

Pathogenresistant and Insertion of Monellin in Sugarcane by Fusion Protein and Particle Bombardment (Sugarcane for Diabetic Patients)

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Abstract: *Sugarcane is tall perennial grass of the genus Sacharum. Sugarcane has a sucrose content of 10-18% and a fiber content of 10-15% at harvest. Sugarcane is the 2nd largest cash crop of Pakistan (Naz., 2003). Sugar cane commercially very important crop. Sugarcane is a primary raw material for production of sugar (M.IrfanShaukat). Sugarcane has been chosen as a crop for improvement because of its potential for high biomass production and therefore application for the production of bioenergy and bio materials. The prevalence of the pathogens constitutes on abnormal condition, affecting plant growth and impairing important physiological process. To increase sugar contents of sugarcane monellin insert because monellin is 3000 time sweeter than sucrose and 1000,000 more sweeter than sugar on molar basis it is suitable for diabetes.(Rosalind kim et al., 1992).For better production of sugarcane it should remove the pathogens and make it pathogenic resistance and for this purpose chitinase use. Chitinase are enzymes that catalyze the hydrolysis of β -1,4 N-acetyl glucosamine linkage present in chitin. As chitin is a major component of fungal cell walls and is absent in plants, chitinase play a role in plant defense against pathogens (Schlumbaum et al., 1986). For this purpose construct form by using 35s promotor and in construct monellin, chitinase and promoter use and when construct complete it can transfer insugarcane by particle bombardment. When construct will beinserting then reporter gene show color then we observed gene has been express in stem of sugarcane*

Keywords: Monellin, fusion protein, particle bombardment, chitinase, 35s promotor, transcription termina

1. Introduction

Sugarcane is tall perennial grass of the genus *Sacharum*, with originally soft, watery culm sugarcane acquired through human selection a distinct feature of partitioning carbon into sucrose in the stem. The striking ability of accumulating levels of sucrose that can reach around 0.7m in mature internodes is an almost unique feature in cultivated plants (Moore, 1995). Sugarcane has a sucrose content of 10-18% and a fiber content of 10-15% at harvest. The stem or stalks develop from buds and are ready for harvesting 10-24th month later (Ahmed et al., 2007). Sugarcane is the 2nd largest cash crop of Pakistan (Naz, 2003) and the 5th largest country in the world in term of area under sugarcane cultivation, 11th by production and 60th in yield. Sugarcane is the commercially important crop that accounts for approximately 65% of the global sugarcane production. More than 120 countries produce sugar, 74% of which is made from sugarcane (worlds sugar statistics., 2006) Sugarcane is a primary raw material for production of sugar (M.IrfanShaukat). Sugarcane is cultivated in more than 20 million hectares in tropical and sub tropical region of the world, producing up to 1.3 billion metric tons of the crushable stems. It has served as a source of sugar since 100 of years, represents an important renewable biofuels source, which could turn into a global commodity and important energy source (Pandey et al., 2000).

Sugarcane has been chosen as a crop for improvement because of its potential for high biomass production and therefore application for the production of bioenergy and bio materials. The prevalence of the pathogens constitutes on abnormal condition, affecting plant growth and impairing important physiological process. As highlighted by FAO (2005), fungus, bacteria, virus, nematodes and many other pathogens are the main disease causing agents resulting in

serious losses to agriculture and also native plants reducing the productivity, nutritional value and overall quality of the produced biomass. To overcome all these problems scientist has been used many techniques such as, use breeding programme (Amaresh et al., 2012) for ethanol production in sugarcane, embryonic culture (Supra et al., 2010) for increase sugarcane production, recombinant DNA (Sharma et al., 2000) for insect resistance, pathogen defense mechanism (Ana caroline, 2011), micropropagation (Ahmed et al., 2006) for effect of sucrose and growth regulators, tissue culture (Sangar et al., 2011) for improving plant quality and producing pathogens free plants, remote sensing technique (Ahmed et al., 2007) for management of sugarcane, RT-PCR (si-liange., 2004) for diagnosis of disease, genetic engineering (Falco et al., 2001) for enhance host resistance to insect pests, quantification of yield gaps in different planting types of sugar cane (Balasaheb., 2013)

