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Agricultural Biochemistry I

- การประยุกต์ชีวเคมีทางด้านการเกษตร
- การหาคุณค่าทางโภชนาการของธัญพืช ถั่ว สัตว์ปีก อาหารสัตว์
- การพัฒนาและใช้ประโยชน์ยีนที่ดีกว่า
- การกำจัดหรือยับยั้งสารพิษ สารต้านโภชนะ ที่พบในธัญพืช ถั่ว ด้วยวิธีปรับปรุงพันธุ์หรือทางเคมี เช่น BOAA ในถั่ว *Lathyrus*, สารยับยั้ง trypsin ในถั่วเหลือง หรือ Aflatoxin ในถั่วลิสง

Agricultural Biochemistry I

- การประยุกต์ชีวเคมีทางด้านการเกษตร (ต่อ)
- เทคโนโลยีในการเก็บรักษาและแปรรูปอาหาร
- สรีรวิทยาหลังการเก็บเกี่ยวผักและผลไม้
- ชีวเคมีของการต้านทานโรคและศัตรูพืช
- ชีวเคมีของการทนแล้ง เช่น บทบาทของ proline และ hydroxy proline ต่อการทนแล้งของข้าวฟ่าง
- แหล่งโปรตีนนอกแบบ เช่น single cell protein, leaf protein

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Review

Review on postharvest technology of banana fruit

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POSTHARVEST BIOCHEMISTRY OF BANANA

The ripening process, at the fruit level involves several biochemical pathways like degradation of starch to sugar, change in the peel and pulp colour, cell wall changes, change in the concentration of volatiles and acids (Gowen, 1995).

Texture

Most fruit soften during ripening and this is a major quality attribute that often dictates shelf life. Fruit

Texture

Most fruit soften during ripening and this is a major quality attribute that often dictates shelf life. Fruit softening could arise from one of the three mechanisms: Loss of turgor; degradation of starch; or breakdown of the fruit cell walls. Loss of turgor is largely a non-physiological process associated with the postharvest dehydration of the fruit, and as such can assume commercial importance during storage. Degradation of starch probably results in a pronounced textural change, especially in those fruit like banana, where starch accounts for a high percentage of the fresh weight (Tucker, 1993; Turner, 2001).

Pigments

There is a decrease in chlorophyll content from between 50 to 100 µg per gram fresh weight to almost zero in ripe fruit, while carotenoid levels (xanthophylls and carotene) remained approximately constant at 8 µg per gram fresh weight. There is reduction in total carotenoid content in the peel during the early stage of ripening followed by carotenoid biosynthesis at the yellow-green to yellow-ripe stage (Seymour et al., 1987).

Enzyme activity

Banana fruit contains several hydrolytic and oxidative enzymes. The relative activities of α-mylase, starch phosphorylase, acid phosphate, and catalase increased considerably in banana fruits stored for 5 weeks at 20°C. The stage of maturity of banana fruit at harvest significantly influenced most of the physical and biochemical constituents and the activities of some enzymes. Banana fruits harvested at mature or early mature stage had a longer storage period and better quality. The potential storage life of banana fruits decreased with an advance in stage of maturity (Salunkhe and Kadam, 1995).

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เรื่อง Review on postharvest technology of banana fruit และ Risk assessment of genetically modified sugarcane expressing AVP1 gene

1. เรื่องเทคโนโลยีหลังการเก็บเกี่ยวของกล้วย หน้า 638 คอลัมน์ Nutrient management กล่าวถึงความสำคัญของ K และ N อย่างไรบ้าง

2. หน้า 641 ระหว่างที่กล้วยสุก รงควัตถุ ผนังเซลล์ กรดอินทรีย์ และเอนไซม์ เปลี่ยนแปลงอย่างไร

3. เรื่อง การประเมินความเสี่ยงอ้อย GMO ที่มียีน AVP1

3.1 ยีน AVP1 ได้มาจากพืชชนิดใด เกี่ยวข้องกับลักษณะอย่างไร

3.2 ประเมินความเสี่ยงของอ้อย GMO ด้านใดบ้าง

3.3 ด้านโภชนาการ ตรวจสอบอ้อย GMO เรื่องใดบ้าง

3.4 สรุปเรื่องความปลอดภัยของอ้อย GMO ว่าอย่างไร

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The aim of this review is in threefold: First, to explore the effect of different preharvest treatments on postharvest quality of fruits and vegetables. Second, the principles of biological, chemical and biochemical changes in banana during development, maturation, ripening and storage were reviewed. Third, postharvest handling and factors affecting quality of banana were examined. These include disinfecting, packaging and storage temperature. Pre- and postharvest treatments were found to have an effect on postharvest quality of banana, suggesting that postharvest quality of produce subjected to preharvest treatments should be assessed from a quality improvement, maintenance and consumer safety point of view. Literature recommends an integrated agro-technology approach towards improving quality at harvest and maintenance of qualities of banana.

Key words: Banana, fruit, postharvest handling, packaging, storage.

INTRODUCTION

Banana, cooking banana and plantain (*Musa spp*. AAA, AAB and ABB groups) are major starch staple crops of considerable importance in the developing world. They are consumed both as an energy yielding food and as a dessert (Dadzie and Orchard, 1997). They are giant perennial herbs that originated in Southeast Asia. They evolved by natural hybridization between the two species *M. acuminata* (contributing genome A) and *M. balbisiana* (contributing genome B). All important bananas and plantains are triploid. Banana and plantain are monocotyledonnous plants belonging to the section Eumusa within the genus *Musa* of the family Musaceae in the order Scitamineae. Most edible-fruited bananas, usually seedless, belong to the species *Musa acuminata* Colla. *Musa balbisiana* Colla of southern Asia and the East Indies bears a seedy fruit but the plant is valued for its disease-resistance and therefore, plays an important role as a "parent" in the breeding of edible bananas (Morton, 1987).

Bananas and plantains are today grown in every humid tropical region and constitute the 4th largest food crop of the world after rice, wheat and maize (Picq et al., 1998; Arias et al., 2003). Moreover, with increasing urbanization, bananas and plantains are becoming more and more important as cash crops, in some cases providing the sole source of income to rural populations. Thus, playing an important role in poverty alleviation. Bananas and plantains are one of the cheapest foods to produce. The cost of production of one kg of plantain is less than that for most other staples, including sweet potato, rice, maize and yam. Consequently, bananas and plantains can be a very cheap food to buy and are, hence, an important food for low-income families (Picq et al., 1998). Bananas and plantains also grow in a range of environments and produce fruit year-round. Thus, it provides energy during the “hungry-period” between crop harvests. They are particularly suited to intercropping systems and to mixed farming with livestock and they are also popular as a backyard crop with urban populations. When grown in perennial production systems, they maintain soil cover throughout the year and if their biomass is used for mulch, soil fertility and organic matter remain stable. In mixed farming systems, bananas are used as a ground shade and nurse-crop for a range of shade-loving crops including cocoa, coffee, black pepper and nutmeg (Picq et al., 1998).

Banana ranks third place in world fruit volume production after citrus fruit and grapes at 64.6 Mt (FAO, 2000), and second place in trade after citrus fruit, at 14.7 Mt

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Also, a relationship between natural production conditions and physical fruit traits (texture and colour) were reported by Bugaud et al. (2007) that the peel of green bananas harvested during the hot humid season was not as hard as that of bananas harvested during the cool dry season. In ripe bananas, a decreasing correlation was also noted between the mean daily temperature and the fruit yellowness. This interaction could be responsible for the yellower colour banana pulp and of bananas harvested during the coolest seasons. The green life of bananas harvested during the hot humid season was shorter than that of bananas harvested during the dry and intermediate seasons (Bugaud et al., 2007).

