Transformation and Transgenic Expression studies of Glyphosate tolerant and Cane Borer Resistance Genes in Sugarcane (Saccharum officinarum L.)

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Abstract Sugarcane is an important sugar and cash crop grown throughout the world. Sugarcane quality and production is mostly affected by biotic and abiotic factors that caused low yield of sugarcane products throughout the world. Using biotechnology and genetic engineering as a complement for traditional breeding methods it is possible to introduce insect/pest, herbicide-tolerant traits into various crop species. It has been found that the absence of insect/pest, abiotic and herbicide tolerant genes in genetic pool of crop plant species makes traditional breeding programs difficult. The varieties that are productive and at the same time has resistance against certain pathogens and diseases, can improve yield. The development of biotic and abiotic resistant sugarcane varieties through transgenic technology will be cost effective in controlling all type of stresses which ultimately improve yield potential. The present review will provide its readers the opportunity to understand the methods of glyphosate tolerant and Bt resistant genes in sugarcane.

Keywords Saccharum officinarum; Gene transformation; Biotic; Abiotic resistance; Stress

Introduction Sugarcane is tall perennial true grasses belongs to the grass family; Poaceae, of the genus Saccharum. Sugarcane is an economically important crop and used for sugar production. They have stout jointed fibrous stalks that are rich in sugar, and measure 6 to 19 feet tall. All sugarcane species interbreed and the major commercial cultivars are complex hybrids. Agriculture is central to economic growth and development in Pakistan. Sugarcane occupies an important position in national economy in order to drive the large sugar industry. Agriculture contributes to 21.4% of GDP and employs 45% of the country’s labor force. During 2012-13, sugarcane was cultivated on an area of 1124 thousand hectares. It adds 3.2% in agriculture and 0.7% in GDP. The production of sugarcane during year 2012-13 was 62.5 million tonnes with 5.9% increase in yield as compared to last year i.e. 58.4 million tonnes (Economic Survey of Pakistan, 2012-13). Sugarcane is indigenous to tropical South and Southeast Asia. The genus Saccharum contains six species; two wild species S. spontaneum and S. robustum; and 4 cultivated species S. edule, S. officinarum, S. barberi and S. sinense (Peter Sharpe, 1998). Different species originated in different locations e.g. S. barberi originated in India; S. edule and S. officinarum in New Guinea. All commercial canes grown today are inter-specific hybrids (Wrigley, 1982). Sugarcane originated in India about 300 B.C and subsequently spread through trade routes to North Africa, the Mediterranean region, Spain and later on to Arabia, China and Persia (Ullah et al., 2011). It was first domesticated as a crop in New Guinea around 6000 BC. Approximately 70% of the sugar produced globally comes from S. officinarum. It is assumed that multiple crossing between S. spontaneum, Erianthus asundinaceus and Miscanthus sinensis resulted in S. officinarum (Daniels and Roach, 1987).

Sugarcane cultivars have a complex aneuploid, highly heterozygous and polyploid genome with chromosome number varying from 80-120 (Joyce et al., 2010). Sugarcane cultivars derive from recent inter-specific hybrids obtained by crossing S. officinarum (2n=8x=80) and S. spontaneum (2n=5x=40 to 2n=16x=128) (Cuadrado et al., 2004). The challenge in sugarcane sequencing is complexity of its genome structure that is highly polyploid and aneuploid with a complete set of homologous genes predicted to range from 8-10 homologous copies of...
most loci (Souza et al., 2011; Mudge et al., 2009). Multiple alleles are expressed although transgenes expressed from the corresponding promoters can undergo efficient silencing (Moyle and Birch, 2013).

Sugarcane is largely grown in tropical and sub-tropical regions of the world and provides around 74 million tonnes of sugar annually and contributes to 2/3rd of world sugar production (Ullah et al., 2011; Weng et al., 2010: Hillocks and Waller, 1997). Sugarcane propagation is through stem cuttings of immature 8-12 months old canes. These are called "setts", "seed", "seed-cane" or "seed-pieces". Cane is grown highest latitude at 34° N in northwest Pakistan and 37° N in southern Spain (Sharpe, 1998). The best setts are taken from the upper 3rd part of the cane because the buds are younger and less likely to dry out.

It takes 12,500-20,000 setts to plant one hectare (Purseglove, 1979). The setts are lightly covered with soil until they sprout in 10-14 days and then the sides of the furrow are turned inward (Sharpe, 1998). Sugarcane is a perennial crop which usually produces crops for about 3-6 years before being replanted. The first crop is called the "plant crop" and takes 9-24 months to mature, depending on location (Purseglove, 1979).

**Sugarcane Production in Pakistan**

Pakistan is the 6th largest cane producing country in the world in term area and ranks 15th in sugar production (FAO, 2011; Ullah et al., 2011; Qureshi, 1998). Sugarcane contains a major source of edible sugars and many by-products are also produced by sugarcane (Table 1).

**Sugarcane Production in World**

Cane is the world’s largest, important and major crop which is very sweet in taste. It is used as raw material as 80% of sugar production is based upon it. It is cultivated in several countries. Brazil is the biggest sugarcane producing country of world with a total production of 734,000,000 tonnes, while Mauritius is the 2nd biggest producer after Brazil and India ranked 3rd (FAO, 2011) (Table 2).

**Sugarcane Uses**

Sugarcane is the main source of sucrose that is deposited in stalk internodes. *Saccharum officinarum* L. is cultivated on a large scale as raw material for sugar and industrial products, such as furfural, dextrans, alcohol, chip board, paper manufacturing, beverages, food processing, confectionery, chemicals, plastics, paints, synthetics, fiber, insecticides and detergents. It accounts for 75% of sugar production worldwide (World Sugar Statistics, 2009; Joyce et al., 2010; Enríquez-Obregón et al., 1998; Patrau, 1989).

