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Editors

Maize: Nutrition Dynamics and Novel Uses

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Foreword

Maize is a globally important cereal with the high production and productivity. It is being cultivated in more than 160 countries under different agro-climatic conditions. In India, it ranks third after wheat and rice. Maize was primarily used as a food, but nowadays it is also used for feed and industrial purposes. Therefore, apart from providing nutrients for humans and animals, it also serves as a basic raw material for a number of industrial products like starch, oil and protein, alcoholic beverages, food sweeteners and biofuel. Because of its worldwide distribution and wider range of uses, maize possesses many advantages over other cereals.

In India, maize occupies a prominent position, as each plant part finds some specific application in feed, food or industrial sector. After harvest of the grain, the dried stems are used as forage for ruminants. It gives the highest average grain yield as compared to other cereals such as wheat and rice. Nutritionally, maize contains about 67–73 % starch, 7–13 % protein and 2–6 % oil. However, the protein quality of maize is poor because of the deficiency of essential amino acids such as lysine and tryptophan. Quality protein maize (QPM) is nutritionally improved as it contains required concentrations of lysine and tryptophan. Among cereals and millets, oil is extracted mainly from maize, and efforts have been made under National Maize Improvement Programme towards the development of high-oil maize hybrids with better oil quality. Maize oil is very popular and widely used for human consumption as vegetable oil due to its cholesterol lowering characteristics. The embryo which forms about 10–12 % of the whole grain is a rich source of oil.

Yellow maize is a rich source of vitamin A. Maize has more riboflavin than wheat and rice and is rich in phosphorus and potash as well. Corn starch is used as a thickening agent in the preparation of many edibles like soups, sauces and custard powder. Corn syrup is used as an agent in confectionary units. Corn sugar (dextrose) is used in pharmaceutical formulations, as a sweetening agent in soft drinks, etc. Gel made from corn starch is used as a bonding agent for ice cream cones due to its moisture retention characteristics.

It is well understood that the nutritional quality of maize is very important, and there is still a need to improve it further. A sound strategy and well-defined action plan is required to address these issues. The book entitled *Maize: Nutrition Dynamics and Novel Uses* will provide valuable information about the nutritional quality as well as researchable issues in the development of nutritionally improved maize.

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Preface

Maize or corn (*Zea mays*) is a plant belonging to *Poaceae*, the family of grasses. It is cultivated globally being one of the most important cereal crop worldwide. Maize is not only an important human food but also a basic element of animal feed and raw material for manufacturing a variety of industrial products. It is a versatile crop grown over a range of agro-climatic zones from 58°N to 40°S, from below sea level to altitudes higher than 3,000 m and in areas with 250 mm to more than 5,000 mm of rainfall per year. Its growing cycle ranges from 3 to 13 months. The global maize production during 2010 was 844 mt from an area of about 162 million hectares. The USA, China, Brazil, Mexico, Argentina, Indonesia, India, France, South Africa and Ukraine are the top ten maize-producing countries. In India, maize is the third most important crop grown after rice and wheat.

Maize is consumed as a staple food in Africa, South America and some parts of Asia. The nutritional quality of maize is poor as it is deficient in some essential amino acids such as lysine and tryptophan. A nutritionally superior maize named quality protein maize (QPM) has already been developed as a result of nearly half a century of research dedicated to malnutrition eradication. Compared with traditional maize types, QPM has twice the amount of lysine and tryptophan, as well as protein bioavailability that rivals milk casein. QPM could be a practical food for alleviating protein malnutrition in the developing countries, particularly sub-Saharan Africa and other parts of the world that are worst affected by protein-energy malnutrition. Maize is one of the most important natural multipliers of starch. Within just 3 months, a single corn kernel produces more than 500 kernels containing about 70 % starch. Maize is also a source of good-quality oil which is highly regarded for human consumption as it contains essential fatty acid (linoleic acid) and vitamin E which is an important antioxidant. Maize is also a rich source of natural pigments, particularly carotenoids, which have diverse health benefits ranging from maintaining normal vision to lowering of oxidative stress. Maize is the most suitable crop for biofortification, particularly iron and zinc, along with provitamin A carotenoids, for alleviating micronutrient deficiencies.

Considering the nutritional role of maize worldwide in alleviating protein-energy malnutrition (PEM), micronutrient deficiencies, vision impairments and oxidative stress, it seems that maize is going to play a major role in human nutrition apart from its utilization as an industrial raw material. Till date, no comprehensive efforts have been made to discuss the nutritive

value of maize. The present book provides a platform to understand the various aspects of maize nutrition as a whole and its correlation with other biotic and abiotic stresses. It also covers the role of natural variability for various traits available in the germplasm and their future significance. The value-added and fermented products of maize have also been discussed thoroughly.

We pay our sincere thanks to Dr. O.P. Yadav, Director, Directorate of Maize Research, for his valuable guidance and suggestions which helped us in improving this book. We are also thankful to Dr. R. Sai Kumar and Dr. Sain Dass, Ex-Directors, Directorate of Maize Research, for their prompt help and motivation. Finally, we thank all the contributors for their painstaking efforts in the timely submission of their chapters.

New Delhi, India

Dharam Paul Chaudhary
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Part I

Introductory Chapter

Nutritive Value of Maize: Improvements, Applications and Constraints

1

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Abstract

Maize is a globally important crop mainly utilized as feed, food and raw material for diverse industrial applications. Among cereals, it occupies the third place after wheat and rice and is a staple food for a large segment of population worldwide, particularly in the Asian as well as African countries. Its nutritional quality is, however, poor due to deficiency of two essential amino acids, viz. tryptophan and lysine. The discovery of *opaque-2* gene has revolutionized the research in enhancing nutritional quality of maize, and subsequent research efforts gave birth to the present-day quality protein maize (QPM). This brings about a twofold increase in the levels of lysine and tryptophan as the zein or prolamine fraction is reduced by about 50 %. Starch is the major nutritional component of maize kernel constituting about 70 % of its weight. Starch composition in maize is genetically controlled, and significant variation has been observed in the amylose to amylopectin ratio which makes it suitable for different industrial purposes. Maize is also a source of oil which is highly regarded for human consumption as it reduces the blood cholesterol concentration. Many value-added products as well as fermented foods have been produced from maize which is consumed in different forms worldwide. Naturally, maize is a rich source of carotenoids such as beta-carotene, zeaxanthin, lutein and cryptoxanthin which have highly diverse health benefits ranging from maintaining normal vision to lowering of oxidative stress. Efforts have been made towards the development of biofortified maize rich in iron, zinc and provitamin A concentration.

