Red Rot Resistant Transgenic Sugarcane

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Sugarcane is a multipurpose commercial crop of Pakistan. Due to its worldwide significance as an agricultural article of trade, a lot of research has been focused on the improvement of sugarcane crop through breeding and recently through biotechnological approaches. Sugarcane is subjected to many diseases caused by fungi, bacteria, nematodes and viruses. Red rot disease caused by a fungus, *Colletotrichum falcatum*, is one of the major, oldest, widely distributed and documented disease of sugarcane in Punjab and Sindh provinces.

The defense response/ PR genes function in a variety of ways to hinder fungal infection and the expression of these genes in transgenic plants has been documented to augment fungal resistance. Literature also supports the co-expression of genes encoding chitinase and chitosanase to enhance plant defense against fungal pathogens. Therefore, we co-expressed the genes for Chitinase and Chitosanase enzymes, in red rot susceptible genotype (S-2003-us-359) of sugarcane to enhance its resistance against fungal pathogens. For this, heterologous expression of a couple of antifungal genes (*HarChit* and *HarCho* encoding Chitinase and Chitosanase enzymes respectively) from *Trichoderma harzianum* was achieved into sugarcane genotype S-2003-us-359 for inducing resistance against *Colletotrichum falcatum*. The philosophy behind the co-expression of these genes is the synergistic enhancement of antifungal activity when these enzymes are used together. In this study, a total of 684 calli were bombarded for delivering these two antifungal genes along with selectable marker gene bar and were put on to the tissue culture regime having Basta as selection pressure. In selection regime, 12 Basta resistant putative transgenic plants were selected on 10 mg/l Basta which makes a transformation frequency of 1.75%, as shown in Figures below.