It is also a valuable renewable source of biofuel/bio-energy for the production of ethanol (Ricaud et al., 2012; Weng et al., 2011; Menossi et al., 2007; Butt et al., 2015). Due to this reason, it has gained importance all over the world in the last 20 years. It is a prime candidate for the future fuel crop

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**Table 1 Area, Production and Yield of Sugarcane in Pakistan**

| Year   | Area (000 Hectare) | % Change | Production (000 Tonnes) | % Change | Yield (Kg/Ha.) | % Change |
|--------|--------------------|----------|------------------------|----------|----------------|----------|
| 2008-09| 1029               | –        | 50,045                 | –        | 48634          | –        |
| 2009-10| 943                | –8.4     | 49,373                 | –1.3     | 52357          | 7.7      |
| 2010-11| 988                | 4.8      | 55,309                 | 12.0     | 55981          | 6.9      |
| 2011-12| 1058               | 7.1      | 58,397                 | 5.6      | 55196          | 1.4      |
| 2012-13| 1124               | 6.2      | 62,472                 | 7.0      | 55580          | 0.7      |

Source: Pakistan Bureau of Statistics, 2012–13

**Table 2 Ten Sugarcane Producers of World**

| Rank | Country   | Production (000 Tonnes) |
|------|-----------|------------------------|
| 1    | Brazil    | 734,000                |
| 2    | Mauritius | 645,600                |
| 3    | India     | 342,382                |
| 4    | China     | 115,124                |
| 5    | Thailand  | 98,950                 |
| 6    | Pakistan  | 55,309                 |
| 7    | Mexico    | 49,735                 |
| 8    | Colombia  | 34,000                 |
| 9    | Philippines | 26,656              |
| 10   | Australia | 25,182                 |

Source: FAO, 2011
due to its efficient biomass production (Bower and Birch, 1992; FAO, 1991).

Sugar Production
The sugar industry is the 2nd largest industry in Pakistan (Ullah et al., 2011). Global production of table sugar is now exceeded up to 165 million tonnes a year. Approximately 70-80% is produced from sugarcane. The remaining is produced from sugar beet, which is grown mostly in the temperate zones of the northern hemisphere. Sugar is produced in 120 countries. 70 countries produce sugar from sugarcane, 40 from sugar beet, and 10 from both (World Sugar Statistics, 2009). The 10 largest sugar producing nations represent roughly 75% of world sugar production and 2/3rd of total world consumption. Brazil alone accounts for almost 25% of world production. White sugar consumption in developed countries can be considered as saturated whereas developing countries are considered as growing markets, particularly in Asia, and to a lesser extent in the Middle-East and Africa. At the beginning of the 20th century, a world population of 1.6 billion people consumed roughly 8 million tonnes of sugar, i.e. 5.1 kg per capita. Today, a world population of 7 billion people consumes roughly 165 million tonnes of sugar i.e. 23 kg per capita on average (World Sugar Statistics, 2009) (Figure 1).

Problems of Sugarcane Industry
Internal disputes between sugarcane growers and processors are big problem the industry. Procurement practices such as buying and purchasing used by processors such as delaying the crushing season, buying cane at less than the support price, and withholding payments hurt the farmers’ profitability. On the other hand, sugar processors complaint that farmers grow unapproved varieties that produce low sucrose content resulting in lower sugar production and recovery rates. As a result of the fluctuations in quantity and quality of raw material, sugar mills have been required to operate at 50% of their installed capacity. Furthermore, the lower sugarcane supplies have also forced most of the mills in cane producing areas to close 1-2 months earlier than normal (www.thebioenergysite.com).

Low Yield of Sugarcane
Wide use of sugarcane and its relevant products have created a challenging situation for sugarcane researchers and growers. In spite of extensive research the average yield of sugarcane in Pakistan is very low as compared to other cane producing countries of the world (Imam, 2001). There are many factors which are responsible for this low yield, among them severe attack of insect pests at early and mature stages of crop are the most significant one. Sugarcane borers are the most damaging among the pests attacking this crop (Ashraf and Fatima, 1980). These include top borer, stem borer, root borer, and Gurdaspur borer. Borers may reduce the yield up to 80% (Kalra and Sidhu, 1955). The damage caused by borers not only reduces the crop yield but also affects the sucrose contents of cane.

Sugarcane Problems
Approximately, 100 diseases have been reported to hinder the growth of sugarcane all over the world (Khurana and Singh, 1975). The most common are fungal diseases which occur as spots and hinder the process of photosynthesis affecting the growth of plant and ultimately the yield (Patil and Bodhe, 2011). A number of bacterial, fungal and viral pathogens attack the crop and cause serious damage.

Bacterial Diseases
Some important bacterial diseases of sugarcane are gumming (Xanthomonas vavculorum pv. vavculorum), leaf scald (Xanthomonas altilineans), mottled stripe (Pseudomonas rubrisubalbicans), ratoon stunting disease (Clavibacter xyli) and red stripe or top rot (Pseudomonas avenae, P. rubrilineans).

Fungal Diseases
Fungal diseases of sugarcane are red rot (Physalospora tucumanes anamorph; Colletotrichum
Pythium graminicola, Rhizoctonia sheath and shoot rot (Rhizoctonia solani), pineapple diseases (Thielaviopsis paradoxo), downy mildew (Peronosclerospora sacchari), wilt (Fusarium sacchari), rust (Puccinia melanocephala, P. kuehni), smut (Ustilago scitaminea) and seedling blight (Alternaria alternata) which are common and most prevalent in cane growing areas. Smut is prevalent in Southeast Asia and Africa. The whip-like black organs emerge from the center of the plant and damage the whole plant gradually (Sengar et al., 2011).

Viral Diseases
There are several known viral diseases the most damaging of which is Mosaic disease. It was first recognized in Java in 1892. Currently, the sub-group SCMV of the genus Potyvirus is known to have seven species out of which SCMV (Sugarcane Mosaic Virus) and SrMV (Sorghum Mosaic Virus) are known to attack sugarcane plants (Alegria et al., 2003; Arencibia, 1997). Other viruses that infect the sugarcane are chlorotic streak virus, sugarcane dwarf virus (SCDV) and sugarcane Fiji disease virus.