1.1 Introduction

Maize (*Zea mays* L.), also known as corn, belongs to the tribe Maydeae of *Poaceae (Gramineae)*, commonly known as grass family. It is a tall monoecious plant domesticated by indigenous peoples in Mesoamerica in the prehistoric times, when grown in the form of a wild grass called

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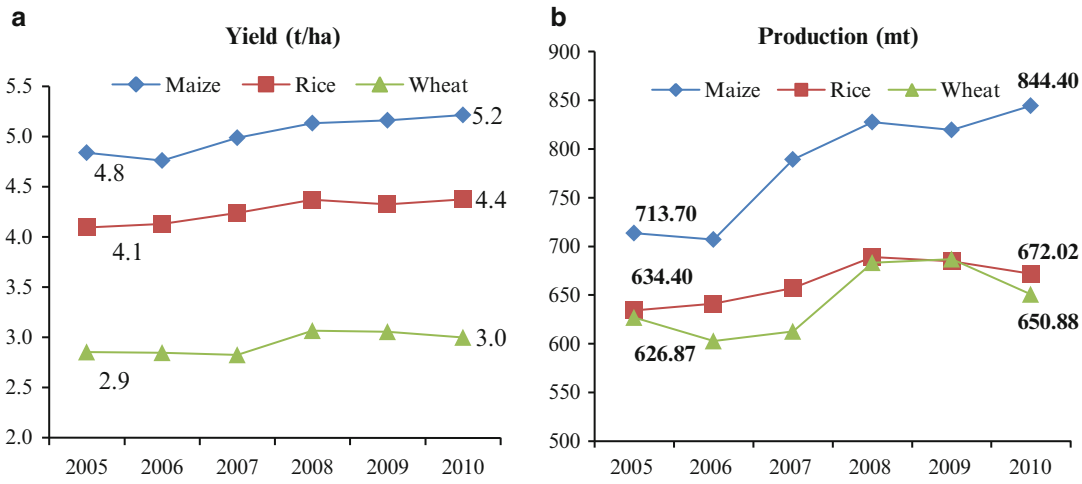

Fig. 1.1 Maize productivity (**a**) and production (**b**) along with other major cereals in the world during 2010 (Source: Based on FAO 2012)

teosinte (Beadle 1939). The history of modern-day maize begins at the dawn of human agriculture about 10,000 years ago. Over time, the systematic selection of certain varieties for their desired traits led to the gradual transformation of *teosinte* to its present-day form known as maize. Today, maize is the world's leading crop widely cultivated as a cereal grain. It is cultivated in more than 160 countries over a range of agroclimatic zones. Being a C_4 plant, maize has a specialized physiology known as kranz anatomy, which makes it well suited to hot and dry climates. However, the extensive breeding interventions made this plant suitable for colder regions as well. It is grown from 58°N to 40°S, from below sea level to altitudes higher than 3,000 m and in areas with 250 mm to more than 5,000 mm of rainfall per year (Dowswell et al. 1996). Its global production is higher than that of rice and wheat (Fig. 1.1). The global maize production during 2010 was 844 mt from an area of about 162 million hectares. The USA, China, Brazil, Mexico, Argentina, Indonesia, India, France, South Africa and Ukraine are the top ten maize-producing countries (FAO 2012). The USA is the largest producer and consumer of maize contributing around 316 mt to the total global maize production during 2010 followed by China with a production of 177 mt (FAO 2012). The maize production in Brazil and India was 56 and 21.72 mt, respectively, in 2010.

Globally, maize is used for feed followed by food and as an industrial raw material. Maize is the main staple food for people in Africa, South America and some parts of Asia and is also used worldwide as a fodder crop for livestock. Recent advancements towards biofortification have led to the development of quality protein maize (QPM) and maize with higher provitamin A activity which can play a major role in alleviating globally the protein-energy malnourishment and vitamin A deficiency, respectively.

This book covers various nutritional aspects of maize including its importance and limitations as food for humans, as livestock fodder, industrial applications along with value addition, importance of its oil in human nutrition, various fermented products and bioethanol production and constraints in increasing its productivity in the changing climatic scenery as discussed in the following sections.

1.2 Composition of Maize Kernel

The maize plant is one of nature's greatest multiplier. It is a high-capacity factory for efficiently converting large amounts of radiant energy

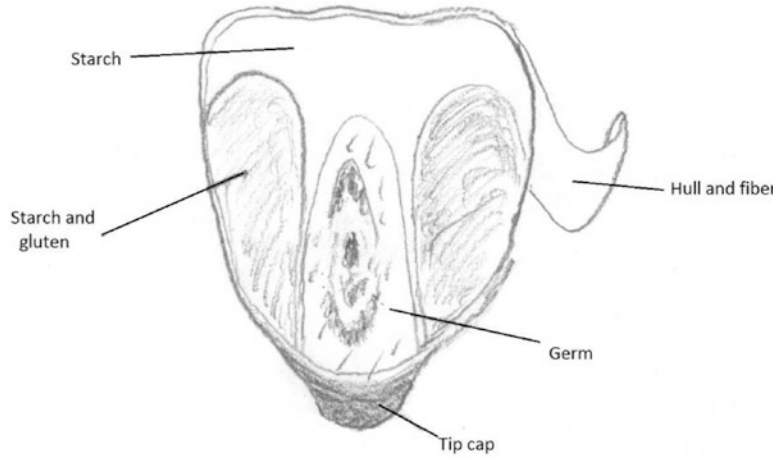

Fig. 1.2 Structure of maize kernel

from the sun into stable chemical energy. This energy is stored as cellulose, oil and starch in the corn plant and its kernel. Approximately 4 months after planting, a single kernel yields from 300 to more than 800 kernels. Maize kernels develop through accumulation of the products of photosynthesis, root absorption and plant metabolism on the female inflorescence called the ear. It consists of four major physical structures: endosperm, germ or embryo, outer seed coat or pericarp and the tip cap (dead tissue found where the kernel joins the cob) as shown in Fig. 1.2. The pericarp is the outermost layer that is characterized by high crude fibre content, mainly consisting of hemicellulose, cellulose and lignin.

Endosperm is the largest component (80–85 %), followed by germ (9–10 %) and pericarp (5–6 %). It is mainly composed of starch (≈70 %) followed by small concentrations (8–10 %) of protein (Lawton and Wilson 1987; Prasanna et al. 2001). Fat content of the endosperm is relatively low. The endosperm is composed of a large number of cells, each packed with starch granules embedded in a continuous matrix of protein. The cell wall consists of non-starchy polysaccharides (β-glucan and arabinoxylan), proteins and phenolic acids. Protein bodies are composed almost entirely of a prolamine-rich protein fraction known as zein. Maize grain has two types of endosperm – floury and horny. Floury endosperm contains loosely packed starch granules surrounding the central fissure, while horny endosperm has tightly packed, smaller starch granules towards periphery. The germ contains high concentrations of fat (≈33 %) and relatively high levels of protein (18–19 %) and minerals (Watson 1987). Germ oil is relatively stable due to the presence of natural antioxidants and highly regarded for human consumption because of its fatty acid composition, which mainly consists of oleic and linoleic acids. Maize contains various bioactive constituents, such as carotenoids, anthocyanins, tocopherols and phenolic compounds that have many health-promoting and disease-preventing properties.

1.3 Nutritional Quality of Maize

Improving nutritional quality in cereal crops is particularly important as the benefits can easily spread to hundreds of millions of people in a most rapid and effective manner without changing the traditional food habits. A significant human population consumes maize as a staple food, worldwide, the nutritional quality of which in turn depends upon the chemical composition of various components of its kernel. Since endosperm accounts for the largest component, the quality of maize kernel as a whole, therefore, depends largely upon its chemical composition.