The effect of high temperature is the function of time in which at 48°C the damage occurred in 3 h and 45 min, at 57°C it took 7 min and at 62°C it took only 1 min (Gowen, 1995). Under peel discolouration, the fruit was observed with air temperature of 33 to 35°C but was associated with high light intensity and moderate to low rainfall. A more widely occurring effect of high temperature is bleaching of the chlorophyll in both leaf and fruits.

Another important effect of temperature is chilling damage during crop development. It includes reddish brown streaking on the skin of the fruit, reduced latex flow, slow colour development of the fruit during ripening, a dull or grayish yellow colour in ripe fruits and brown skin in advanced stages. More subtle changes are reduced production of volatiles during ripening and disturbances to the evolution of CO_2 and ethylene (Gowen, 1995). In addition, Crane et al. (2005) reported that a tem-perature below 16°C but above 0°C resulted in failure of the flowering stalk or fruit bunch to emerge from the pseudostem (called choking) and an increase in fruit rotting during ripening.

Water management

Banana plant must be irrigated immediately after planting and about 30 to 40 weekly irrigation is required. Inadequate irrigation to banana leads to delayed flowering, bunch size, delayed maturity of reduced fingers, induces physiological disorders and also poor keeping quality of fruits (Lopez, 1998). However, excess water also has detrimental quality consequences for plant. The photosynthetic rate decreases with overly high water availability and low transpiration rates. High moisture content in fruit also tends to dilute the soluble solids leading to low flavor intensity. During dry periods, soluble solids accumulate rapidly inside plant structure. When the water supply increases suddenly, by irrigation or rainfall, water moves quickly to the plant cells in response to the osmotic gradient. The rapid rise in hydro-static pressure can rupture cell membranes and walls, leading to splitting of fruits and other structures (Shewfelt and Prussia, 1993). Furthermore, a high relative humidity during fruit development shortens the storage life and increases the incidence of finger drop and crown rotting (Munasque et al., 1990). Generally, the water regime in the early stage of growth had a more pronounced effect both on dry matter accumulation and nutrient uptake than it did after bunch emergence (Gowen, 1995).

Nutrient management

For growth and fruit production, bananas require high amount of nutrients which are often supplied only by the soil. Since large amount of nutrients are removed from the soil and these nutrients have to be replaced to permit continuous production of high yield and this is achieved through application of organic manure and/or mineral fertilizers (Gowen, 1995).

Nitrogen is considered second only to potassium for growth and production. Beyond its effect on growth and production, it also affects bunch maturation period and fruit quality in which as nitrogen level increases the length of the maturation period also increases. In addition, nitrogen also affects bunch weight through its effect on number of hands and fingers, fruit circumference and weight. It also affects fruit quality by reducing total sugars and total soluble solids, and increasing acidity (Gowen, 1995).

On the other hand, potassium is found to be the key element in banana nutrition. It increases both the yield and quality of fruits. High potassium and calcium will give high dry matter and glucose content in the peel and pulp of banana fruit (Caussiol, 2001). Respiration was lower in potassium deficient plants and therefore the main effect of low potassium supply on dry matter production would be through reduction of photosynthesis. On the contrary, low levels of nitrogen, phosphorus and magnesium give high dry matter in the pulp. Low potassium produces thin fruits and fragile bunches. Excess potassium over nitrogen causes a premature ripening of fruits (yellow pulp). In addition, a potassium supply above that which influence growth and yield, changes in reducing and total sugars have reported. As potassium supply increases, the amount of sugar will increase and the acidity decreases. Thus potassium supply has an effect on fruit quality over and above its influence on yield (Gowen, 1995).

Phosphorus requirement of banana is not large as opposed to its demand for potassium and nitrogen, this can be explained by the facts that bananas accumulate the phosphorus they require over extended period of time; and a relatively small quantity of phosphorus is exported with the fruit and that phosphorus is easily redistributed from old to young leaf (Gowen, 1995).

Stage of maturity and ripeness

The bananas are harvested at a specific maturity stage in which the maturity indices are based on the age of the bunch, the interval between flowerings and harvesting, the filling of the fingers or the colour of the skin and pulp

vest, physical injuries, disease incidence, increased temperatures up to 30°C and water stress (Pesis, 2004). On the other hand, ethylene production rates by fresh horticultural commodities are reduced by storage at low temperature, by reduced O_2 levels, and elevated CO_2 levels around the commodity (Irtwange, 2006). Exposure of climacteric fruits to ethylene advanced the onset of an irreversible rise in respiration rate and rapid ripening. Various packages can delay the onset of climacteric and prolong shelf life of fruits by reducing ethylene production and sensitivity.

Ethylene appears to be intimately involved in the initiation of ripening in banana, as in other climacteric fruit, but its mode of action is unknown (Seymour, 1993). Unripe banana show a constant, but low level of ethylene production until the onset of ripening. Ethylene production then increased and is followed by a rise in the rate of respiration. During ripening peak ethylene is normally reached while the rate of respiration is still increasing. As the rate of ethylene production declines, the rate of respiration reaches its maximum at around 125 mg CO_2 Kg^{-1} and then declines slightly, but remains at high level (Seymour, 1993). So to achieve optimum fruit quality, postharvest techniques like the application of controlled atmospheric storage and modified atmospheric packaging are used in order to modulate the physiological processes of ripening of banana fruits.

Transpiration loss

According to the study of Irtwange (2006) transpiration is the evaporation of water from plant tissues. Water loss is a very important cause of produce deterioration, with severe consequences. Water loss is, first, a loss of marketable weight and then adversely affects appearance (wilting and shriveling). Also, the textural quality is reduced by enhanced softening, loss of crispness and juiciness, and reduction in nutritional quality. The nature of the epidermal system of the commodity governs the regulation of water loss that is affected as well by environmental factors. Eventually, transpiration is a result of morphological and anatomical characteristics, surface-to-volume ratio, surface injuries and maturity stage on the one hand, and relative humidity, air movement and atmospheric pressure on the other hand. As a physical process, it can be controlled by applying waxes and plastic films as barriers between the produce and the environment, as well as by manipulating relative humidity, temperature and air circulation.

The banana skin bears stomata and transpiration continues after the bunches has been cut. The magnitude of transpiration depends on temperature and relative humidity and absolute values for it are not worth reporting here for this reason. In trend, however, it shows a very marked relation to ripening which may briefly be described as follows. The green fruit, immediately after cutting, shows an initial fall in transpiration rate and then settles down to a steady state at level depending upon temperature and humidity; at the climacteric there is a sharp peak followed, as the fruit ripens, by the attainment of a new steady state (Simmonds, 1959).

There is usually a final rise in water loss which is related to degenerative changes of the skin caused by fungal attack; since the skin in this stage is senescent the loss can hardly be described as transpiration. These facts were established for individual fruits; because the bunches ripens progressively from the top hand to the bottom, the climacteric transpiration peaks of individual fingers are concealed and whole bunches (or bigger bulks of fruit) show simply a steady rising curve of water loss during ripening (Simmonds, 1959).