Insect Pests of Sugarcane
Insect pests are the major problem in agriculture which are responsible for the low yield of sugarcane include termites, borers, pyrilla, white flies, mealy bugs and termites which cause heavy losses to the crop in terms of quality and yield (Kleter et al., 2007). Globally, 1300 species of insects were recorded to be responsible for attacking sugarcane while 61 were recorded to be present in Pakistan (Hashmi, 1994). One of the major restriction factors that affect sugarcane production is the damage caused by insects such as Lepidopterous stem borers (Weng et al., 2010; Fauconnier, 1993). These sugarcane insects are often hard to control using chemical insecticides because of their ability to tunnel into and feed inside the sugarcane stems. Moreover, the insect-resistant germplasm is not available in sugarcane varieties (Christy et al., 2009). The annual loss caused by insects has been estimated to be more than 10% of the sugarcane yield losses worldwide (Ricaud et al., 2012).
In mainland China, sugarcane borer Walker (Proceras venosatus) is one of the major pest which alone causes more than 7% of sugarcane yield losses per year (Weng et al., 2010; Weng et al., 2006). According to Gupta & Singh (1997), 25% reduction in weight was observed by the 3rd and 4th brood of the sugarcane borers. Insecticides are extensively used to combat losses from insect pests. In 2011-12, 112,928 metric tons of pesticides were consumed which cost 11242 million rupees (Department of Plant Protection, Ministry of Food and Agriculture, Pakistan).

Major Lepidopteran insect pests of sugarcane are stem borer (Diatraea saccharalis) (Rossato et al., 2010), root borer (Emmalocera depressalis), sugarcane top borer (Chilo terrenellus) (Goebel and Way, 2003), pink borer (Sesamia inferens) and Mexican rice borer (Eoreuma loftini) (Naidu, 2009). Borers may reduce the yield up to 80% (Kalra and Sidhu, 1955). The damage caused by borers not only reduces the crop yield but also affects the sucrose contents of cane. Naqvi et al. (1978) have reported a gross negative correlation between the intensity of infestation and sucrose contents and recorded reduction of sucrose beyond 10% borer infestation on internodes basis. The sugarcane beetles (Tomarus subtropicicus), giant termite (Mastotermes darwiniiensis) and ants can affect sugarcane yield by damaging the roots and this damage can be minimized by using appropriate insecticide like imidacloprid or chloropyrifos. The other pests that damage sugarcane yield are moth or butterfly, sugarcane thrips (Fulmekiola serrate), cane aphid (Melanaphis sacchari), grasshopper (Oxya chinensis), sugarcane whitefly (Aleurolobus barodensis) and burrowing bug (Scaptocoris talpa) (Naidu, 2009). Sap-sucking insects include woolly aphid (Cerato Vacuna lanigeria) is predominant in Asia (Srikanth, 2004). Overall, 10% of the yield reduction is observed all over the sugarcane growing countries (Ricaud et al., 2012; Srikanth and Kurup, 2011). Non-insect pests include rats which also damage sugarcane crop.

Weeds of Sugarcane
Weeds are considered undesirable or detrimental to other plants. Weeds are the plants that are not part of the intended crop and compete for nutrients, water, light and other factors integral to crop growth (Ross and Lembi, 1999). The presence of undesirable plants in sugarcane plantations reduces crop yield. Weeds can cause major losses in terms of quality and quantity. They can reduce cane yield by competing for moisture, nutrients, and light during the growing season (Rainbolt and Dusky, 2006). Ahmad et al., (2012)
reported that 15-30% reduction in yield of sugarcane is due to weeds in Pakistan. Furthermore according to Netafim (2010), weeds are estimated to cause 12 to 72% reduction in cane yield worldwide. In Pakistan, national average yield of sugarcane is about 58.0 tons/ha as compare to world average production of 65 tons/ha (Economic Survey of Pakistan, 2011-2012).

Reduction in the cane yield with the uncontrolled weeds was recorded up to 14-74%. However, the intensity and the weed flora cause variation in the loss of yield (Singh and Moolani, 1975). Weeds can be controlled by mechanical, chemical and biological methods. Now a day, herbicides are used world widely to eradicate the weeds to minimize crop competition. Weed control by traditional method in growing field of sugarcane requires lots of manpower, moreover it tear the surface of soil resulting in uprooting of plants and cause injuries to the people involved in mechanical uprooting of weeds. Also many of the weeds have deep and extensive roots that cannot be uprooted by traditional method and majority of them can regenerate (Nasir et al., 2014). Using genetic engineering as a complement for traditional breeding methods it is possible to introduce herbicide-resistant traits into Saccharum germplasm (Enríquez-Obregón et al., 1998).

**Transgenic Sugarcane Crop**

Sugarcane is an attractive crop for improvement aimed at sustainable biomaterial production, because of high biomass productivity and an efficient gene transfer system (Birch, 2007). This has been true even for input traits such as insect and herbicide resistance that need only coarse control over the level of constitutive transgene expression (Mudge et al., 2009; Finnegan and McElroy, 1994). The required precision is expected to be higher in metabolic engineering that diverts metabolic flows into novel and high-value products, because diversion of these flows in inappropriate tissues will interfere with plant development (Mudge et al., 2009).

With the advent of genetic transformation techniques based on recombinant DNA technology, it is possible to insert genes into genome of the plant that confers resistance to insects (Romeis et al. 2006). Engineering crop plants for enhanced resistance to insect pests has been one of the successes of transgenic technology. As a trait, insect resistance, either alone or combined with herbicide resistance is currently ranked second in terms of the global area occupied by biotech crops during 1996-2009 (James, 2009). Several sugarcane promoters have been isolated and shown to drive transgene activity in callus or young plants but not in the expected pattern in mature plants (Hansom et al., 1999; Wei et al., 2003). Several heterologous and synthetic promoters have also been shown to drive strong expression in callus but little or no expression in mature plants, with evidence for both transcriptional and post-transcriptional gene silencing (PTGS) effects. In contrast, the maize Ubi-1 promoter has driven sustained reporter transgene expression in sugarcane (Hansom et al., 1999).