Now we want to make pathogenic resistance sugarcane and also insert monellin to enhance sugar contents and this transgenic sugarcane can be useful for diabetic patients as well as fatty. Monellin is sweeter than sucrose & just give sweetness not energy as sucrose do. Monellin is a protein that is found in the fruit of an African plant that is called "Dioscoreophyllum cumminsii". Monellin is approximately 3000 time sweeter than sucrose on a weight bases. Monellin is a dimer of two chains, one chain (A) contain 45 amino acids and other (B) contain 50 amino acids. The chains are held together by weak non covalent bond. Unfortunately, the fact that monellin compound of two separate chains limits its usefulness as a sweetener because it is readily dissociate and consequently losses its sweetness, when it is either heated during cooking or exposed to acid. To circumvent this problem a monellin gene that encode both A & B chains as a single peptide was chemically synthesized, the fusion protein was produced. In fusion protein we also use chitinase

transgene that can disrupt the cell wall of fungi and kill are remove pathogens. Chitinase are enzymes that catalyze the hydrolysis of β -1, 4 N-acetyl glucosamine linkages present in chitin. As chitin is a major component of fungal cell walls and is absent in plants, chitinase play a role in plant defense against pathogens (Schlumbaum et al., 1986). Important applications of fungal chitinase include the possibility for improving plants resistance with the help of genetic manipulation techniques. The **Chi42 gene** of *T.harzianum* encodes a powerful endochitinase, which has a much stronger anti fungal activity against a number of phytopathogenic fungi and is expressed constitutively in different plants. These transgenic plants thereby show a high level of resistance against phytopathogenic fungi and many other insects. In fusion protein promoter, transcription terminator monellin chains, chitinase and GUS reporter marker used because GUS reporter system (GUS B-Glucuronidase) is a reporter gene system, particularly useful in plants. The purpose of this is to analyze the activity of a promoter in terms of expression of gene. When incubated gene then some specific colorless, non fluorescent substrates can transform them into colored or fluorescent products. When construct has been completed then we used particle bombardment technique to insert that construct in the stem of sugarcane. In which we shoot the resistance gene into the cells of sugarcane stem cutting. This transgenic sugarcane is resistant plants and cost effective also because the discovery has the potential to save the world sugarcane breeding industry million of dollars.

2. Review of Literature

There are many journals that have already done sufficient work on transgenic sugarcane. Everyone has used different techniques in the completion of their work.

Application of remote sensing technique to sugarcane production (Ahmed et al., 2007). This technique was provided chemically, up-to-date and relatively accurate information for the management of sugarcane. The aim was to provide accurate and fundamental information related to spectral properties of sugarcane to its genomic, health and nutritional states characteristics that were important for farmers and farm managers. This technique was suitable for the classification of varieties, yield prediction, detection of diseases, nutrients and water deficiencies.

Biotechnological interventions in sugarcane improvement, strategies, methods and progress (supraanna., 2010). In this study somatic embryogenesis used to plant regeneration in sugarcane current research was centered on developing innovative in vitro culture system with potential, for rapid propagation and generating novel germplasm with desirable traits. In this study accelerate seed germination, improvement in seedling establishment, stimulate vegetative growth and crop yield in many field crops and was perform under salinity conditions.

Effect of various amino acids on shoot regeneration of sugarcane (Asad et al., 2007). Amino acids have been found critical to induce somatic embryogenesis in plant tissue culture medium. By somatic embryogenesis ornamentals produced like: orchard grass, embryos formed on amino

acids containing medium should high percent age of conversion and considerably less incidence of precious germination. In somatic embryogenesis amino acids were inserted in plants embryos and white callus changed into yellow callus by adding amino acids at different stages. In result observed that somatic embryogenesis and shoot regeneration was high on three of the five amino acids treatment.

Prospects for using transgenic resistance to insects in crop improvement, (Sharma et al., 2000). Recombinant DNA technology offers the possibility of developing entirely new biological insecticides that retain the advantages of classical biological control agents. The ideal technology was commercially feasible, environmentally biodegradable and was easily to use in diverse agro ecosystems as well as show a wide spectrum of activity against the crop pests. It was also be harmless to the natural enemies. In this technique Bt gene was introduce against pests/ insects and then transgenic insects resistance sugarcane was formed.