POSTHARVEST BIOCHEMISTRY OF BANANA

The ripening process, at the fruit level involves several biochemical pathways like degradation of starch to sugar, change in the peel and pulp colour, cell wall changes, change in the concentration of volatiles and acids (Gowen, 1995).

Texture

Most fruit soften during ripening and this is a major quality attribute that often dictates shelf life. Fruit softening could arise from one of the three mechanisms: Loss of turgor; degradation of starch; or breakdown of the fruit cell walls. Loss of turgor is largely a non-physiological process associated with the postharvest dehydration of the fruit, and as such can assume commercial importance during storage. Degradation of starch probably results in a pronounced textural change, especially in those fruit like banana, where starch accounts for a high percentage of the fresh weight (Tucker, 1993; Turner, 2001).

Carbohydrates

Starch forms about 20 to 25% of the fresh weight of the pulp of unripe bananas. During ripening this starch is degraded rapidly and the sugars sucrose, glucose and fructose accumulate; traces of maltose may also be present. Sugars are present in the green fruit only very small amounts, average about 1 to 2% of the fresh pulp; they increase to 15 to 20% at ripeness, the beginning of the increase coinciding with the respiration climacteric. Starch disappears concurrently, dropping from about 20 to 25% in the green fruit to about 1 to 2% in the ripe fruit; it is higher in the ripe plantain (6%) than in the dessert bananas (Simmonds, 1959). In the banana pulp sucrose is the predominant sugars, at least at the start of ripening, and its formation precedes the accumulation of glucose and fructose. The peel tissue also contains starch, about 3% fresh weight, and appears to show similar changes in

carbohydrate during ripening. These characteristic patterns of carbohydrate metabolism can be altered under certain environmental conditions such as exposure to elevated temperatures during ripening (Seymour, 1993; Turner, 2001).

Pigments

There is a decrease in chlorophyll content from between 50 to 100 µg per gram fresh weight to almost zero in ripe fruit, while caroteniod levels (xanthophylls and carotene) remained approximately constant at 8 µg per gram fresh weight. There is reduction in total caroteniod content in the peel during the early stage of ripening followed by caroteniod biosynthesis at the yellow-green to yellow-ripe stage (Seymour et al., 1987).

Cell wall change

Softening of fruits appears to be closely linked with changes in their cell walls structures. In bananas, the changes in texture of the fruit during ripening probably result from alterations in both cell wall structure and the degradation of starch (Seymour, 1993).

Hemicellulose and pectin are components of cell wall and their concentration during ripening follows a trend similar to that of starch, dropping from 7 to 8% of the fresh pulp in the green fruit to about 1% at ripeness. There are indications of interconversion of starch and hemicellulose in the early phases of storage of green fruit. In behavior, however there is an important difference from starch, for the hemicellulose disappear whether or not the fruit is normally ripened; in fruit kept in prolonged cool storage, starch concentration declines slowly so that a high starch content is characteristic of “chilled” fruit until a very advanced stage of ripeness. The hemicelluloses, however, disappear at the normal rate under such treatment and their fate is still mysterious and they may be connected with loss of dry weight of the fruit which, it will be remembered, excess the loss that can be accounted for by respiration (Simmonds, 1959).

Organic acids

Pulp pH and total titratable acidity are important postharvest quality attributes in the assessment of fruit ripening quality. Generally, when fruits are harvested at matured green stage, the pulp pH is high but as ripening progresses pH drops. Thus, the pulp pH could be used as an index of ripening (Dadzie and Orchard, 1997). The skin of the fruit shows similar trend but is slightly delayed with respect to the pulp; this is not surprising in view of the fact that ripening proceeds from the core of the pulp outwards. Various measurements of pH range between about 5 to 5.8 for the pulp of the green fruit and between about 4.2 and 4.8 for post climacteric fruit (Simmonds, 1959).

The levels of organic acids in a fruit can markedly affect its taste. Generally, in banana the pulp shows an increase in acidity during ripening and the main organic acid present are malic, citric and oxalic. As ripening advances, acidity declines presumably due to the utilization as respiratory substrates. The astringent taste of unripe bananas is probably attributed at least partly to their oxalic acid content, which undergoes significant decarboxylation during ripening probably by the action of oxalate oxidase (Seymour, 1993). Despite the increase in acid with ripening, is less that the rate of increase of sugars and total soluble solids (Gowen, 1995; Abbas et al., 2012).

Enzyme activity

Banana fruit contains several hydrolytic and oxidative enzymes. The relative activities of α-mylase, starch phosphorylase, acid phosphate, and catalase increased considerably in banana fruits stored for 5 weeks at 20°C. The stage of maturity of banana fruit at harvest significantly influenced most of the physical and biochemical constituents and the activities of some enzymes. Banana fruits harvested at mature or early mature stage had a longer storage period and better quality. The potential storage life of banana fruits decreased with an advance in stage of maturity (Salunkhe and Kadam, 1995).

POSTHARVEST MICROBIOLOGY OF BANANA

Postharvest diseases can cause serious losses of fruits both in terms of quantity and quality. Fruits infected with disease have no market value. There are many postharvest diseases of banana, cooking banana and plantain. Among these important ones are crown rot, anthracnose and cigar-end rot (Dadzie and Orchard, 1997).

Crown rot

Crown rot is one of the most important postharvest diseases of banana/plantain. It is characteristically a disease complex caused by several fungi, sometimes in association with other micro-organisms such as bacteria. The most common pathogens associated with crown rot are *Colletotrichium musae* (*Gloesporium musarum), Fusarium roseum, Fusarium semitectium and Botryodiplodia theobromae.* When the hands are cut from the stems, the massive open wound is an ideal weak spot for crown rot fungi to enter and grow. Fungal spores on the fruit in the field are carried along (after harvesting bunch) to the packing house. Spores follow the fruit right into delatexing baths, where they are drawn deeply into the weak spot, the wound on the crown tissue (due to dehanding). Spores also remain on the outside of the fruit and are packed (Dadzie and Orchard, 1997; Dionisio, 2012). Symptoms of crown rot include softening and blackening

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Risk assessment of genetically modified sugarcane expressing *AVP1* gene

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ABSTRACT

Biosafety is a multidisciplinary approach that encompasses social, societal, ethical issues and policies for the regulations of genetically modified (GM) organisms. The potential health risks associated with GM sugarcane containing *AVP1* gene confers resistance against drought and salinity were evaluated by animal feeding studies and some genotoxicity assays. Acute and sub-chronic toxicity examinations were carried out via oral dose administration of GM sugarcane juice supplemented with the normal diet (modified from certified rodent standard diet) on Wistar rats. AVP1 protein concentration in sugarcane juice was 1mg/1 mL. Biochemical, haematological blood analyses were performed and the results revealed that there were non-significant differences among all the treatment groups; GM sugarcane juice, non-GM sugarcane juice and the control group (normal diet and water). Genotoxicity assessment based on the comet assay and the micronucleus assay data exhibited that *AVP1* GM sugarcane was not genotoxic or cytotoxic in rat's peripheral blood. These research findings supported the conclusion that GM *AVP1* sugarcane was non-toxic in experimental animals. Therefore, data generated through this research work would be helpful for the commercial release of GM *AVP1* sugarcane.