**Sugarcane Breeding**

The progress in traditional breeding of sugarcane, a highly polyploid and frequently aneuploid plant, is impeded by its narrow gene pool, complex genome, poor fertility and the long breeding and selection cycle. These constraints make sugarcane a good candidate for molecular breeding. In the past decade considerable progress has been made in understanding and manipulating the sugarcane genome using various biotechnological and cell biological approaches. The creation of transgenic plants with improved agronomic or other important traits; advances in genomics and molecular markers and progress in understanding the molecular aspects of sucrose transport and accumulation are notable aspects of transgenic crops. More recently, substantial effort has been directed towards developing sugarcane as a bio-factory for high-value products (Lakshmanan et al., 2005).

Traditional plant breeding techniques have been widely used to enhance important economic traits in agronomic crops, but this approach is laborious and time-consuming, especially in vegetatively propagated species like sugarcane (Liu et al., 2003). Moreover, various important traits such as resistance to insects, viruses, and herbicides are often absent from the normal sugarcane germplasm. The conventional breeding method of crossing between various germplasms has been used successfully for maximizing the productivity of sugarcane (Weng et al., 2011; Fauconnier, 1993). However, due to the biological complexity of sugarcane crop and
non-availability of desirable germplasms, this valuable traditional approach has also had its limitations, in particular, in breeding insect-resistant sugarcane varieties. Rapid progress of plant genetic engineering in the last two decades suggests that sugarcane breeding could benefit a lot from the use of non-conventional methods. In particular, genetic engineering may not only be able to shorten the period of breeding time and reduce costs for producing an improved sugarcane line. It can also introduce new agronomic traits that are absent in the natural sugarcane germplasms e.g. transgenic sugarcane plants were shown to produce sorbitol, gentiobiose and gentiobitol (Chong et al., 2010; Jain et al., 2007; Chong et al., 2007), and have resistance to herbicides or pest (Weng et al., 2006; Hernandez, 2000). Since 1990s, genetic transformation using insecticidal Bt genes is regarded as an effective method to develop insect-resistant transgenic plants (Valderrama et al., 2007).

Transformation Methods in Sugarcane

Transformation methods to introduce resistance genes into a plant genome have allowed the generation of new sugarcane varieties resistant to herbicide spray (Enriquez-Obregon et al., 1998) or insect attack (Arencibia et al., 1997; Gonzalez-Cabrera et al., 1998). Genetic transformation using insecticidal Bt genes is regarded as an effective method to develop insect-resistant transgenic plants (Weng et al., 2011; Valderrama et al., 2007). Particle bombardment and Agrobacterium co-cultivation are the two most common methods for delivery and expression of transgenes, but a direct comparison of these systems have been limited in sugarcane and other plant species (Liu et al., 2003). Since 1990s, several methods for generation of transgenic sugarcane plant have been established, such as micro-projectile bombardment and electroporation of embryogenic cells (Weng et al., 2011; Arencibia et al., 1995; Bower and Birch, 1992). The progress in transformation methodology has facilitated incorporation of several useful genes into sugarcane genomes, including a bacterial toxin degradation gene (Weng et al., 2011), three virus resistance genes (Gilbert et al., 2009; McQualter et al., 2004; Ingelbrecht et al., 1999), a bovine pancreatic trypsin inhibitor gene (Christy et al., 2009), and several insect resistance genes (Weng et al., 2006; Arencibia et al., 1997; Shan et al., 2015; Zameer et al., 2015). However, a common problem associated with these transgenic sugarcane plants is the low expression levels of transgenes (Zhang et al., 2007), which might account for the less than satisfactory performance of elite transgenic sugarcane lines in field trials (Weng et al., 2011; Arencibia et al., 1999).

Micro-Projectile Bombardment

The efficiency of gene transfer into embryogenic calli of sugarcane has been increased tenfold by optimization of particle bombardment conditions and increase in stable transformation frequencies. Genes co-precipitated on separate plasmids are co-transformed at high efficiency. The development of a genetic transformation system opens the way for sugarcane molecular improvement by introducing useful foreign genes, blocking the expression of undesired genes, moving isolated genes between varieties, or tailoring the patterns of expression of key sugarcane genes. Transgenic sugarcane plants were produced by bombardment of embryogenic callus with high-velocity DNA-coated micro-projectiles, followed by a selection and regeneration procedure designed for this target tissue. Optimal bombardment conditions for embryogenic callus required higher micro-projectile velocities for sugarcane suspension culture cells (Bower and Birch, 1992). Transgenic sugar-cane plants have been successfully recovered from cell suspensions and embryogenic calli transformed by particle bombardment (Gonzalez-Cabrera et al., 1998; Arencibia et al., 1995; Bower and Birch, 1992).

Agrobacterium-mediated DNA Transformation

The most frequently used and most efficient technique to genetically transform dicot cells employs the Gram-negative soil bacterium, Agrobacterium tumefaciens; without verified success in the production of transgenic plants (Potrykus, 1991; Klee et al., 1987). However, successful Agrobacterium-mediated uptake of DNA by monocot cells, especially the economically important cereals, has been limited (Raineri et al., 1990). Optimization of the Agrobacterium-mediated DNA transfer to sugarcane meristems has recently been reported (Enriquez-Obregon et al., 1998).

Electroporation

The naked DNA molecules can be introduced into protoplast by electrically induced changes in the
membrane permeability. This has been used successfully for transformation of a wide range of crop species. Electroporation treatment of protoplasts has allowed regeneration of transgenic rice and maize plants (Rhodes et al., 1988), but this approach is limited by severe difficulties with plant regeneration from protoplasts for most cultivars. Transgenic sugarcane plants have been successfully recovered from electroporated intact cells (González-Cabrera et al., 1998).

