of development differentiated from unfertilized ovules from C762-17 in tissue culture media.

To develop a tissue culture-derived pure breeding line, a self fertilized (S1) individual plant from germplasm C762-17 (PI560130) was used as a donor of floral buds for tissue culture. C762-17 was developed by the USDA-ARS sugarbeet program at Salinas, CA and released in 1989 as a curly top-resistant parental line, but is genetically variable and not all plants produce viable pollen. To select the most suitable plant part for tissue culture, we tested pollen and unfertilized ovules from sugarbeet. Tissue culture of the ovule proved the most successful and was used for producing haploid plants (Fig. 1). Twenty-eight plants were produced and all were tested for chromosome number using DNA quantification equipment known as flow cytometry. We used a specific tissue culture media in which the plant parts (ex-plant) we chose were expected to spontaneously develop the correct number of chromosomes without applying special chemicals such as colchicine and oryzalin. Three of the plants that developed were confirmed to be true diploids (2n=18) because they had the correct number of chromosomes. These three plants were considered to be genetically different breeding lines, and were advanced separately as three DHLs named KDH04, KDH09 and KDH13(where K signifies Kimberly).

The USDA-ARS Kimberly sugarbeet program, in cooperation with the Beet Sugar Development Foundation, has released the pure genetic stock KDH13 (PI663862) to public breeders and seed companies. The stock has been deposited at the Western Regional Plant Introduction Station (WRPIS) in Pullman, WA. KDH13 is the first publically available sugarbeet genetic stock that is highly resistant to curly top. It has performed better than the resistant check, Hilleshog PM90, in two greenhouse experiments (9 replications per treatment and 6 leaf hoppers per plant in a clip-cage) and same as the resistant check in the 2011 Curly Top Nursery in Kimberly, ID. Leaf samples were collected from plants in the greenhouse experiment to

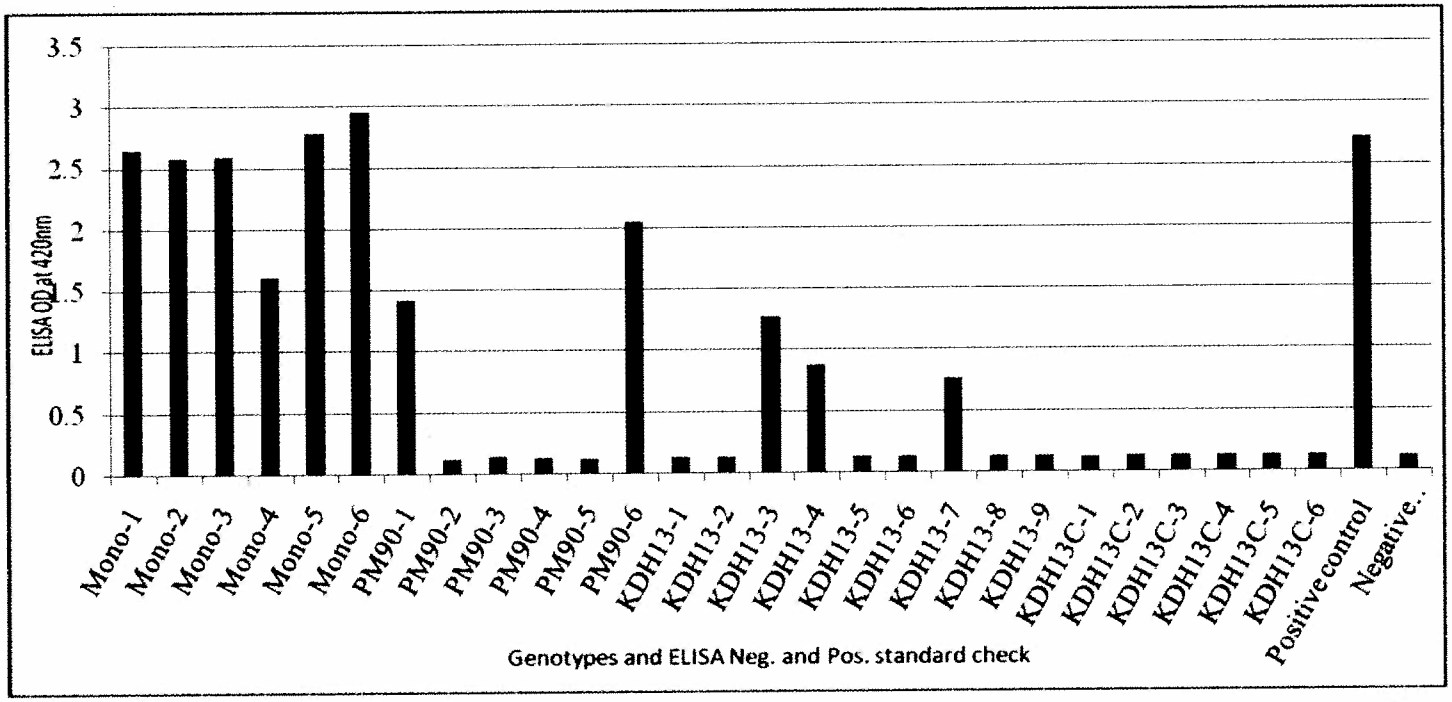

Fig. 2. ELISA results show level of virus accumulation in susceptible check (Mono = Monohikari), resistant check (HM PM90), and KDH13 (inoculated). KDH13C is control not subjected to beet leafhopper feeding.

determine virus accumulation in the plants using the Enzyme Linked Immunosorbent Assay (ELISA) analysis (performed by Dr. Alex Karasev, University of Idaho, Moscow, ID). Though the plant performance data were similar to the ELISA data, there was variation in the amount of virus particles within the resistant check and the KDHLs (Fig. 2). This observation is a very important one because we know this variation is not due to genetic variation within a DHL. Therefore, the variation might be due to the specific virus species that infected the plant, as well as to the interaction among the three virus species. To answer these research questions, we currently are using KDH13 to develop beet leafhopper populations that carry a single virus species. Having a genetically uniform line eliminates the normal genetic variation between plants when we are testing for specific virus species.

In summary, KDH13 is monogerm, self-fertile, and highly resistant to bolting. It has light green, upright, narrow leaves, and a small compact canopy. It's susceptible to rhizomania, caused by the beet necrotic yellow vein virus (BNYVV), and powdery mildew (caused by ***Erysiphe polygoni***). KDH13 can be an ideal donor of curly top resistance in hybrids, or used for backcrossing and inheritance studies of other economically important traits. The initial sugarbeet ovule culture work was performed under contract by a private company.