1. Introduction

Sugarcane is one of the most important receptors of solar energy, which is converted to fermentable sugars and fibres (FAO, 1988). Although unprocessed sugarcane juice is normally used to produce crystalline sugars, sometimes sugarcane juice (extracted from pressed cane) is used as a beverage (owing to its nutritional importance) in many countries particularly where it has been commercially developed such as Pakistan, India, Southeast Asia, Egypt, and Latin America. The evaporated sugarcane juice as a component in prepared beverages and food represents a sugar (sweetener) derivative of cane syrup (FDA, 2009). Sugarcane juice is sold nationally among the street sellers in Indonesia and Malaysia, whereas in Singapore it is sold in food courts only (Satran, 2013). It is a national drink of Pakistan.

Genetically modified (GM) crop plants have been developed and grown on a commercial scale on approximately 1.96 billion hectares during the past 19 years in 28 countries of the world (James et al., 2015). Food and feed obtained from GM crops must be assessed for potential harm to animals, humans, and the environment prior to commercial release (Domingo, 2016). The methods of safety/risk assessment are referred to as comparative safety assessments (Bartholomaeus et al., 2013; Codex Alimentarius Commission, 2009). Biosafety assessments differentiate GM crops from their parental counterparts and this process is based on a "weight of evidence" law where new information is available regarding potential distinctions and their impact on food or feed safety (Cockburn, 2002; Herman et al., 2009; Herman and Price, 2013; König et al., 2004; Kuntz and Ricroch, 2012; Parrott et al., 2010; Ricroch, 2013). However, the variations do not automatically mean that any harmful effects would result. If deviations are identified then the likelihood that they would potentially harm the animal or human health must be thoroughly assessed (Garcia-Alonso, 2010).

Transgenic sugarcane expressing the *Arabidopsis* vacuolar proton-pump pyrophosphatase (*AVP1*) gene has been developed at the National Institute for Biotechnology and Genetic Engineering (NIBGE) and sugarcane plants expressing higher levels of *AVP1* are more tolerant to drought and salinity (Raza et al., 2016).

GM crops expressing a particular protein resulting from a genetic alteration are evaluated via acute (short-term) and chronic (long-term repeated dose) experiments on animals (Bartholomaeus et al., 2013; Delaney et al., 2008b; EFSA, 2008; Hammond and Cockburn, 2008; Rice et al., 2007). Acute (short-term) toxicity studies performed (OECD, 2001) with single or multiple doses administration of a purified protein in a short period of time not exceeding from 24 h. Sub-chronic (long-

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term repeated dose) rodent feeding studies of GM crops recommended (FAO/WHO, 1996) to determine if there are any harmful effects based on any toxic health effect, potential allergenicity/animal studies, nutritional compositional changes and inserted gene integrity (Codex Alimentarius Commission et al., 2003; WHO, 1995). Different ways to identify the risks/adverse effects associated with GM crops have been documented (EFSA, 2008). Various genetically altered crops have been assessed by these methods, such as tomato (Noteborn et al., 1995), soybean (Appenzeller et al., 2008; Zhu et al., 2004), rice (Poulsen et al., 2007; Poulsen et al., 2007a; Schrøder et al., 2007; Wang et al., 2002), maize (Hammond et al., 2004, 2006a; He et al., 2008; MacKenzie et al., 2007; Malley et al., 2007), and cotton (Dryzga et al., 2007).

Genotoxicity assays have been performed to identify DNA reactive molecular compounds (Maluszynska, 2005) through both *in vitro* and *in vivo* means. These assays are designed to recognize substances that cause mutation, DNA damage, and chromosomal aberrations. The substances have proven to be genotoxic (positive) after experimentation are considered carcinogens (may stimulate cancer cell proliferation) or mutated by interrupting DNA molecules (Auffan et al., 2006; Colognato et al., 2008; Fenech, 2008). The GM plants are strictly regulated in the form of an organised network. For example, in the United States of America, the Food and Drug Administration (FDA) works with federal agencies, including the Environmental Protection Agency (EPA) and the United States Department of Agriculture (USDA) that demand full risk assessment evaluation studies for newly discovered chemical active ingredients or GM food before their commercialization. In Pakistan, GM crops are regulated by the National Biosafety Committee (NBC) which emphasizes that genetically modified organisms (GMOs) should be properly evaluated both for toxicity and allergenicity following the Cartagena Protocols on Biosafety (World Health Organization, 2017).

The comet assay (single-cell gel electrophoresis) is a technique for the evaluation of direct DNA damage by the exposure of any chemical compound, radiation, or recombinant protein in any eukaryotic organism. When it was first introduced (Ostling and Johanson, 1984), the name “Comet” was used because the images produced from the assay resemble a comet with a discrete head consisting of intact DNA and a smear of damaged DNA giving the impression of the tail of a comet. The amount of DNA in the comet tail is directly proportional to the amount of DNA damage.

A micronucleus test is a tool for the genotoxicity assessment of a potentially toxic chemical or compound (a carcinogen) caused by genetic damage both *in vivo* and *in vitro* means (OECD, 2010). It is an inexpensive, simple, rapid, and reliable method. A micronucleus is a third (erratic) nucleus, which is produced during anaphase of mitosis or meiosis. Micronuclei were first used to measure chromosomal damage (Evans et al., 1959) in root tips of *Vicia faba* (Heddle, 1973; Schmid, 1975); thereafter, further researchers (Jaszczak et al., 2008) performed a micronucleus test and comet assay in mice fed on genetically modified (GM) triticale (bar transgene).

The present research was conducted to assess the risks associated with GM *AVP1* sugarcane based on acute and sub-chronic toxicity studies in Wistar rats. Biochemical, haematological analyses and genotoxicity assays were performed which revealed that GM *AVP1* sugarcane did not exhibit any toxic effect on Wistar rats.

2. Materials and methods

2.1. Maintenance of laboratory animals

Wistar albino rats (male and female) aged 6–8 weeks were obtained from the National Institute for Health (NIH) and acclimatized (22 ± 3°C; 12 h light and 12 h dark; 60% relative humidity) for one week in properly labelled polypropylene cages in the animal room at NIBGE prior to the start of experiment. They were fed on a modified pelleted diet (modified from certified rodent diet 5002*) and drinking water *ad libitum*. Paddy husk (Al-Murtaza Wood Crafts, Jhang Road, Faisalabad, Pakistan) was used as bedding material and was changed twice a week. The selection of rats was made because of their similarity in various cellular, enzymatic functions, and 95% genome identity with human beings.

2.2. Nutritional evaluation of GM and non-GM sugarcane juice

The nutritional evaluation was performed for both non-transgenic and transgenic *AVP1* sugarcane juice. The percentage values of proximates were calculated in different laboratories of NIBGE and Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan.

2.3. Extraction, elution and quantification of AVP1 protein from AVP1 GM sugarcane juice

AVP1 protein was extracted from *AVP1* sugarcane juice according to the procedure of Cullis et al. (2014). The specific AVP1 protein from the crude mixture was run in an SDS-PAGE (10% and stained with Coomassie Brilliant Blue) and the exact 85 kDa band of AVP1 protein was excised from the gel and eluted by following the Thermo Fisher Scientific procedures (Thermo Fisher Scientific, 2009). The eluted specific AVP1 protein was quantified using the standard Bradford's method, where the concentration (1 mg/mL) of GM *AVP1* protein in sugarcane juice was determined (Bhattacharyya et al., 2007; Bradford, 1976).