Polyethylene Glycol (PEG) Mediated Transformation
The development of monocot transformation systems has involved direct DNA transfer methods such as PEG treatment of protoplasts (Mass and Werr, 1989). This involved use of chemicals like PEG and divalent cations designed to increase the membrane permeability in freshly isolated protoplasts to DNA. PEG-mediated DNA uptake typically transforms 0.1-0.4% of the total protoplast treated (Hayashimoto and Murai, 1990).

Microinjection
This involves immobilization of protoplast and microinjecting DNA directly into the nucleus. Transformation efficiencies have ranged from 12-66% (Miki et al., 1987) with the highest efficiency being obtained in tobacco. However, DNA-pollen mixtures or DNA injection into young floral tillers, have not proved generally applicable (Bower and Birch, 1992).

Insect Resistance in Plants
Biotechnology efforts have focused on developing GM crops that resist abiotic and biotic stress and on increasing yields. Genetically modified (GM) crops were grown on 160 million ha globally in 2011 (James, 2011). Among them, transgenic cotton expressing insecticidal proteins from Bacillus thuringiensis (Bt) has been one of the most rapidly adopted GM crops in the world. Transgenic insect-resistant crops carrying genes from Bacillus thuringiensis were grown commercially for the first time in 1996 and increased quickly, with 14 million ha grown worldwide in 2002 (James, 2002). The insecticidal toxin gene of Bt, a known insecticidal gene, is one of the most commonly used genes in the development of GM crops. Bt cotton is considerably effective in controlling Lepidopteran pests, and is highly beneficial to the grower and the environment by reducing chemical insecticide sprays and preserving population of beneficial arthropods (Gianessi and Carpenter, 1999). Crops contain Cry genes such as Cry1Ac, Cry1Ac + Cry2Ab or Cry1Ac + Cry1F. Larvae from the resistant strain of European corn borer (Ostrinia nubilalis) did not survive on transgenic corn that produces Cry1Ab or Cry1Ac (Huang et al., 2011). Enríquez-Obregón et al., (1998) report with evidence of the first transgenic sugarcane lines resistant to stem-borer attack.

Bacillus thuringiensis
Bacillus thuringiensis (Bt) is a Gram-positive, spore-forming, soil-dwelling bacterium with entomo-pathogenic properties. Bt produce insecticidal proteins during the sporulation phase as parasporal crystals that produces crystalline protein inclusions known as δ-endotoxins (delta-endotoxin). The number of sequenced crystal proteins in Bt is more than 100, encoding Cry and Cyt proteins (Schneef et al., 1998). Crystal (Cry) and cytolytic (Cyt) protein families are a diverse group of proteins with activity against insects of different orders e.g. Lepidopterans (Schneef et al., 1998; Hofte and Whitley, 1989), Coleopterans (Herrnstadt et al., 1986), Dipteran insects (Andrews et al., 1987) and also against other invertebrates such as nematodes. These genes are generally safe for human consumption (Bakhsh et al., 2010; Bently, 2003). These are being widely used to develop insect resistance in various crops. The traditional breeding program could successfully accomplish pyramiding the foreign Bt genes with native insect resistant trait, in a single genetic background.

Mechanism of Bt Endotoxins
The mechanism of action of the Bt Cry proteins involves solubilization of the crystal in the insect midgut, when ingested by larvae, toxin proteins bind to specific receptors in the midgut region and toxin binding in susceptible insects disrupts midgut epithelium; thereby causing overall toxic effects and ultimately resulting in death of the larvae. Their primary action is lysis of midgut epithelial cells by inserting into the target membrane and forming pores in the apical microvilli membrane of the cells (Bravo et al., 2005; Aronson and Shai, 2001). Among this group of proteins, members of the 3-domain Cry family are used worldwide for insect control. Like other PFTs that affect mammals, Cry toxins interact with specific receptors located on the host cell surface.
and are activated by host proteases following receptor binding resulting in the formation of a pre-pore oligomeric structure that is insertion competent. In contrast, Cyt toxins directly interact with membrane lipids and insert into the membrane (Bravo et al., 2007).

Zhang et al. (2006) recently suggested that toxicity could be related to G-protein mediated apoptosis following receptor binding. Cry proteins pass from crystal inclusion pro-toxins into membrane-inserted oligomers that cause ion leakage and cell lysis. The crystal inclusions ingested by susceptible larvae dissolve in the alkaline environment of the gut and the solubilized inactive pro-toxins are cleaved by midgut proteases yielding 60–70 kDa protease resistant proteins (Bravo et al., 2007; Bravo et al., 2005).

Bt Cry and Cyt toxins belong to a class of bacterial toxins known as pore-forming toxins (PFT) that are secreted as water-soluble proteins undergoing conformational changes in order to insert or translocate across cell membranes of their host. There are two main groups of PFT:

1. The α-helical toxins, in which α-helix regions form the trans-membrane pore
2. The β-barrel toxins, that insert into the membrane by forming a β-barrel composed of β-sheet hairpins from each monomer (Bravo et al., 2007; Parker and Feil, 2005)

PFT-producing bacteria secrete their toxins and these toxins interact with specific receptors located on the host cell surface. In most cases, PFT are activated by host proteases after receptor binding inducing the formation of an oligomeric structure that is insertion competent. Finally, membrane insertion is triggered by a decrease in pH that induces a molten globule state of the protein (Parker and Feil, 2005).