Insight on pathogen defense mechanism in the sugarcane transcriptome (Nougveira et al., 2012) recognition of pathogens and activation of defense mechanism is a common feature known from all multi cellular organisms. Among higher plants, systematic acquired resistance (SAR) is known to activate pathogenic-related (PR) genes after recognition of the pathogen mediated by resistance genes. Both gene classes (R, PR) represent the main mechanism against biotic and sometimes also a biotic stresses. Defense mechanism is a mechanism of plant it activate when any pathogen/ insect attack on plant and protein related gene of plant activate and protect themselves. Besides R and PR genes, hormones are also important signaling molecules, playing important regulatory role on plant development and inducing the expression of PR proteins.

Deterrmination of flavonoids in cultivated sugar (Colombo et al., 2005). A high performance liquid chromatography method with photo diode array (DAD) detection was developed to separate and quantify the flavonoids in sugarcane leaves and bagasses and in sugarcane juices. Sugarcane flavonoids consist of a complex mixture of Aglycane and glicoside and the HPLC-UV method, here in proposed is suitable for thier quantification as total flavonoids. Due to considerable contents of flavonoids found in sugarcane juice and it's by products, studies are in progress in laboratory to obtain additional data to evaluate the potential use of sugarcane as a dietary source of flavonoids including its possible utilization as a functional food or as a nutritional products.

3. Materials and Method

3.1 Gene introduce

The technical steps required introducing genes into sugarcane and regenerate plants containing transgene have been developed and widely used to introduce a range of novel traits (Lakshmanan et al., 2005). 35s promoter, transcriptional terminator, chitinase, monellin chains and reporter marker will require to form construct. Genetically modifeid sugarcane field trials have however, occurred in

many countries including some of the largest producers of sugarcane and also in Pakistan.

3.2. Fusion

Now, in Pakistan fusion technique will use for production transgenic sugarcane. This technique will use for the insertion of monellin to enhance sweetness and chitinase to produce pathogenic resistance sugarcane.

3.3. Construct formation

Then we will construct fusion protein under the control of 35s promoter from cauliflower mosaic virus, each construct have transcription terminator- polyadenylation site from a Ti plasmid. Then we will add chains of monellin (A, B), chitinase and use GUS as a reporter gene to examine the gene expression, it help in detection either gene has been inserted or not. When the construction will be complete then we will insert that construct by particle bombardment.

3.4. Particle bombardment

Particle bombardment is also called biolistics. In this technique we used gold/ tungsten spherical particles that will be coat with fusion construction that will beprecipitate with calcium chloride; the coated particles are high speed (300-600 ms⁻¹) with a special apparatus called particle gun. The construct will incorporate into the cutting stem of the sugarcane to make it more sweet and pathogenic resistance.

3.5. Gene expression

When construct will be inserting then reporter gene show color then we observed gene has been express in stem of sugarcane.

4. Tentative Timetable

Almost in 2 years proposal will be complete. In 2 months chitinase gene will isolate, 2 months will require in monellin isolation, in 2 months reporter gene will isolate and in 4 months fusion construction will be complete and 2 months will require for particle bombardment technique and in 1 year sugarcane will grow.

5. Possible outcomes

Transgenic sugarcane that has fusion construction, that will be sweeter than sucrose that will increase sugar contents than wild type. Due to presence of monellin diabetic patients can use it because its metabolic energy is not like sucrose. The fatty people can use this transgenic sugarcane because sucrose may increase the weight of patients and can cause many other serious problems. This transgenic sugarcane will be pathogenic resistance because in fusion construction chitinase gene present that is important for the removal of pathogens and kill insects that can increase the productivity of crop than wild type. Transgenic sugarcane will improve the economy of our country because major part of economy is based on cultivation of sugarcane. The germination of this transgenic sugarcane will be longer than the wild type and it can grow for long time.

6. Future Perspectives

In future it is possible to increase sugar contents in sugarcane by artificial flavor and that sugar cane will use diabetic patients. The production of sugarcane will improve in future due to insect resistance. When production increase then income of our country will increase and it will be helpful for nation and in country income.

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