1.3.1 Protein Quality

Protein quality, from a nutrition perspective, is a comparative term used to describe how well a food protein fulfils the body's requirements and, therefore, how useful the protein is for the biological system. This is determined by the building blocks which make up the protein, called amino acids. Among the 20 primary amino acids, nine are considered "essential" as these cannot be synthesized by our body and so must come from the diet. The best quality protein is one which provides essential amino acid pattern very close to that of the tissue proteins. Usually, milk and egg proteins serve as reference protein, because of their superior quality. As per Joint FAO/WHO Expert Consultation (1991) recommendations, the essential amino acid composition (mg/g protein) of reference protein required for 2–5-year-old children is as follows: lysine 58, threonine 34, tryptophan 11, methionine + cysteine 25. Various methods have been introduced to measure protein quality including biological value (BV), net protein utilization (NPU) and protein efficiency ratio (PER). The maize protein quality is, however, poor due to deficiencies of two main essential amino acids, lysine and tryptophan, and excess of leucine in the endosperm protein of its kernel (Osborne and Mendel 1914). The endosperm protein is classified into various fractions such as albumin (3 %), globulin (3 %), zein/prolamine (60 %) and glutelin (34 %). Based on their solubility, genetic properties and the apparent molecular masses, zeins have been classified into α- (22 and 19 kDa), the most abundant β- (14 kDa), γ- (27 and 16 kDa) and δ-zein (10 kDa) (Wilson et al. 1981). The α-zein fraction is rich in cysteine while β and γ fractions are rich in methionine. Generally in normal maize, zein fraction contains higher proportion of leucine (18.7 %), phenylalanine (5.2 %), isoleucine (3.8 %), valine (3.6 %) and tyrosine (3.5 %), but low amounts of other essential amino acids such as threonine (3 %), histidine and cysteine (1 %), methionine (0.9 %) and lysine (0.1 %), but is devoid of tryptophan. The non-zein protein fraction is balanced and rich in lysine and tryptophan. The high proportion of zeins in the endosperm is the primary reason for the poor protein quality of maize protein (Vasal 2000).

The discovery of *opaque-2* gene and its association with higher lysine and tryptophan content in the maize endosperm led to the beginning of the development of QPM (Mertz et al. 1964). High-lysine mutants such as *opaque-2* were developed and introduced into normal maize which brings about a twofold increase in the lysine and tryptophan levels. Further, lysine-deficient zein or prolamine fraction is reduced dramatically by about 50 %, while other fractions, such as albumins, globulins and glutelins which are rich in lysine, show a marked increase. The soft endosperm *opaque-2* maize was, thus, evolved. However, the *opaque-2* maize varieties could not become popular due to its soft and chalky grains texture, inferiority in terms of yield and agronomic performance and susceptibility to insects and pest infestation. This was the big setback for researchers involved in the development of nutritionally improved maize. However, later on, some partially hard endosperm or "modified" grains had been observed which possess desired concentrations of essential amino acids. The modified *opaque-2* maize with hard endosperm is known as QPM.

1.3.2 Carbohydrate Profile

Major carbohydrate of maize kernel is starch, which contributes approximately 70 % of the kernel weight. In fact, maize is one of the greatest manufacturers of starch. The starch molecule is a homopolymer of repeating anhydroglucose units joined by α-glycosidic linkages, the aldehyde group of one unit being chemically bound to a hydroxyl group on the next unit through hemiacetal linkages. The 1,4-linkages yield straight-chain starch molecules called amylose, while the 1,6-linkages serve as the branching point in branched-chain starch molecules called amylopectin. The starch composition in maize is genetically controlled. In normal maize, amylose makes up 25–30 % of the starch and amylopectin up to 70–75 %. Wide genetic variability exists in

maize with respect to starch profile. On the basis of starch composition, maize is categorized in three classes: (1) waxy maize, which contains almost 100 % amylopectin starch; (2) high-amylose maize, with starch containing amylose content between 40 % and 70 %; and (3) sugary maize, containing lower starch but higher level of sucrose (Nelson and Pan 1995). Maize with amylose as high as 85 % (amylomaize) is also reported. These variants have different industrial applications. Waxy maize is having large stake in food industry, whereas high-amylose maize is preferably required by textile industry. Waxy mutants starch has been reported to be more crystalline than regular cereal starches (Singh et al. 2003; Vandeputte et al. 2003). Dietary starch varies greatly in digestibility and its effects on the utilization of other nutrients. Maize possesses wide genetic variability with respect to digestibility of its starch molecule. Starch is classified into three groups depending upon the rate of release and absorption of glucose in the gastrointestinal tract: rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). RDS is the group of starches that can be rapidly hydrolyzed by digestive enzymes; SDS is the group that is digested at a relatively slow rate (Englyst et al. 1992). However, a part of it is not digested in the small intestine and reaches the large intestine (colon) where it is fermented by the gut microflora that produces short-chain fatty acids as end products which are known to promote the optimal function of the viscera (Topping and Clifton 2001). This indigestible portion of starch is known as resistant starch. Waxy maize starch is more rapidly digested than high-amylose starch, attributed to more surface area per molecule of the amylopectin than amylose. Many health benefits such as improved cholesterol metabolism and reduced risk of type II diabetes and colon cancer have been associated with the consumption of RS (Hoebler et al. 1999).

Major food uses for starch include sweeteners, brewing adjuncts, chemicals/pharmaceuticals, viscosity control agents in canning, starch-based confectionery products and bakery applications. Non-food industrial applications include adhesives in paper and building materials and coatings and sizings in textiles and paper products (Kulp 2000). The application of starch in various industries is primarily determined by its functional properties such as viscosity, swelling, solubility, gelatinization, pasting and retrogradation, which vary considerably from crop to crop and with ecological and agronomic influences (Riley et al. 2006; Yuan et al. 2007). Raphael et al. (2011) compared the functional properties of two major botanical sources of starches, maize and cassava for East African starch industries, and found applications in different industries. Most of the corn starch is extracted from the kernel through wet milling process along with other by-products. Subsequently, the starch fraction can be processed through chemical or biochemical procedures to enhance its applicability in food and industrial products. For example, oxidized starches are used in laundry starches and paper manufacturing. Dextrins (dry heating and roasting of starch with or without an acid or alkaline catalyst and process known as dextrinization) are widely used as quick-setting pastes in paper products. Instantized products such as instant puddings are produced from pregelatinization of starches. Starches are treated with enzymes to produce high-fructose corn sweeteners found in soft drinks. It can also be fermented to produce alcohol. Starch can be derivatized by a number of agents reacting with hydroxyl groups, cross-linking and stabilization derivatives and other derivatives depending upon required functional properties such as increased water-combining capacity, impeded retrogradation, etc. (Jackson 1992; Kulp 2000). Further, as starch is totally biodegradable, it can be used for developing bioplastics in the form of packaging material, fast-food service ware, etc.

Maize kernel also possesses some complex carbohydrates. The complex carbohydrate content of the maize kernel comes from the pericarp, tip cap, endosperm cell walls and to a smaller extent the germ cell walls. Maize bran is composed of 75 % hemicellulose, 25 % cellulose and 0.1 % lignin on a dry-weight basis (Sandstead et al. 1978; Van Soest et al. 1979). These constituents, though indigestible, help in

normalizing the digestive process. Other carbohydrates are simple sugars present as glucose, sucrose and fructose in amounts ranging from 1 % to 5 % of the kernel weight. Immature kernels contain relatively high levels of sugars and fewer amounts of other nutritional components which accumulate during development (Boyer and Shannon 1982). The sugar content makes the most clearly recognizable component of sweet maize quality as sweet corn is eaten at an immature stage of development (Evensen and Boyer 1986).