2.4. Acute toxicity studies of GM AVP1 sugarcane juice

Male and female Wistar albino rats (200–250 g) were selected for a two-week study with GM *AVP1* sugarcane juice. Animals were randomly distributed in three treatment groups consisting of 10 animals per each group (5 male and 5 female). Five animals were maintained per cage. They were supplied with standard rodent feed (5002*) and water *ad libitum* (Bhattacharyya et al., 2007). Animals of all the treatment groups were administered to repeated doses (after 24 h) of 0, 15 mL (30% juice), and 30 mL (60% juice)/body weight/day or 0, 1050 and 2100 mg/kg body weight respectively with *AVP1* GM and non-GM sugarcane juice for 14-day. Group 1 (G1) animals were dosed with GM *AVP1* sugarcane juice, animals of group 2 (G2) were dosed with non-GM sugarcane juice and group 3 (G3) animals were fed with normal rodent diet and water, serving as the control. GM and non-GM sugarcane fresh juice were mixed in distilled water according to their daily consumption (35 mL water + 15 mL juice for 30% and 20 mL water + 30 mL juice for 60%). The animals were observed for 48 h carefully, after hourly intervals, and after every 12 h till the day of sacrifice. The following physical examinations such as skin and eye irritation, urination, salivation corneal reflex, spontaneous responses in behavioural or autonomic activities mortality were observed (OECD, 2001).

2.5. Sub-chronic toxicity studies of GM AVP1 sugarcane juice

A 90-day sub-chronic study on Wistar albino rats (male and female) was conducted according to OECD guidelines 408 to assess the effect of fresh GM *AVP1* sugarcane fresh juice (harvested and crushed daily). Wistar rats approximately 200–250 g of weight were assigned to three groups of 10 animals (5 males and 5 females). The juice of GM *AVP1* sugarcane plants was used throughout this study. One selected dose of 12 mg or 12 mL (24% juice)/body weight/day or < 5000 mg/kg body weight and two control treatments, one with non-GM sugarcane (12 mL/kg body weight/day) and the other with distilled water were administered orally. GM and non-GM sugarcane fresh juice were mixed in distilled water according to their daily consumption (38 mL water + 12 mL juice). Fresh rodent pelleted modified diet was provided daily as required. Animals were observed daily to note any changes in behavioural or autonomic activities (irritation, urination, salivation corneal reflex, spontaneous responses and mortality).

2.6. Body weights and food consumption

Body weight and food consumption (g) were recorded daily during the first week of the exposure time and weekly thereafter.

2.7. Haematology and blood biochemistry

Blood samples were collected from the external jugular vein at the end of the experiment (EDTA-coated and Heparin vacutainers for biochemical and haematological, evaluations respectively). The blood samples were sent to a clinical pathology laboratory (PINUM, ISO 9001–2008 certified hospital, Faisalabad) for analyses. Haematological parameters such as haemoglobin, erythrocyte sedimentation rate (ESR), total lymphocyte count (TLC), differential leukocyte count (DLC), neutrophils, lymphocytes, monocytes and eosinophils were observed. Biochemical parameters such as blood sugar, blood urea, serum creatinine, serum uric acid, cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, total bilirubin, direct bilirubin, indirect bilirubin, alanine aminotransferase (ALT or SGPT), aspartate aminotransferase (AST or SGOT), alkaline phosphatase, serum proteins, serum albumin, serum globulin, albumin to globulin ratio were analyzed.

2.8. Relative organ weight

At the end of the experiment, the animals were euthanized. Various organs such as the heart, lungs, liver, spleen, kidneys, gonads and brain were carefully dissected out and weights were recorded. The relative organ weight of each animal was calculated as

$$\text{Relative organ weight (\%)} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat on sacrifice day (g)}} \times 100$$

2.9. Histopathological examination

Rat liver and kidney tissue samples were fixed in 15% neutral formalin and organised for histopathological examination. Risen embedded sections were stained with toluidine blue in 1% boric acid for microscopic examination at 10X and 100X magnification.

2.10. Genotoxicity assessment

Genotoxicity was tested by performing the comet assay (DNA damage) and the micronucleus test (cytotoxicity).

3. Statistical analyses

Statistical analyses were performed with SigmaPlot 13.0 (SYSTAT software). Data were analyzed using ANOVA, to account for differences in data distribution and variance. The pairwise comparison was made using Tukey's test (Tukey, 1993).

4. Results

The rat's diet was nutritionally balanced and there were no obvious diet-related toxic effects observed in all treatment groups. GM and non-GM sugarcane juice proximate nutritional evaluations showed non-significant differences as presented in Table 1.

4.1. Weekly body weight in acute toxicity test

No mortalities were observed during the period of 14-day acute toxicity evaluations. There were no statistically significant differences in the mean body weights and food consumption data of both male and female rats after 14-day oral administration of GM *AVP1* sugarcane juice at the dose of 2100 mg/kg body weight (Fig. 1A and B respectively).

Table 1
Nutritional composition analyses of *AVP1* GM and non-GM sugarcane juice.

Proximates	GM sugarcane juice	Non-GM sugarcane juice
Protein (%)	0.45 ± 0.01	0.45 ± 0.02
Ash (%)	0.43 ± 0.02	0.43 ± 0.01
Moisture content (%)	78.67 ± 1.53	79.00 ± 1.00
Crude fat (%)	0.81 ± 0.02	0.81 ± 0.01
Crude fibre (%)	6.20 ± 0.20	6.17 ± 0.12
Brix content (%)	23.93 ± 1.01	24.00 ± 0.20
Total dry matter (%)	19.97 ± 0.15	20.10 ± 0.20
pH	5.60 ± 0.10	5.60 ± 0.10
Sucrose (%)	32.10 ± 0.20	32.03 ± 0.15
Glucose (%)	1.59 ± 0.02	1.57 ± 0.03
Fructose (%)	2.41 ± 0.01	2.40 ± 0.01
Sodium (%)	0.27 ± 0.06	0.27 ± 0.06
Potassium (%)	0.03 ± 0.01	0.02 ± 0.01
Calcium (%)	0.02 ± 0.01	0.02 ± 0.01
Magnesium (%)	0.02 ± 0.01	0.01 ± 0.01
Iron (%)	1.10 ± 0.10	1.07 ± 0.06
Zinc (%)	1.17 ± 0.06	1.13 ± 0.06
Chromium (%)	0.03 ± 0.01	0.03 ± 0.01
Lead (%)	0.01 ± 0.01	0.01 ± 0.01
Copper (%)	0.17 ± 0.01	0.18 ± 0.01
Manganese (%)	1.43 ± 0.01	1.43 ± 0.02

Values presented (Mean ± SE) for GM and non-GM sugarcane juice.

Values presented (mean ± SD) body weight of male rats. (G1): rats fed with GM sugarcane juice, (G2): rats fed with non-GM sugarcane juice, (G3): rats fed with water and normal diet. Significance checked at $P \leq 0.05$.

4.2. Weekly body weight in sub-chronic toxicity studies

The male and female maximum body weight gains were up to 364.6 g and 270 g respectively and there were non-significant variations ($P \geq 0.05$) among all the treatment groups under sub-chronic (90-day) toxicological evaluations. The overall weights of male rats were greater than the female that was considered negligible regarding any adverse effect (Fig. 2A and B respectively).