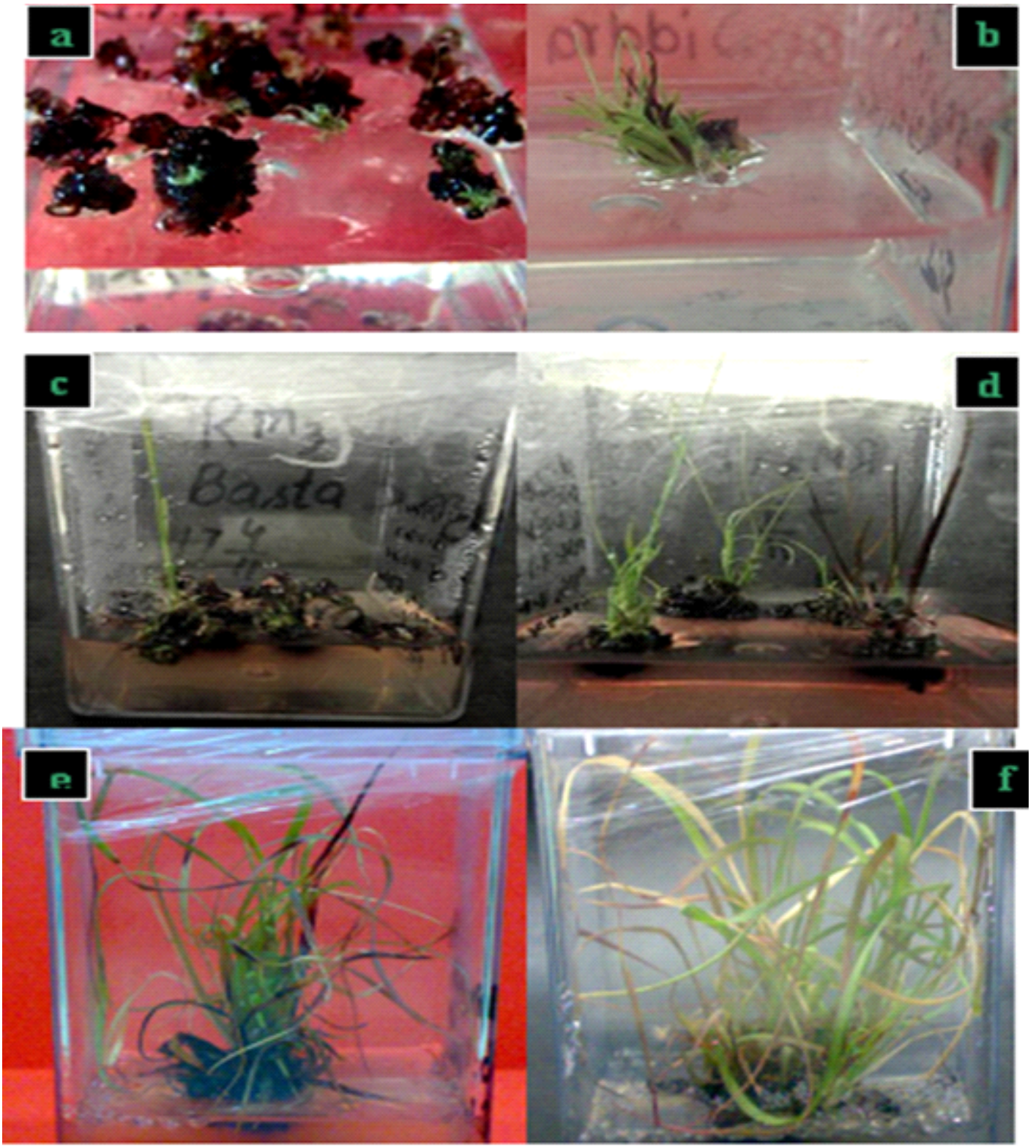

Figure: Various stages in transgenic selection: Basta resistant plants on medium RM3 with 10 mg/l Basta and ½ MS medium with 10 mg/l Basta for rooting. (a) selection on regeneration medium (b, c & d) Advanced selection stage on regeneration medium (e & f) multiplication and rooting.

After the selection of putative transgenic plants on Basta, these plants were analyzed and confirmed at genomic level using PCR, Southern blotting and reverse transcriptase PCR. Gene expression of transgene in the form of protein is required for a transgenic plant thus these transgenic plants were then analyzed for protein expression by *in vitro* (Leaf extract assays) and *In Planta* assay.

For determining the effectivity of these expressed proteins against pathogen of interest (*Colletotrichum falcatum*), we developed this new protocol which tells not only about the presence of desired protein but also gives information about the effectivity of the expressed proteins. This method has advantage over Western blot analysis and ELISA, because both these methods just give information for the presence of specific protein, but this protocol will help to identify the presence as well as the effectivity of expressed proteins where disease resistance related proteins are over-expressed or down-regulated. From in vitro assay, it was concluded that in such experiments if the proteins could be kept intact by isolating in required buffer, they can be functional and show their performance against fungal pathogens in question, as shown in figure below. Such assays are highly valuable for scientist working in developing countries. In both assays P9 showed 56.71% resistant to *C. falcatum* compared to wild type plant. P1 and P5, showed 57.9 % and 65.92% resistance to *C. falcatum*, respectively. P7 showed more resistance to *C. falcatum* as compared to others, and revealed 75.12% resistance against *C. falcatum*.

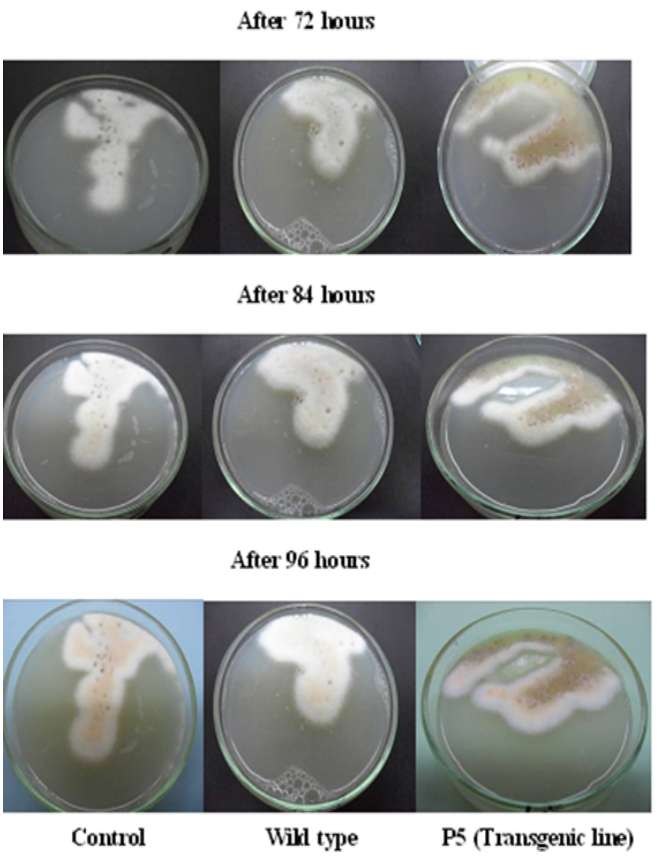

Figure: In vitro fungal assay: Control: without leaf extract, only spores in sodium acetate buffer. **WT:** Leaf extract of Wild type in sodium acetate buffer plus spores**, P5:** Leaf extract of transgenic lines in sodium acetate buffer plus spores. Comparison of mycelial development of *C. falcatum* was made in the presence of leaf extract of wild type and transgenic plants. Fungal growth was documented up to 96 hours post culture. Retarded growth of fungus was observed in the presence of leaf extract of transgenic plants.