1.3.3 Oil Composition

Oil is mainly confined to the germ which provides around 85 % of the total kernel oil. The rest of the oil is dispersed in endosperm and hull fractions. Maize oil is mainly used in cooking and has high smoke point which makes it valuable for frying purposes (Katragadda et al. 2010). It is also a key ingredient in some margarines. Oil is an important by-product of starch industry. Normal maize provides around 2–6 % of oil, whereas high-oil maize contains more than 6 % of oil (Lambert 2001). High-oil maize differs in kernel composition as its germ size is found to be larger as compared to normal maize. Since most of the oil is contributed by the germ, therefore, increasing oil content in maize is directly proportional to the germ size (Motto et al. 2005). Increased germ size, however, is associated with reduction in starch as both oil and starch are negatively correlated (Yang et al. 2013). Maize genotypes with higher oil concentrations are of interest to many livestock feeders, primarily because of the higher calorific value of the grain (Lambert 2001). High-oil varieties of maize were developed at the University of Illinois (Dudley and Lambert 1992). Although these genotypes possess higher energy content, they showed poor agronomic characteristics.

Maize oil is valued because of its low levels of saturated fatty acid, i.e. on an average 11 % palmitic acid and 2 % stearic acid. On the other hand, it contains high levels of polyunsaturated fatty acids (PUFA), mainly linoleic acid (≈24 %), and small concentrations of linolenic acid. Corn oil plays an important role in human nutrition. The PUFAs help in regulating blood cholesterol and lowering the elevated blood pressure (Hauman 1985; Dupont et al. 1990). Corn oil is a rich source of linoleic acid which is an essential fatty acid that the body cannot synthesize. The term "essential fatty acid" refers to the fatty acids, required for biological processes. Only two fatty acids are known to be essential for humans: alpha-linolenic acid (an omega-3 fatty acid) and linoleic acid (an omega-6 fatty acid). The Food and Agriculture Organization and World Health Organization recommend about 2–4 % of energy in the form of essential fatty acids, with an additional 3 % energy for pregnant or breast-feeding women. A tablespoon serving of corn oil is sufficient to satisfy the daily essential fatty acid requirement of a healthy child or an adult. Corn oil is also recognized as an excellent source of tocopherols. Tocopherols function as antioxidants and provide a good source of vitamin E. Like essential fatty acids, vitamin E also represents an essential component of human diet as it cannot be synthesized in the body. It is a class of strong antioxidants which protect polyunsaturated fatty acids in membranes against degradation by reactive oxygen species such as ozone, singlet oxygen, peroxides and hyperoxides (Dormann 2003). So, the antioxidant activity of tocopherols is important not only from health point of view but also in terms of oil quality as it helps in increasing its shelf life by retarding the development of rancidity. The four major tocopherols found in corn oil are alpha-, beta-, gamma- and delta-tocopherol. In commercially available corn oil, gamma-tocopherol is the most abundant, followed by alpha-tocopherol and delta-tocopherol. The tocopherol that exhibits the greatest antioxidant effect is delta-tocopherol, whereas alpha-tocopherol has the highest vitamin E activity.

1.4 Natural Pigments

The colour of maize ranges from white to yellow, red, blue, purple, etc. Blue-, purple- and red-pigmented maize kernels are rich in anthocyanins with well-established antioxidant

and bioactive properties (Adom and Liu 2002). Anthocyanin, carotenoid and phenolic contents in maize vary with the colour. The highest concentration of anthocyanin pigments in maize is present in the pericarp portion, whereas the aleurone layer contains small amounts (Moreno et al. 2005). Maize exhibits considerable natural variation for kernel carotenoids with some genotypes accumulating as high as 66.0 μg/g (Harjes et al. 2008). The predominant carotenoids in maize kernels in decreasing order of concentration are lutein, zeaxanthin, β-carotene, β-cryptoxanthin and α-carotene. Yellow maize has more carotenoids than floury maize. Generally, provitamin A carotenoids constitutes only 10–20 % of total carotenoids in maize whereas 30–50 % represent by zeaxanthin and lutein each. In typical maize, concentrations of provitamin A carotenoids, i.e. α-carotene, β-carotene and β-cryptoxanthin, range from 0 to 1.3, 0.13 to 2.7 and 0.13 to 1.9 nmol/g, respectively (Kurilich and Juvik 1999).

Maize is also known to contain a wide range of phenolic acids. Ferulic acid is an important phytochemical in maize and its concentration varies in different maize types. The high-carotenoid maize contains higher amount of total ferulic acid compared to white, yellow, red and blue maize. Most of the ferulic acid in maize is present in bound form (Adom and Liu 2002). The bulk of phenolics (phenolic acids, flavonoids and conjugated amines) are concentrated in the pericarp and aleurone layers as well as the germ, with traces in the endosperm (Sen et al. 1994).

1.5 Mineral Composition

Iron deficiency is the most common and widespread nutritional disorder in the world. Apart from affecting a large number of children and women in developing countries, it is the only nutrient deficiency which is also significantly prevalent in industrialized countries. The numbers are staggering: two billion people – over 30 % of the world's population – are anaemic, many due to iron deficiency, and in resource-poor areas, this is frequently exacerbated by infectious diseases. It can retard mental development and learning capacity and impair physical growth during childhood and adolescence, while in adults it reduces the capacity to do physical labour (Bouis 2002). Deficiency of zinc, another common micronutrient, lowers the intestinal absorption of fat and fat-soluble vitamins including retinol (Ahn and Koo 1995a, b; Kim et al. 1998). Similarly, other mineral elements, including calcium, copper, magnesium, manganese, phosphorus and potassium, are also considered essential for various physiological activities including growth and development of bones, teeth, blood, nerves and skin; synthesis of vitamins, enzymes and hormones; as well as for healthy functioning of the nervous system, blood circulation, fluid regulation, cellular integrity, energy production and muscle contraction (MacDowel 2003; O'Dell and Sunde 1997).

Mineral composition of cereal grains can be affected by a number of factors such as soil type and fertility, soil moisture, environmental factors, crop genotype and interactions among nutrients (Arnold et al. 1977; Feila et al. 2005). Wide natural variability for various minerals exists in the maize germplasm. Menkir (2008) evaluated a vast set of tropical-adapted inbred lines for mineral concentrations. The best inbred lines identified from each trial had 32–78 % more Fe and 14–180 % more Zn than their trial average. The first two principal component axes, which accounted for 55–64 % of the total variation in kernel mineral concentrations, stratified the inbred lines in each trial into four groups based on differences in their grain mineral compositions. None of the correlations of Fe and Zn with Mn, Cu, Ca, Mg, K, P and S were significant and negative in the various trials, while the correlations of Fe with Zn were positive and significant. Ullah et al. (2010) also analysed grains of ten maize varieties for mineral composition and reported the range of Na (540.30–620.41 ppm), K (2915–3471 ppm), Ca (410–590 ppm), Fe (38.02–56.14 ppm), Zn (37.05–52.4 ppm), Mg (985.2–1125.3 ppm) and Cu (11.02–14.25 ppm). High levels of phytate (myo-inositol hexaphosphate) are present in unrefined cereals, which is a powerful inhibitor of iron and zinc absorption in both adults (Egli et al. 2004; Mendoza et al. 1998) and

children (Davidsson et al. 2004). Any reduction in dietary phytate can have a positive effect on zinc absorption (Lonnerdal 2002) and iron absorption (Mendoza et al. 1998).