Values presented (mean ± SD) body weight of male rats. (G1): rats fed with GM sugarcane juice, (G2): rats fed with non-GM sugarcane juice, (G3): rats fed with water and normal diet. Significance checked at $P \leq 0.05$.

4.3. Weekly food consumption during sub-chronic toxicity study

Weekly food consumption of male and female rats under sub-chronic toxicity studies was increased with time and correlated with body weight in all treatment groups (Fig. 3A and B respectively) with non-significant variations ($P \leq 0.05$). Male and female rats consumed an average of 27.08 and 25.7 g of fresh pelleted diet, respectively.

Values presented (mean ± SD) body weight of male rats. (G1): rats fed with GM sugarcane juice, (G2): rats fed with non-GM sugarcane juice, (G3): rats fed with water and normal diet. Significance checked at $P \leq 0.05$.

4.4. Weekly water consumption during sub-chronic toxicity study

Weekly water consumption was consistent throughout the sub-chronic toxicity studies both in male and female Wistar rats (supplementary data).

4.5. Biochemical and haematological analyses after acute and sub-chronic toxicity

There were no statistically significant ($P \geq 0.05$) differences observed in the mean values of measured clinical chemistry variables

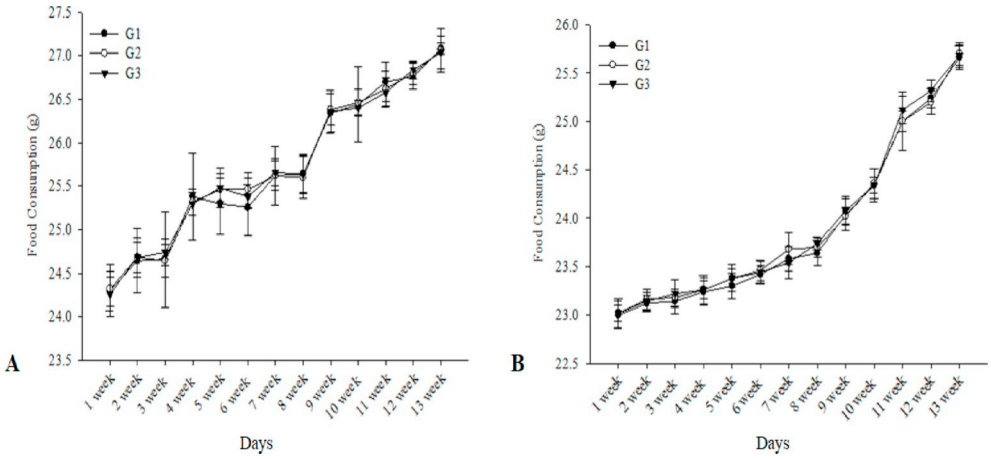

Fig. 3. Weekly food consumption (g) male (A) and female (B) Wistar rats after sub-chronic toxicity studies.

Table 2
Biochemical and haematological analyses for male Wistar rats after 14-day acute toxicity.

Biochemical Parameters				
	Units	G1	G2	G3
Blood Sugar	(mg/dL)	70.20 ± 5.54[a]	70.60 ± 12.22[a]	69.80 ± 9.44[a]
Blood Urea	(mg/dL)	40.00 ± 3.81[a]	41.40 ± 2.88[a]	40.10 ± 0.74[a]
Serum Creatinine	(mg/dL)	0.60 ± 0.07[a]	0.64 ± 0.05[a]	0.66 ± 0.09[a]
Serum Uric Acid	(mg/dL)	1.66 ± 0.29[a]	1.63 ± 0.38[a]	1.61 ± 0.07[a]
Cholesterol	(mg/dL)	64.60 ± 4.34[a]	65.40 ± 4.51[a]	62.80 ± 0.84[a]
Triglycerides	(mg/dL)	73.00 ± 16.64[a]	74.00 ± 2.45[a]	73.80 ± 5.93[a]
HDL Cholesterol	(mg/dL)	23.20 ± 1.30[a]	22.40 ± 1.62[a]	22.90 ± 4.72[a]
LDL Cholesterol	(mg/dL)	38.00 ± 6.20[a]	38.60 ± 3.05[a]	39.50 ± 0.50[a]
Total Bilirubin	(mg/dL)	1.02 ± 0.19[a]	1.08 ± 0.19[a]	1.17 ± 0.08[a]
Direct Bilirubin	(mg/dL)	0.22 ± 0.08[a]	0.22 ± 0.04[a]	0.25 ± 0.05[a]
Indirect Bilirubin	(U/L)	0.77 ± 0.15[a]	0.80 ± 0.18[a]	0.78 ± 0.08[a]
SGPT	(U/L)	5.20 ± 0.84[a]	5.40 ± 0.89[a]	5.20 ± 0.84[a]
SGOT	(U/L)	3.20 ± 0.84[a]	3.200.45[a]	3.52 ± 0.48[a]
Alkaline Phosphatase	(mg/dL)	483.60 ± 94.95[a]	481.00 ± 88.99[a]	482.80 ± 63.82[a]
Serum Proteins	(mg/dL)	7.08 ± 0.43[a]	7.02 ± 0.13[a]	7.05 ± 0.11[a]
Serum Albumin	(mg/dL)	3.24 ± 0.09[a]	3.18 ± 0.22[a]	3.12 ± 0.04[a]
Serum Globulin	(mg/dL)	3.86 ± 0.34[a]	3.88 ± 0.19[a]	3.87 ± 0.16[a]
A/G Ratio		0.78 ± 0.04[a]	0.78 ± 0.10[a]	0.77 ± 0.04[a]
Haematological Parameters				
Haemoglobin	(g/dL)	16.60 ± 0.35[a]	16.18 ± 0.19[a]	16.32 ± 0.31[a]
ESR	(mm/h)	2.60 ± 0.55a	2.02 ± 0.04[a]	2.00 ± 0.00[a]
TLC	(mcL)	8300.00 ± 692.82[a]	8360.00 ± 1566.21[a]	8360.00 ± 1757.27[a]
Neutrophils	(%)	15.80 ± 3.03[a]	15.20 ± 0.84[a]	15.86 ± 3.57[a]
Lymphocyte	(%)	77.80 ± 7.19[a]	77.40 ± 4.22[a]	77.60 ± 3.58[a]
Monocytes	(%)	1.12 ± 0.16[a]	1.16 ± 0.47[a]	1.22 ± 0.44[a]
Eosinophils	(%)	1.66 ± 0.65[a]	1.66 ± 0.30[a]	1.60 ± 0.38[a]

Values presented mean ± SD. Significance checked at $P \leq 0.05$. (G1): group 1 rats fed with GM sugarcane juice, (G2): rats of group 2 fed with non-GM sugarcane juice, (G3): rats of group 3 fed with water and normal diet (n = 10). Similar alphabets represented non-significant variation among different treatment groups.

from 6.72% to 6.74% whereas in non-transgenic sugarcane juice and the control (normal feed) it was ranged from 6.72 to 6.74% both in female and male rats respectively. The micronuclei frequency after the 90-day feeding of transgenic sugarcane juice was ranged from 7.70% to 7.72% whereas in non-transgenic sugarcane and in the control (normal diet) it was 7.72% both in male and female Wistar rats respectively. The micronuclei frequency in blood erythrocytes revealed that there were non-significant ($P \geq 0.05$) signs of toxicity among all the treatment groups (supplementary data).