Need to Develop Bt Transgenic Crops
The crystal protein “Cry endotoxins” produced by *Bacillus thuringiensis* are among the most specific and potent insecticides known to man. Unfortunately, these toxins must be eaten by the target insect and persist for only a few days after application sprays produced with Bt toxins have had limited use. It was realized more than a decade ago that problem with persistence, complete coverage of the plant which is impossible with sprays, especially for the control of internal feeders like stem borers and therefore ingestion could be overcome by transferring the Cry genes to plants. Plants able to defend themselves effectively even against high densities of insects were produced by the early 1990s and finally commercialized in the USA and Australia in 1996. Bt-transgenic varieties are referred to by the US-EPA as “plant pesticides”. Bt-transgenic crops can facilitate the use of under-utilized management tactics such as crop rotation and pheromone disruption that are not generally sufficiently effective alone at moderate-to-high pest densities (Roush, 1997).

Benefits of Bt Transgenic Crops
One of the most successful applications of Bt has been the control of Lepidopteran defoliators. The development of transgenic crops that produce Bt Cry proteins has been a major breakthrough in the substitution of costly and laborious spraying of chemical insecticides by environmental friendly alternatives (Bakhsh et al., 2010; Bravo et al., 2007). In transgenic plants the Cry toxin is produced continuously, protecting the toxin from degradation and making it reachable to chewing and boring insects. Cry protein production in plants has been improved by engineering Cry genes with a plant biased codon usage, by removal of putative splicing signal sequences and deletion of the carboxyl-terminal region of the pro-toxin (Schuler et al., 1998).

Glyphosate Tolerance in Plants
Glyphosate-resistant technology offers broad-based weed control with flexibility in application timing. Herbicide-tolerant crops have been widely and rapidly adopted by farmers in several countries due to enhanced weed control, lower labor and production costs, increased environmental benefits and gains in profitability. The huge diversity of perennial and annual weeds is found to occur in the crop fields. However, some particular weeds pre-dominate different cropping systems and zones. Due to these reasons both broad spectrum (selective and non-selective) herbicides are applied in weed rich fields. It is reported that continuous application of some herbicides has induced resistant in weed plants and has provoked weed problems, e.g. *Phalaris minor* developed resistance against isoproturon, a phenylurea herbicide. Herbicides using a mechanism of blocking
specific metabolism pathways do not differentiate between crop plants and weeds. These non-selective herbicides are commonly applied before sowing of crop plants and their residual effects may affect crop seeds germination and growth of seedlings. There is very limited flexibility in the timings of the herbicide use and their application demand lot of precautions. However, few crop plants surprisingly possess gifted resistance to specific herbicides. For example, 2,4-dichlorophenoxyacetic acid (2,4-D) causes death only to broad-leaved weeds. Thus it can act as the best type of selective herbicide in monocot crops like sugarcane, wheat and maize. In the same way, maize shows resistance to atrazine and simazine (Bowler et al., 1992).

Glyphosate (N-phosphonomethyl glycine) is a well-known broad-spectrum systemic herbicide used to kill weeds, especially annual broad-leaf weeds and grasses, which compete with commercial crops. It is quite effective in killing a wide variety of weed plants, including broadleaf, grasses and woody plants. On the contrary, it has a relatively small effect on some clover species. It is one of the most widely used herbicides. It was initially patented and sold by Monsanto Company in the 1970’s under the trade name Roundup, and its US patent expired in 2000. In 1983, scientists of Monsanto and Washington University isolated the common soil bacteria, Agrobacterium tumefaciens strain CP4, which is highly tolerant to glyphosate because its EPSPS is less sensitive to inhibition by glyphosate than EPSPS found in plants (Watrud et al., 2004). By 1986, they had successfully inserted the CP4 EPSPS gene into the plant genome and obtained glyphosate resistance (GR) plants. The initial GR crops were the most quickly adopted technology in the history of agriculture. In 2007, 12 million growers in 23 countries planted 114.3 million ha of biotech crops (Baum et al., 2007). Growers chose GR crops because glyphosate made weed control easier and more effective, increased profit, required less tillage, and did not restrict crop rotations. Before the introduction of GR crops, growers routinely used many different herbicides with many different modes of action. Now, many growers rely only on glyphosate (Foresman and Glasgow, 2008; Gustafson, 2008). Applying glyphosate alone over wide areas on highly variable and fertile weeds made the evolution of resistant weeds inevitable (Gower et al., 2003).

Weeds and their Control
Many types of weeds are present that crowd the sugarcane cultivated areas together with grasses, broadleaf weeds and sedges. Grasses that present in cane growing districts are Bermuda grass (Cynodon dactylon), wild sorghum (Sorghum spp.) and Guinea grass (Panicum maximum). Field grasses in particular can be challenging when cane has to grow in territory consequently. Broadleaf weeds are more regional and soil specific unlike sedges and present in all sugarcane growing areas and soil types (McMahon et al., 2000). Some other weeds can also present in the field and can be problematic such as hymenachne and giant sensitive plant. Hymenachne an aquatic grass and can be 2.5 meters tall and declared as “weed of national significance” in Australia. It can block irrigation and drainage channels in cane growing field and pollute the sugarcane crop. Spraying with herbicide is used three months periodically to control hymenachne (Anonymous, 2003).

Glyphosate
Glyphosate (N-phosphonomethyl glycine) is a broad-spectrum, non-selective herbicide that has little residual soil activity and is non-toxic to animals. Glyphosate inhibits aromatic amino acid synthesis. It is a potent inhibitor of 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS). Plant DNA encodes gene for glyphosate-tolerant enzyme 5-enolpyruvyl-shikimate-3-phosphate (EPSP) synthase. The DNA encodes glyphosate-tolerant EPSPS modified by substitution of an alanine residue for a glycine residue in a sequence found between positions 80 and 120, and a threonine residue for an alanine residue in a second conserved sequence found between positions 170 and 210 in the mature wild type EPSPS (Eichholtz et al., 2001).