1.6 Biofortification of Maize

Globally, approximately one-third of preschool-age children and 15 % of pregnant women are estimated to be vitamin A deficient (WHO 2009). The problem becomes more severe particularly in the developing countries whose poor populations rely on a single staple crop for their sustenance, e.g. Africa and Southeast Asia have the highest burden of vitamin A deficiency (WHO 2009). The consequences of vitamin A deficiency include blindness, reduced growth in children and increased morbidity and mortality (Sommer and West 1996; Shankar et al. 1999; Rice et al. 2004; Maida et al. 2008). Further, interaction of vitamin A content with both iron as well as zinc content has been well documented (Hess et al. 2005). Iron metabolism is negatively affected by vitamin A deficiency in the diet, and iron is not incorporated effectively into haemoglobin (Hodges et al. 1978). The co-occurrence of iron and vitamin A deficiencies has been found in infants in South Africa (Oelofse et al. 2002), preschool children in the Marshall Islands (Palafox et al. 2003), school-age children in Côte d'Ivoire (Hess 2003) and pregnant women in India, Nepal and Malawi (Pathak et al. 2003; Dreyfuss et al. 2000; van den Broek and Letsky 2000). Similarly, zinc intake has been considered to be inadequate for an estimated 30 % of the populations in 46 African countries (Hotz and Brown 2004). Zinc deficiency, as already, discussed lowers the intestinal absorption of fat and fat-soluble vitamins including vitamin A retinol in rats. Various implications associated with iron and zinc deficiencies have already been discussed along with other minerals under mineral composition part.

Biofortification is the development of micronutrient-dense staple crops using the best traditional breeding practices and modern biotechnology. To alleviate the vitamin A and micronutrient, particularly iron and zinc deficiencies from rural areas of developing countries, biofortification of major staple food crops of respective areas or countries is the only feasible way, since this will better ensure targeting and compliance. As the third most important cereal staple food crop worldwide (FAPRI 2009) and a major cereal staple for African consumers (FAOSTAT 2010), maize qualifies as a suitable crop for biofortification. Before devising a strategy to enhance the provitamin A carotenoids and iron and zinc micronutrients in maize, one question that must be addressed is the target quantity for biofortification. This is related to the bioavailability or the fraction of an ingested nutrient that becomes available to the body for utilization in physiological functions or for storage (Jackson 1997; Fraser and Bramley 2004). There are numerous factors that influence bioavailability. After considering all these factors, it has been decided that at least 15 μg β-carotene/g dry kernel weight, 60 mg/kg iron and 55 mg/kg zinc are required for biofortified maize to have an impact on nutrition (Graham et al. 1999).

1.7 Value Addition

A broad definition of value addition is to economically add value to a product and form characteristics more preferred in the market place. Value addition in maize has a great potential. There are several value-added products of maize, particularly QPM and baby corn, which not only increase the farm income but also provide employment to rural youth and farm women. QPM has been used for the development of traditional products, baked products, extruded products, convenience foods, infant food, health foods, snacks and savoury items, specialty foods, etc. which can meet the nutritional need of vulnerable section. Further, maize was blended with soya bean/green gram in ratio 70:30 and the product developed includes cake, biscuit, *halwa*, *upma*, *vada*, etc. (Kawatra and Sehgal 2007). A number of value-added products have been developed from specialty corns taking in care the various age groups and their requirements as discussed in detail in related chapter of value addition.

1.8 Fermented Maize Foods

Fermented foods are considered as palatable and wholesome foods prepared from raw or heated raw materials and appreciated for their attributes such as pleasant flavour, aroma, texture, improved processing properties and better digestibility. Maize is a good source of dietary fibre and protein while being very low in fat and sodium. The endosperm consists of approximately 70 % starch embedded in a protein matrix that makes the maize an excellent substrate for fermentation. Maize is processed, fermented and consumed in various ways. Some of the fermented maize products include Chicha (a clear, yellowish, effervescent, alcoholic beverage having alcohol content between 2 % and 12 %, most popular in South and Central America), Tesguino (a slurry-like alcoholic beverage prepared from germinated maize or maize stalk juice commonly consumed in Northern and Northwestern Mexico), *Umqombothi* (a pink, opaque, mild alcoholic drink having yoghurt-like flavour with thin consistency, prepared from maize and sorghum popular in South African population), *Busaa* (a Kenyan opaque maize beer having 2–4 % ethanol and 0.5–1 % lactic acid), Atole (a sour porridge-type product of Southern Mexico), Pozol (fermented maize dough formed into balls of various shapes and sizes which is an important recipe of Southeastern states of Mexico), Ogi (prepared from submerged fermentation of maize or sorghum and millet is a traditional weaning food common in West Africa), *Mahewu* (a non-alcoholic sour beverage made from corn meal and is mostly consumed in Africa and some Gulf countries), Pito (a traditional beverage, very popularly consumed throughout Nigeria and Ghana) and Uji (a sour maize gruel from East Africa).

1.9 Industrial Aspects

Maize is an important raw material for the production of a number of industrial products such as starch, oil, ethanol, malt and animal feed. Two types of industrial processes are common for maize processing: wet milling and dry grinding. Wet milling involves separation of grain kernels to its constituent components such as germ, bran (fibre), protein and starch. In this process, the grains are steeped or soaked into water for 24–48 h to facilitate the separation of kernel components. These components are then sequentially removed through a series of industrial processes to obtain the final products. The germ is removed and processed for the extraction of oil which is then purified and sold for human consumption. Bran (fibre components) is used in the animal feed. The starch as well as gluten is separated through centrifugation processes. Maize is an important industrial raw material for the production of starch which is utilized in a number of industries such as paper industry, textile industry, pharmaceutical industry, food industry and leather industry. It can be processed further to synthesize a number of components such as dextrose, fructose and high-fructose corn syrups. Corn gluten meal is a by-product which is sold to poultry sector as a high-protein feed.

In the dry grind process, the entire grain is ground into flour (Butzen and Haefele 2008). Dry grind process is usually employed for the production of ethanol from maize. In this process, kernels are ground and mixed with water to form slurry called "mash" which is further cooked during which the starch is enzymatically converted to sugars. Sugars are fermented to ethanol by the addition of suitable yeast. The ethanol is extracted and the solid remains are dried to produce distiller's dried grain soluble (DDGS) which is used as a high-protein ingredient in animal diet. A significant part of maize produced is utilized for the production of biofuels in the USA. In India, policy makers are seriously considering the exploration of maize for the synthesis of biofuels to meet the ever-increasing demand of fuels for vehicular traffic. The increasing production of maize throughout the world has necessitated finding economically viable industrial processes for its conversion to ethanol for its use as a biofuel. Another important use of maize could be its conversion to malt to be used for the production of beer. Presently, maize is used as an adjunct along

with barley which is the most preferred cereal for malting purposes. The issue has been addressed in a separate chapter.

1.10 Specialty Maize

Plant breeders have altered through genetics the starch, protein and oil content of corn to meet the needs of the human population, livestock feeder, the food industry and other industrial users of corn in a better way. As a result of their modifications of ordinary dent types, new specialty corns have been created, including sweet corn, waxy, high-amylose, high-oil and baby corn. Sweet corn and baby corn are two important special types which are gaining wide-scale popularity in the recent times. Sweet corn is a variety of maize with high sugar content. Unlike other normal maize, which are harvested when the kernels are dry and mature, sweet corn is harvested when the kernels are immature (milk stage) and eaten as a vegetable. Immature standard sweet corn is considered desirable when kernels are succulent because of a mutant recessive *sugary-1* gene (su-1) that retards the conversion of sugar into starch during endosperm development. In comparison, sucrose produced in leaves from photosynthesis in dent corn is passed on to developing kernels, where it is rapidly converted to dextrin (a non-sweet, water-soluble polysaccharide) and then to starch. The *sugary-1* gene slows down this process.