The results of the comet assay and micronuclei assays revealed that feeding *AVP1* GM sugarcane didn't show any cytotoxicity or DNA damage in the peripheral blood of Wistar rats fed with transgenic sugarcane juice.

5. Discussion

GM crops are subjected to risk assessment for the safety of animals and humans owing to their consumption as feed and food respectively (Delaney et al., 2018; Ferrante and Conti, 2018). The methods and data have been developed over many years for the safety assessment studies by various international environmental protection authorities such as the Organization for Economic Cooperation and Development (OECD), Food and Agriculture Organization (FAO) and World Health Organization (WHO) (Garcia-Alonso, 2013). In the current research, potentially toxic and genotoxic risks effects associated with transgenic *AVP1* sugarcane were assessed. Similarly, Badawy et al. (2008) carried out risk assessment studies on GM sugarcane (insect resistant) by following the international standard protocols of testing.

Although, there are some intended benefits of GM crops, their safety

Table 5
Biochemical and haematological data for female Wistar rats after 90-days sub-chronic toxicity.

Biochemical Parameters				
	Units	G1	G2	G3
Blood Sugar	(mg/dL)	71.00 ± 5.87[a]	70.40 ± 4.62[a]	70.00 ± 1.87[a]
Blood Urea	(mg/dL)	49.20 ± 11.26[a]	49.60 ± 2.41[a]	48.82 ± 2.93[a]
Serum Creatinine	(mg/dL)	0.62 ± 0.04[a]	0.60 ± 0.01[a]	0.60 ± 0.01[a]
Serum Uric Acid	(mg/dL)	2.26 ± 0.22[a]	2.21 ± 0.53[a]	2.23 ± 0.28[a]
Cholesterol	(mg/dL)	102.00 ± 22.31[a]	101.20 ± 7.60[a]	103.40 ± 5.03[a]
Triglycerides	(mg/dL)	112.60 ± 1.67[a]	113.40 ± 5.13[a]	113.95 ± 2.66[a]
HDL Cholesterol	(mg/dL)	31.40 ± 4.88[a]	30.00 ± 1.58[a]	30.30 ± 1.57[a]
LDL Cholesterol	(mg/dL)	54.00 ± 12.92[a]	55.00 ± 3.32[a]	55.10 ± 3.47[a]
Total Bilirubin	(mg/dL)	0.75 ± 0.14[a]	0.74 ± 0.13[a]	0.74 ± 0.04[a]
Direct Bilirubin	(mg/dL)	0.23 ± 0.04[a]	0.23 ± 0.04[a]	0.23 ± 0.04[a]
Indirect Bilirubin	(U/L)	0.52 ± 0.11[a]	0.52 ± 0.09[a]	0.52 ± 0.11[a]
SGPT	(U/L)	5.37 ± 0.41[a]	5.38 ± 1.06[a]	5.37 ± 0.55[a]
SGOT	(U/L)	7.40 ± 2.19[a]	7.42 ± 1.53[a]	7.40 ± 0.38[a]
Alkaline Phosphatase	(mg/dL)	645.60 ± 20.96[a]	646.20 ± 171.99[a]	645.30 ± 31.22[a]
Serum Proteins	(mg/dL)	7.69 ± 0.26[a]	7.68 ± 0.24[a]	7.67 ± 0.06[a]
Serum Albumin	(mg/dL)	3.57 ± 0.38[a]	3.56 ± 0.50[a]	3.57 ± 0.07[a]
Serum Globulin	(mg/dL)	4.13 ± 0.59[a]	4.12 ± 0.44[a]	4.13 ± 0.04[a]
A/G Ratio		0.92 ± 0.07[a]	0.91 ± 0.08[a]	0.92 ± 0.05[a]
Haematological Parameters				
Haemoglobin	(g/dL)	15.02 ± 0.23[a]	15.04 ± 0.88[a]	15.00 ± 0.81[a]
ESR	(mm/h)	3.37 ± 0.71[a]	3.33 ± 0.41[a]	3.26 ± 0.54[a]
TLC	(mcL)	10120 ± 3455[a]	10108 ± 678.47[a]	10145 ± 1218.40[a]
Neutrophils	(%)	20.40 ± 2.07[a]	20.40 ± 1.82[a]	21.80 ± 3.11[a]
Lymphocyte	(%)	73.00 ± 0.71[a]	73.20 ± 1.48[a]	73.60 ± 6.11[a]
Monocytes	(%)	4.56 ± 1.04[a]	4.56 ± 0.43[a]	4.54 ± 0.36[a]
Eosinophils	(%)	3.29 ± 0.85[a]	3.26 ± 0.43[a]	3.28 ± 0.81[a]

Data presented mean ± SD. Significance tested at $P \leq 0.05$. (G1): group 1 animals fed with GM sugarcane juice, (G2): animals of group 2 fed with non-GM sugarcane juice and (G3): animals of group 3 fed with water and normal diet (n = 10). Similar letters represented non-significant variation among different treatment groups.

assessment related to nutritional and toxicological evaluations are required prior to commercial release. A survey report (summarising 147 agronomical studies) has been published based on the performances of GM crops at various geographical regions in different countries predict that the benefits of biotech crops have increased the farmer's profit by 68% and the yield has been risen up to 22%. Although the seed cost of GM varieties can be higher, the farmer gets extensive benefits and that is the major reason for selecting biotech crops over the conventionally bred lines (Klümper and Quaim, 2014). So, there is a necessity to increase crop yield by 70% especially in the developing countries to fulfil the demand of food for increasing world's population, So, there is the need to cultivate the crops with novel traits and better performances (Delaney, 2015). The Product Biosafety Commission (KKHPRG), Indonesia (James, 2013) and the National Biosafety Technical Commission (CTNBio), Brazil (ISAAA, 2017) approved the GM drought tolerant and insect resistant sugarcane respectively, for commercialization.

The main goal of the risk assessment studies of GM *AVP1* sugarcane was to provide the scientific information that demonstrates it would not cause any toxic effect when consumed as a food. This enables the risk managers to determine if any additional measures are needed when making a well informed decision. Proximate compositional analyses from sugarcane juice revealed that the GM *AVP1* sugarcane was substantially equivalent to its non-GM counterpart. Pelleted rodent diets were nutritionally balanced for all dosed groups as reported by Delaney et al. (2014). The measured quantity of dose was orally administered for 90-days to the experimental animals according to the 90-day sub-chronic (OECD, 1988) and 14 days sub-acute toxicity guidelines (OECD, 2001), in accordance with studies recommended in research areas (FAO/WHO, 1996).

The Implementing Regulation (EU) needs a mandatory 90-day rodent feeding study as a part of the toxicological assessment in order to identify potential risks associated with GM food, even when no particular risk hypothesis might be expected. The European Food Safety Authority (EFSA) incorporate the recommendations into a legal text (EFSA, 2011). The results obtained from the current sub-acute and sub-chronic toxicological assessment in Wistar rats indicated that there

Table 6
Relative organ weights (%) of rats (male and female) after two weeks of oral administration of *AVP1* GM sugarcane juice, non-GM sugarcane juice and normal diet treatments.