Mechanism of Glyphosate Action
Glyphosate is registered for use on more than fifty crops and has been used for more than 30 years to manage annual, perennial, and biennial herbaceous grass, sedge and broadleaf weeds as well as unwanted woody brush and trees. Many glyphosate formulations and salts are commercially available; the most common salts are the mono-potassium and iso-propylamine. The type and amount of adjuvant included in the various formulations differ greatly and strongly influence weed control. This molecule is an
acid, which dissociates in aqueous solution to form phyto-toxic anions. Several anionic forms are known. The term “glyphosate” refers to the acid and its anions (Eichholtz et al., 2001). Glyphosate strongly competes with the substrate phosphoenolpyruvate (PEP) at the EPSP enzyme-binding site in the chloroplast, resulting in the inhibition of the shikimate pathway. Glyphosate inhibits the shikimic acid pathway which provides a precursor for the synthesis of aromatic amino acids. Specifically, glyphosate inhibits the conversion of phosphoenolpyruvate (PEP) and 3-phosphoshikimic acid to 5-enolpyruvyl-3-phosphoshikimic acid by inhibiting the enzyme 5-enolpyruvyl-3-phosphoshikimate synthase (EPSPS) which is the very important enzyme of the pre-chorismate part of the shikimate pathway (Nasir et al., 2014; Eichholtz et al., 2001; Steinrucken and Amrhein, 1980). Early kinetic characterization established that glyphosate is a reversible inhibitor of EPSPS, acting by competing with PEP for binding to the active site. The products of the shikimate pathway include the essential aromatic amino acids tryptophan, tyrosine, and phenylalanine and other aromatic compounds and important plant metabolic products in plants, fungi, bacteria, and apicomplexan parasites (Senseman and Armbrust, 2007). EPSPS reaction proceeds through a tetrahedral intermediate formed between S3P and the carbocation state of PEP, followed by elimination of inorganic phosphate (Eichholtz et al., 2001).

Over-expression of EPSPS gene in cells renders glyphosate tolerance in transformed plants (Pline-Smic, 2006; Castle et al., 2004). Two mechanisms have been reported underlying the glyphosate tolerance i.e.:

1. Over-expression of EPSPS gene
2. Herbicide-insensitive enzyme (Nasir et al., 2014)

Transgenic Glyphosate Resistance Crops

It has been shown that glyphosate-tolerant plants can be produced by inserting into the genome of the plant the capacity to produce a higher level of EPSP synthase. In plants including weeds, glyphosate inhibits chloroplast localized EPSP synthase (Castle et al., 2004). The present invention provides a means of enhancing the effectiveness of glyphosate-tolerant plants by producing variant EPSP synthase enzymes which exhibit a lower affinity for glyphosate while maintaining catalytic activity (Eichholtz et al., 2001). The introduction of herbicide resistant crops has dramatically changed weed management in crop production systems (Vencill et al., 2012; Owen, 2008). Using genetic engineering as a complement for traditional breeding methods it is possible to introduce herbicide-tolerant traits into *Saccharum* germplasm. Absence of herbicide tolerant genes in genetic pool of wild relatives of sugarcane makes traditional breeding programs difficult. The varieties that are productive and at the same time has resistance against certain pathogens and diseases, can improve yield. The development of herbicide resistant sugarcane varieties through transgenic technology will be cost effective in controlling weeds which ultimately improve yield potential. Several crops have been genetically modified for resistance against herbicide. The most significant was Roundup ready soybean which is the most widely adopted biotech crop that contained a version of EPSPS gene (glyphosate) from the *Agrobacterium tumefaciens* (Nasir et al., 2014).

Need for Glyphosate Resistance Crop

Herbicide-tolerant crop plants could reduce the need for tillage to control weeds, thereby effectively reducing costs to the farmer. Currently, weeds in sugarcane crop are being controlled by the application of certain organo-phosphorus herbicides like Roundup. The active ingredient of roundup is glyphosate which is a non-selective, broad spectrum herbicide that translocated symplastically to the meristems of the growing plants and is well known for its high effectiveness, low toxicity, low residues in organisms and soil and overall limited environmental impact. However, heavy use of glyphosate based herbicides increases the emergence of glyphosate resistant weeds and also it is reported that once glyphosate travels to a plant's roots, it is released into the rhizosphere where it is immobilized at the soil matrix (Tefamariam et al., 2009) which leads to disruption of soil and root microbial community (Busse et al., 2001). About 1-2% of glyphosate runoff in rainfall after glyphosate is applied (Giesy et al., 2000). Paganelli et al., (2010) indicated that low doses of glyphosate cause birth defects in frogs and chickens.

Promoters

Sugarcane is a crop of great interest for engineering of sustainable biomaterials and bio-fuel production.
Promoters isolated from sugarcane have generally not maintained the expected patterns of reporter transgene expression. This could arise from defective promoters on redundant alleles in the highly polyploid genome, or from efficient transgene silencing (Mudge et al., 2009). In eukaryotic systems the expression of genes is directed by a region of the DNA sequence called the promoter. In general, the promoter is considered to be that portion of the DNA, upstream from the coding region that contains the binding site for RNA polymerase II and initiates transcription of the DNA. The promoter region also comprises other elements that act as regulators of gene expression. These include a TATA-box consensus sequence in the vicinity of about -30bp and often a CAAT-box consensus sequence at about -75bp, 5’-end relative to the transcription start site, or cap site (Messing, 1983; Breathnach and Chambon, 1981). In plants the CAAT-box may be substituted by the AGGA-box (Messing, 1983).

The promoter is a key DNA regulatory element that directs appropriate strength and patterns of gene expression in a constitutive or specific manner, and therefore, plays a crucial role in successful transformation studies. Moreover, the number and types of promoters that drive strong, constitutive expression of transgenes are relatively few in sugarcane. For example, the viral Cauliflower Mosaic Virus 35S (CaMV 35S) promoter has been widely used in the transformation of many dicot and monocot crops, but activity in sugarcane has been low as demonstrated in various studies (Liu et al., 2003; Elliott et al., 1998; Chowdhury et al., 1992). Nearly all transgenic crops around the world utilize the CaMV 35S promoter, or similar promoters from closely-related viruses to drive transgenes. It is only now becoming clear that this promoter is not as robust as laboratory and glasshouse studies have suggested and its function is influenced by as yet undefined physiological and perhaps environmental factors (Sunilkumar et al., 2002). Foreign gene expresses in transgenic plants, it is necessary use efficient transcriptional promoters that allow a constitutive expression of the transgene. Optimization of transgene expression has been reported for other monocotyledonous plants such as maize and rice, obtaining the highest expression levels when reporter gene was fused to an untranslated intron under the control of a strong promoter (González-Cabrera et al., 1998).