Baby corn is the young ear, harvested when the silks have just emerged and no fertilization has taken place. Baby corn is a very delicious and nutritious vegetable and has been considered to be a high-value agriculture produce in national and international markets. It is free from the harmful effects of pesticides as baby corn is covered tightly with husk and due to early harvesting; it is devoid of harmful pests and diseases. A number of products with additional value from baby corn have been developed, and there is a vast scope of developing and promoting value-added products from baby corn.

1.11 Maize as Fodder for Livestock

Green fodders are the most important component of animal husbandry as they provide cheap and nutritious feed for livestock. The economics of milk production depends primarily upon the availability of nutritious green fodders. Maize is rated as one of the best non-legume fodder. It is considered ideal forage because it grows quickly, produces high biomass, is palatable, is rich in nutrients and helps to increase body weight and milk quality in cattle (Sattar et al. 1994). As fodder for livestock, maize is an excellent, highly nutritive and sustainable source (Hukkeri et al. 1977; Iqbal et al. 2006). Moreover, maize is grown round the year and is also free from anti-quality components. It contains high concentrations of protein and minerals and possesses high digestibility (Gupta et al. 2004). Specialty corn such as sweet corn and baby corn serves dual purpose as they provide fodder along with special purpose maize. Maize also possesses excellent ensiling characteristics as it contains sufficient quantities of soluble sugars required for proper fermentation (Allen et al. 2003). In a recent study carried out at Directorate of Maize Research, excellent silage was produced from baby corn stalks (after the harvest of baby corn) after 45 days of ensiling (unpublished data). The nutritional quality of maize as compared to other forages, the silage-making technology and the potential of specialty corn as fodder for livestock are discussed in detail in a separate chapter.

1.12 Maize Under Changing Climatic Scenario

Rise in atmospheric CO_2 with parallel increase in temperature is posing to be a serious threat to the farming community. During the last 12 years, the increasing rate of CO_2 has been 1.9 $\mu l\, l^{-1}\, year^{-1}$ and is predicted to reach the level of 570 $\mu l\, l^{-1}$ by the middle of this century (IPCC 2007). All the physiological processes are affected by the change in the environment that finally limits

productivity of the crop. Elevated CO_2 causes partial stomatal closure thereby decreasing leaf transpiration, while at the same time, the carbon assimilation is increased (Morison 1998). C_4 plants possess two types of cells, mesophyll and bundle sheath cells, and possess a mechanism that concentrates CO_2 in the bundle sheath cells to levels that have been estimated to be 3–8 times more than the atmospheric CO_2 concentration (Kanai and Edwards 1999). A differential response to increased CO_2 levels was observed in maize with studies showing no enhancement in growth (Hunt et al. 1991) to 50 % stimulation (Rogers and Dahlman 1993). High temperature denatures RuBisCO activase rendering it unable to fit correctly onto RuBisCO. Consequently, inactive RuBisCO is not converted to active form (Crafts-Brandner and Salvucci 2000). Further, high temperature reduces grain size due to steady decrease in the duration of grain filling combined with a failure of compensation by increased rate of dry matter accumulation above a threshold temperature. In respective chapter, various components affecting the growth and yield of maize and other cereal crops under elevated CO_2 and temperature are discussed in detail.

1.13 Insect–Pest Scenario

The grain yields of traditional maize genotypes in India are quite low, and in spite of sincere efforts, the yield potential of Indian maize genotypes has not reached even half the mark to that in the USA. Furthermore, the gap in yield potential of maize genotypes under experimental conditions and that under farmer's fields is huge, due to different stress factors; mainly, the insect pests during vegetative stage need greater attention to break this yield gap. Different insect pests, viz. maize stalk borer, pink stem borer, sugarcane leafhopper, shoot bug, armyworm, shoot fly, corn leaf aphid, cob borer and termites, have constrained the increase in yield potential of the maize genotypes deployed in India. It is likely that with the increase in concentration and quality of nutritional compounds, particularly the protein quality in QPM, maize might favour the proliferation of insect pests as they too prefer quality food for their growth and development and could be a major constraint to increasing production and productivity of QPM. The maize stalk borer, *Chilo partellus,* alone causes 26.7–80.4 % yield loss under different agroclimatic conditions in India (Reddy and Zehr 2004). Host plant resistance is one of the effective means of minimizing losses due to insect pests. However, most of the maize varieties and hybrids released for cultivation are potentially susceptible to *C. partellus* during vegetative stage (Kumar 1997) and maize weevils, *Sitophilus* spp., under field and storage conditions (Arnason et al. 1993; Hossain et al. 2007). Damage potential of different insect pests, status of host plant resistance and the mechanisms of resistance involved and the management of major insect pests have also been discussed in the related chapter.

1.14 Disease Scenario

Maize crop is affected by a total of 62 diseases worldwide. Out of these 62, 16 have been identified as a major constraint in increasing production and productivity of maize in India. Banded leaf and sheeth blight (BSLB), pythium stalk rot, bacterial stalk rot, post-flowering stock rot (PFSR), polysora rust and downy mildews are the major threat to the potential yield of maize. Global losses in maize due to diseases were estimated about 9 % in 2001–2003 (Oerke 2005). These losses varied significantly by region with estimates of 4 % in northern Europe and 14 % in West Africa and South Asia (http://www.cabicompendium.org/cpc/economic.asp). Losses have tended to be effectively controlled in high-intensity agricultural systems where it has been economical to invest in resistant germplasm and pesticide applications. However, in areas like Southeast Asia, hot, humid conditions have favoured disease development, while economic constraints prevent the deployment of effective protective measures. Since maize is affected by a number of diseases with different pathogenic nature, no single management strategy is sufficient to manage such complex group of diseases.

Damage potential of different diseases, status of host plant resistance and the mechanisms of resistance involved, and the management of major disease are needed to be addressed by an integrated disease management approach.

1.15 Future Strategies for Nutritionally Enriched Maize

Maize industry is growing and will attain more importance in the future due to its diverse applications. Maize could serve as an important food source for the increasing human population worldwide. However, research interventions are still needed to improve the nutritional quality and to popularize the nutritionally improved maize. The nutritional superiority of QPM over normal maize is well established. QPM can become a boon to vulnerable groups particularly from rural poor populations worldwide. QPM rather tastes sweet compared to normal maize and, therefore, can be consumed without any change in the traditional food habits. Recently, the Indian Government has set aside a significant amount in its annual budget towards the development of nutrifarms in the country ensuring that the farmers growing QPM should get remunerative price for their produce. There is a need to educate the masses that QPM is a product of conventional breeding and does not involve any transgene. A lot of value-added products from QPM such as QPM Mix-I, QPM Mix-II, QPM ladoo, honey maize chocolate, maize coconut chocolate, maize coconut toffee, maize groundnut toffee and choco maize bar have been developed, and efforts are needed to popularize these products. The vulnerable groups, i.e. infants, preschool children, pregnant and lactating mothers and elderly people, are going to get the maximum benefit from QPM and its products. Efforts should be made towards the development of maize germplasm containing high methionine which is an important ingredient in the poultry feed. Moreover, efforts should be made towards understanding the mechanism of action of modifier genes in the QPM kernel, the expression of which is affected by genetic and environmental factors.