Groups	G1		G2		G3	
Organ	Male	Female	Male	Female	Male	Female
Heart	0.29 ± 0.02[a]	0.31 ± 0.03[a]	0.28 ± 0.03[a]	0.30 ± 0.03[a]	0.27 ± 0.02[a]	0.30 ± 0.03[a]
Lungs	0.57 ± 0.07[a]	0.60 ± 0.08[a]	0.56 ± 0.03[a]	0.60 ± 0.06[a]	0.55 ± 0.11[a]	0.61 ± 0.08[a]
Liver	2.92 ± 0.11[a]	3.01 ± 0.31[a]	2.86 ± 0.28[a]	3.03 ± 0.48[a]	2.74 ± 0.27[a]	2.97 ± 0.70[a]
Spleen	0.16 ± 0.02[a]	0.19 ± 0.03[a]	0.16 ± 0.04[a]	0.18 ± 0.02[a]	0.16 ± 0.02[a]	0.17 ± 0.03[a]
Kidney	0.65 ± 0.05[a]	0.69 ± 0.14[a]	0.65 ± 0.07[a]	0.69 ± 0.11[a]	0.63 ± 0.05[a]	0.67 ± 0.04[a]
Gonads	1.07 ± 0.08[a]	0.07 ± 0.01[b]	1.03 ± 0.12[a]	0.07 ± 0.01[b]	0.94 ± 0.18[a]	0.07 ± 0.01[b]
Brain	0.65 ± 0.02[a]	0.71 ± 0.02[b]	0.65 ± 0.08[a]	0.70 ± 0.06[b]	0.63 ± 0.07[a]	0.68 ± 0.05[b]

Data presented mean ± SD values for each organ relative to body weight (%). Similar superscripts for each organ relative weight presented the non-significant differences ($P \geq 0.05$) and vice versa among all the treatment groups (male and female). (G1): group 1 animals fed with GM sugarcane juice, (G2): animals of group 2 fed with non-GM sugarcane juice and (G3): animals of group 3 fed with water and normal diet.

Table 7
Relative organ weights (%) of rats (male and female) after 90-days oral administration of *AVP1* GM sugarcane juice, non-GM sugarcane juice and normal diet treatments.

Groups	G1		G2		G3	
Organ	Male	Female	Male	Female	Male	Female
Heart	0.21 ± 0.04[a]	0.28 ± 0.02[a]	0.21 ± 0.01[a]	0.28 ± 0.05[a]	0.21 ± 0.04[a]	0.28 ± 0.03[a]
Lungs	0.56 ± 0.01[a]	0.76 ± 0.08[a]	0.55 ± 0.11[a]	0.75 ± 0.04[a]	0.55 ± 0.09[a]	0.75 ± 0.05[a]
Liver	3.05 ± 0.32[a]	4.18 ± 0.30[a]	3.05 ± 0.26[a]	4.17 ± 0.10[a]	3.05 ± 0.17[a]	4.16 ± 0.16[a]
Spleen	0.15 ± 0.02[a]	0.21 ± 0.01[a]	0.15 ± 0.01[a]	0.21 ± 0.01[a]	0.15 ± 0.01[a]	0.20 ± 0.01[a]
Kidney	0.53 ± 0.04[a]	0.71 ± 0.03[a]	0.53 ± 0.02[a]	0.71 ± 0.02[a]	0.52 ± 0.01[a]	0.72 ± 0.02[a]
Gonads	0.74 ± 0.03[a]	0.06 ± 0.01[b]	0.74 ± 0.02[a]	0.06 ± 0.00[b]	0.73 ± 0.01[a]	0.07 ± 0.01[b]
Brain	0.46 ± 0.02[a]	0.63 ± 0.01[b]	0.46 ± 0.01[a]	0.62 ± 0.02[b]	0.45 ± 0.01[a]	0.62 ± 0.01[b]

Values presented mean ± SD values for each organ relative to body weight (%). Similar superscripts for each organ relative weight presented the non-significant differences ($P \geq 0.05$) and vice versa among all the treatment groups (male and female). (G1): group 1 animals fed with GM sugarcane juice, (G2): animals of group 2 fed with non-GM sugarcane juice and (G3): animals of group 3 fed with water and normal diet.

were not statistically significant or treatment-related differences based on daily physical behaviour or signs, body weight, feed consumption, serum biochemistry, haematology, relative organ weight, and histopathology. The GM *AVP1* sugarcane juice exposure at a rate of 2100 mg/kg/body weight (OECD, 2001) for the acute toxicity evaluation and more than 5000 mg/kg/body weight (OECD, 1988) for the sub-chronic toxicity determination to rats was found to be non-toxic and safe. Similarly, Delaney et al. (2014) observed that there was no statistically significant or treatment-related differences after a 13-week (sub-chronic) exposure of herbicide tolerant (DP-Ø73496-4) canola on rats (male and female). These findings were similar to previously published reports on 13-week (sub-chronic) and 2-week (acute) rodent feeding studies for GM glyphosate-tolerant corn, corn borer-protected corn and corn rootworm-protected corn (Hammond et al., 2004, 2006a, 2006b) maize grain event DAS-Ø15Ø7-1 (Malley et al., 2007) maize grain event DAS-59122-7 (Appenzeller et al., 2008, 2009) (soybean DP-356Ø43–5, GM stacked trait lepidopteran and coleopteran resistant (DAS-Ø15Ø7-1xDAS-59122-7) maize grain and herbicide-tolerant maize DP-Ø9814Ø-6 respectively (He et al., 2008) event DAS-59122-7 of maize (Healy et al., 2008) corn rootworm-protected, glyphosate-tolerant MON 88017 corn (Arjó et al., 2012) genetically engineered multivitamin (β-carotene, ascorbate and folate) corn in mice (Harrison and Bailey, 1996); glyphosate-tolerant soybean and Bt-resistant corn (Huang, 2017; Juberg et al., 2009), Bt rice (Cao et al., 2010; Huang, 2017) proved that there were no adverse health effects.

AVP1 GM sugarcane juice did not show any cytotoxicity based on the results of the micronucleus test in the peripheral blood of rats after 90-day and 14-days toxicity experiments. The comet assay results revealed that *AVP1* GM sugarcane juice was not related to DNA damage in the blood (< 5000 mg/kg/body weight) in sub-chronic and in the acute (< 2000 mg/kg/body weight) studies. Similarly, Jaszczak et al. (2008) performed a micronucleus test and comet assay on mice fed with a dose containing GM triticale and reported that there were no statistically significant differences in the micronuclei frequency and DNA damage between the control and experimental groups of mice in all the treatment groups. They concluded that the diet based on GM triticale (*bar* transgene) did not reveal any chromosomal damage and had no role in the creation of DNA breaks or lesions.

This is the first report on biosafety assessment of GM sugarcane expressing the *AVP1* gene. This research work revealed that under the conditions of acute and sub-chronic toxicity evaluations, the *AVP1* (drought tolerant) GM sugarcane juice was found to be non-toxic in Wistar albino rats (male and female) when they were administered orally for 14-day and 90-day. Scientific data generated through this research work would be valuable and provides some important information that will support the safety assessment of GM *AVP1* sugarcane a pre-requisite for commercialization.

6. Conclusions

The present research was conducted to evaluate the potential risks associated with GM *AVP1* sugarcane juice via different biosafety assessment protocols. The results exhibited that *AVP1* (drought tolerant) GM sugarcane juice was non-toxic to Wistar rats after the acute and the sub-chronic toxicity studies. The data generated through this research will be valuable for the commercialization of *AVP1* GM sugarcane.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Conflicts of interest

We all the authors declare no conflict of interests either financial or personal relationships regarding this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2019.05.034.

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