CaMV 35S Promoter

Transcript mapping experiments have identified two viral promoters, designated 19S and 35S. During the virus life cycle, the 35S promoter is transcribed from the viral DNA minus-strand to produce an 8kb transcript referred to as the 35S RNA. The 35S promoter is at least 30 times stronger than the nopaline synthase (NOS) promoter. The strength of 35S promoter accounts for its widespread use for high level expression of desirable traits in transgenic plants (Hemenway et al., 1988). The CaMV 35S promoter is the most commonly used promoter for driving transgene expression in plants. Though it is presumed to be a constitutive promoter, some reports suggest that it is not expressed in all cell types (Sunilkumar et al., 2002; Yang and Christou, 1990; Benfey and Chua, 1989; Williamson et al., 1989). In addition, the information available on its expression profile in all possible cell and tissue types and during early stages of development is incomplete. It is effective in driving transgene expression in both dicots and monocots.

Ubiquitin Promoter

Ubiquitin, a 76 amino acid protein, is present in all eukaryotic cells and is one of the most conserved proteins, differing in only three of 76 amino acids between higher plants and animals. Ubiquitin is encoded by small multigenic families containing two types of genes: polyubiquitin and ubiquitin extension-fusion genes. The best characterized function of ubiquitin is its conjugation to target proteins as a recognition signal for protein degradation. Ubiquitin is also involved in other cellular processes, such as ribosome biosynthesis, chromatin structure and cell cycle control (Christensen and Quail, 1989; Callis et al., 1988). The polyubiquitin genes were reported to be constitutively expressed in all plant organs tested with an increased level in young tissues (Burke et al., 1988).

The Ubi-1 promoter has been shown to be highly active in monocots; these constructs may be useful for generating high-level gene expression of selectable markers to facilitate efficient transformation of monocots, to drive expression of reference reporter genes in studies of gene expression and to provide expression of biotechnologically important protein.
products in transgenic plants. The general availability of strong promoters active in all or most cell types of monocotyledonous plants would be useful in a variety of applications in gene transfer studies with this plant group.

The polyubiquitin promoter has been isolated from several plants including Arabidopsis (Callis et al., 1990; Norris et al., 1993), maize (Christensen et al., 1992), potato (Garbarino and Belknap, 1994), Nicotiana tabacum (Genschik et al., 1994), peas (Xia and Mahon, 1998), and sugarcane (Wei et al., 2003; Yang et al., 2003). The levels of expression were higher for the polyubiquitin promoter than the CaMV 35S promoter in maize, rice, and N. tabacum (Joung et al., 2006). The maize ubiquitin promoter Ubi-1 has been used to drive constitutive transgene expression in sugarcane studies (Falco et al., 2000; Bower et al., 1996). The control of gene expression appears to differ for the cereal and some of the floral and non-cereal monocots. In sugarcane, the maize Ubi-1 promoter showed higher levels of transient expression than the rice Act1 and CaMV 35S promoters (Joung et al., 2006; Gallo-Meagher and Irvine, 1993). The maize Ubi-1 promoter has been used most frequently for developing transgenic plants of cereal monocots that show high constitutive levels of expression, but this promoter is not useful for high levels of expression in Gladiolus (Joung et al., 2006).

In both rice and sugarcane, these assays found that Ubi-1 produced more expression foci than other tested promoters, including the rice Act1 promoter, the pEmu promoter, and some configurations of the CaMV 35S promoter. Ubi-1 has been used to produce stable transgenic sugarcane (Gallo-Meagher and Irvine 1996, Ma et al. 2000). For these reasons, Ubi-1 is often used as a standard of comparison when characterizing new monocot promoters (Wei et al., 2003). The early commercial transgenic traits in plants e.g. resistance against herbicides, insect pests or viruses were achieved through the use of 'constitutive' promoters that drive expression across most plant tissues. Notable examples are the virus-derived CaMV 35S promoter and the maize Ubi-1 promoter (Christensen et al., 1992), widely used in dicotyledonous and monocotyledonous plants, respectively (Moyle and Birch, 2013).

GUS activity from constructs containing RUBQ1 and RUBQ2 promoters in rice suspension cells was 10-15 fold greater than those using the Cauliflower Mosaic Virus 35S (CaMV 35S) promoter, and two to threefold greater than constructs with the maize polyubiquitin Ubi-1 promoter (Liu et al., 2003). Although the widely-used CaMV 35S promoter is active in monocot cells, its relative strength is substantially less than in dicot cells and it is inactive in some cell types, e.g. pollen (Christensen et al., 1992). Because of their expected involvement in fundamental processes in all cell types, the genes for rice actin (Act-1) (McElroy et al., 1990) and maize ubiquitin (Ubi-1) (Christensen et al., 1992) have been investigated as potentially useful alternatives to the CaMV 35S and Adhl sequences. Both of these monocot promoters have been shown to be significantly more active than the CaMV 35S promoter in monocot cells (McElroy and Brettell, 1994; Gallo-Meagher and Irvine, 1993; Christensen et al., 1992) with the Ubi-1 promoter being somewhat stronger than the Act-1 promoter where compared directly (Wilmink et al., 1995; Gallo-Meagher and Irvine, 1993).

Conclusion

It was concluded form all above discussions that the use of GMO (genetically modified organisms) technology, the quality and production of sugarcane may be improved. The resistance against insects/pests, diseases and glyphosate may be induced in sugarcane to increase sugar production. It may be suggested that there is need to increase the use of GMO crops to fulfill world food requirements.

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