Biofortification technology is highly sustainable and cost-effective as the once developed fortified seeds can be grown time and again and could easily reach the undernourished populations in relatively remote rural areas, delivering naturally fortified foods to people with limited access to commercially marketed fortified foods that are more readily available in urban areas. Maize biofortification for provitamin A carotenoids benefits human health and also adds to commercial value of food as these are natural colourants. National germplasm collections hold untapped potential for maize improvement. Biofortification of maize requires large-scale germplasm screening and utilization of identified trait-specific material into the breeding programme. Further, the developed biofortified maize material using trait-specific germplasm should not only contain higher quantities of provitamin A carotenoids and minerals but also have all other crucial traits including higher yield, insect pest and disease resistance and better nutritional quality. So, there is a need to tag new genes/loci for various traits. Further, mutational studies can also provide an insight about the regulatory points in the metabolic pathways. Similarly, stage-specific metabolite profiling and its correlation with candidate gene expression could provide important information regarding regulation chemistry and expression patterns. So, an interdisciplinary approach including biochemistry, genetics, plant breeding and nutrition is required for tagging of new genes and identification of rate-limiting steps of the pathways along with gene expression patterns with respect to time and allozymic diversity, which in turn might provide important information for deciding futuristic strategies regarding overall maize improvement.

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Part II

Protein Quality of Maize

Breeding Challenges and Perspectives in Developing and Promoting Quality Protein Maize Germplasm

2

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Abstract

The chapter discusses the development of nutritionally enhanced maize popularly known as quality protein maize (QPM). The focus will be on describing ever-evolving breeding options and strategies in developing this maize. Most of the examples will be quoted from CIMMYT's work where much of the research and practical breeding has been attempted. Initially, approaches of CIMMYT breeders were not very different from other researchers. High-lysine mutants such as *opaque-2*, *floury-2, opaque-7,* and a few double mutant combinations of different endosperm mutants were tried. Experiences, difficulties, and manipulation of different mutants will be presented having relevance to the development of high-lysine maize genotypes. The strengths and weaknesses of early breeding efforts will be critically discussed revealing complexity of interrelated problems affecting acceptance at producer, consumer, and industrial processing level. Initial research efforts at CIMMYT and elsewhere were confined to developing soft endosperm opaque versions of counterpart normal maize varieties and hybrids. Extensive testing of such materials exposed a series of problems of poor agronomic performance, unacceptable kernel appearance, and some problems of specific nature under certain environmental conditions. High hopes and early optimism of mid-1960s and early 1970s received a major setback in research efforts and brought lots of frustration and declining interest in high-lysine maize types. Funding declined resulting in reduced breeding efforts and abandoning of research partially or completely in several parts of the world. Only a handful of institutions and breeding programs continued research in a persistent and a systematic manner. Continuing efforts demanded exploring other options with and without high-lysine mutants. Several approaches were tried and critically examined for their merits and

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demerits. Different approaches were selected and emphasized in different institutions. These will be briefly discussed, but emphasis will be given as to why these approaches failed to produce desired results. CIMMYT's approach has proven to be viable and has been successfully tried in programs still maintaining interest in this particular maize. Selection of appropriate strategy(ies) and breeding method(s) was the key to CIMMYT's success. One-shot strategy as opposed to several individual problem-related strategies was a must. Based on experience and available information, the choice was to use a combination of two genetic systems involving the *opaque-2* gene and genetic modifiers of the *opaque-2* locus. At the very outset, there was a hope that this system can resolve complexity of problems plaguing such materials. A series of steps were practiced to implement this strategy and to start using it on a larger scale. Variation in kernel modification had to be encountered and donor stocks had to be built up initially. Some chance events will be mentioned which greatly facilitated rapid development of QPM donor stocks. The next challenge was to develop QPM germplasm adapted to different ecologies. A wide array of breeding approaches was used to accomplish this needed task. These will be fully discussed as to their relevance then and now.

Huge QPM germplasm volume was built through these varied efforts. By mid- to late 1970s, improvement of QPM populations through international testing had already begun. Germplasm merging process was initiated at this stage to form manageable number of QPM populations and pools so that systematic improvement process could be started. At this point, an important decision was also made to work in homozygous *opaque-2* backgrounds. The next turning point in QPM breeding efforts was in mid-1980s when hybrid development was started. Combining ability information and heterotic patterns of important classes of QPM germplasm were established. Other aspects of hybrid development such as inbred development, hybrid formation, and testing were initiated on a modest scale. Even with a few hybrids formed and tested, the results were quite encouraging. Several QPM hybrids had performance levels quite similar or superior to better performing normal hybrids. The past 15 years or so have witnessed much greater emphasis on QPM hybrid formation and testing at CIMMYT. The international testing of QPM hybrids has substantially increased, and many national programs are finding these hybrids quite satisfactory in performance to the available commercial hybrids. Several countries have released hybrids while others are on the verge of releasing soon. Future strategies as well as traditional and modern methodologies and tools that need to put in place will be discussed to have broader QPM germplasm base available worldwide for breeding efforts. One point, at the end, which I want to highlight is that support from biochemical laboratory was an important factor for the success of QPM work at CIMMYT. I consider QPM research as an excellent example of interdisciplinary team effort at CIMMYT where biochemists and breeders have worked in complete coordination to produce desired and acceptable QPM products.

2.1 Introduction

Improving nutritional quality of agricultural crops is a noble goal. Nutritional goals in general are difficult and the success stories are relatively few. To achieve good results requires long-term sustained investments, patience, deep commitment, and perseverance in pursuing the desired goals. Improving nutritional quality in cereal crops is particularly important as the benefits can easily spread to hundreds of millions of people in a most rapid and effective manner without changing the traditional food habits. About maize, biochemists had demonstrated several decades ago that maize protein is nutritionally deficient because of the limiting quantities of two essential amino acids: lysine and tryptophan (Osborne and Mendel 1914). In the absence of specific genes that could elevate protein profile with respect to these amino acids, serious research efforts by and large were lacking and limited to screening elite and bank maize accessions in the hope of finding useful genetic variation for these traits (Aguirre et al. 1969; Paez et al. 1969b; Tello et al. 1965; Zuber 1975; Zuber and Helm 1975; CIMMYT 1970). As expected, variation was encountered, but breeding efforts were not pursued to make use of these materials in developing high protein quality strains. Research initiatives had to wait almost 50 years until three distinguished Purdue researchers discovered high-lysine mutants such as *opaque-2* (Mertz et al. 1964) and *floury-2* (Nelson et al. 1965). The discovery of the biochemical effects of these two mutants paved the way for genetic manipulation in breeding to improve the nutritional quality of maize endosperm protein. Introduction of mutant genes into normal maize brings a twofold increase in the levels of lysine and tryptophan. Lysine-deficient zein or prolamine fraction is reduced dramatically by about 50 %, while other fractions—albumins, globulins, and glutelins—rich in lysine show a marked increase. Most maize scientists envisioned this report with great optimism and high hopes. Worldwide conversion programs were initiated producing a wide array of *opaque-2* varieties and hybrids. These high-lysine conversions were tested extensively in the developed and the developing world. Yield and agronomic performance of these materials did not match the normal counterparts (Alexander 1966; Harpstead 1969; Lambert et al. 1969; Gevers 1972; Glover and Mertz 1987; Sreeramulu and Bauman 1970; Vasal 1975; Vasal et al. 1979, 1980, 1984a, b; Bjarnason and Vasal 1992). In general, these materials had 10–15 % less grain yield, the kernel appearance dull and chalky, slower drying, greater kernel rots, and greater vulnerability to stored-grain pests. The reasoning and causes behind these problems are discussed in earlier publications (Bjarnason and Vasal 1992; Vasal 1993, 2000, 2002; Prasanna et al. 2001). Thus in the very first decade, this maize did not pass the test of its strength. Frustration and declining interest was a consequence of its lacking competitive performance. Funding support was either reduced or completely withdrawn. Most national maize programs in the developing countries reacted in a similar fashion. Following mid-1970s, only a few institutes and programs showed continuing interest and enthusiasm. CIMMYT had been one of them. The other institutions and programs which sustained and persisted in their breeding efforts were Purdue University, Crow's Hybrid Seed Company in Milford, Illinois, and the South African program at the University of Natal.

CIMMYT's work on quality protein maize (QPM) is considered significant. It has achieved scientific breakthrough in developing germplasm products that are competitive and meet acceptance of farmers' and consumers' preference (Vasal 2000). Several elements have contributed to this success including sustained funding, strong administrative support at DG and director's level, readiness to deploy alternate sound options in breeding when the rest of the world was witnessing declining interest, continuous evolving breeding methodologies and strategies, and making constant changes and adjustments as and when needed during different phases of germplasm development.

2.2 Turning Points in Developing Nutritionally Enhanced Maize

In developing nutritionally enhanced maize with superior protein quality, the breeding program at CIMMYT has evolved continuously. Breeding tactics and strategies were modified or changed to

better options depending on the situation. As these changes were made, the workload in protein laboratory and the ability to perform rapid and timely analysis were given due attention (Villegas et al. 1992). Sequence of events in developing QPM germplasm are discussed below in a brief manner.

2.3 Development and Improvement of Soft Opaques

In the early period from 1966 to 1973, the emphasis was on developing high-lysine soft endosperm versions of normal materials. Initially, both *opaue-2* (*o2o2*) and *floury-2 (fl2fl2)* mutants were used extensively, but very soon the use of *floury-2* was discontinued in the breeding program. Being semi-dominant, the kernel expression and quality depended on dosage of *fl2* allele in the triploid endosperm. *Floury-2* conversion programs were thus difficult to handle, and deployment of any double mutant combination strategy was even more difficult. In contrast, the *opaque-2* mutant had some obvious advantages. It was inherited in a simple recessive manner, the conversion process was easy, and no dosage effects were encountered in the endosperm. Soft chalky phenotype associated with the *opaque-2* kernels was additional help as it served as a marker and greatly facilitated the conversion process. All potentially important maize materials in the maize program were converted to *opaque-2* with varying numbers of backcrosses. A few *opaque-2* materials were also introduced from other countries such as Thai *opaque*-2 composite, Venzuela-1 *opaque*-2, and Ver.-Antigua *opaque*-2. In addition, at least three broad-based *opaque-2* composites K, J, and I were formed to serve lowland, mid-altitude, and highland agroecologies. Population improvement efforts were undertaken in some populations and population crosses were also developed. It is fair to say that a larger share of the resources was invested in developing and improving soft opaques. The materials so developed were tested nationally and internationally in OMPT-11 trials. In the absence of hybrid program, no efforts were made to develop and convert maize lines. This was, however, attempted in many countries with emphasis on hybrid maize as was the case with Brazil and Colombia. It may be interesting to point out that partially modified kernels were observed during the conversion process, but their significance and importance were not realized and appreciated until the first published report (Paez et al. 1969a). In addition to large-scale research effort on soft opaques, the program during this period was also engaged in a small and insignificant way to address some issues of exploratory nature. These included screening bank accessions for lysine and tryptophan content as well as developing genetic isolation mechanisms to prevent contamination in opaques. Double mutant combinations of *o-2* and *fl-2* with waxy were also developed. A beginning in modified opaques had already begun considering the wisdom and vision of Dr. Lonnquist and Dr. Asnani. By early to mid-1970s, the problems plaguing soft opaques were well recognized and documented in the developing and the developed world.

2.4 Correcting First-Generation Problems and Exploring New Alternatives

It was mentioned earlier that soft opaques were confronted with complex and interrelated problems. It thus became imperative to consider other alternatives in developing high-quality protein maize. Several new ideas emerged suggesting options with and without the use of mutant genes. In approaches not involving mutant(s), three options were considered and tried. These were recurrent selection for high lysine in normal maize, selection for multiple aleurone layers, and increasing the size of germ. Recurrent selection merits consideration but only limited use has been made of this approach. It requires well-established laboratory facilities to perform precise analysis. Other constraints in this approach include lack of wide range in lysine values, difficulty of transferring the improved trait to other genetic backgrounds, and perhaps a long time span in realizing lysine values close to *opaque-2* and with no certainty of enhanced biological value. The size of germ can be exploited favorably to enhance both protein

quantity and quality. It will, however, be difficult to achieve lysine levels matching *o-2* allele. The selection process may affect other traits adversely and may have a negative effect on shelf life of corn. Double mutant combinations of *o-2* and *fl-2* with waxy were also developed but had the problem of unacceptable kernel phenotype.

In the second category, options involving mutant genes, also three options were investigated. These were search for new and better mutants, developing double mutant combinations, and using a combined *o-2* and genetic modifier approach. The search for new mutants has continued, and a few additional mutants—*opaque-7* (Mcwhirter 1971), *opaque-6* (Ma and Nelson 1975), and *floury-3* (*fl3fl3*) (Ma and Nelson 1975)—have been discovered. Unfortunately, none of these mutants offer any advantage over *opaque-2* gene. The double mutant combination *su2o2* appeared promising as it had vitreous kernels and protein quality marginally better than *opaque-2* gene alone (Paez 1973). The kernels are, however, smaller in size reflecting reduced grain yield of about 15–25 % depending on the genetic background. The yield gap can be narrowed down through recurrent selection, but it is highly improbable that the gap can be eliminated completely (Vasal et al. 1980; 1984b; Vasal 1993, 2000, 2002). The double mutant *o2fl2* has resulted in vitreous kernels, but unfortunately it happens in only rare backgrounds. In addition, the double mutant shows effects on lysine, protein, and light transmission (Paez et al. 1970). The combined *o2* and genetic modifier approach appeared promising in altering kernel phenotype and to remedy some of the defects associated with the introduction of *o2* gene. This approach has been used widely in developing agronomically acceptable germplasm.

Enough information and confidence in this approach were already available when CIMMYT maize researchers wanted to make a switch over in the breeding program. Another important point which I wish to make is that effort devoted to developing soft opaques was not a complete waste. It is from these materials we had to look desperately for variation in modified kernel behavior and got initial hints as to the types of genetic backgrounds which will enhance our chances of success in developing QPM (Vasal 2000).

2.5 Genetic Modifiers and Their Use as a Successful Strategy

Earlier several breeding options were mentioned and briefly discussed. Unfortunately, most, if not all, had shortcomings of different nature and found no practical use in breeding program. The approach that seemed to have promise involved exploitation of genetic modifiers of *opaque-2* locus. It offered hope in improving phenotype of soft opaques to hard and in remedying other problems confronting these materials. CIMMYT scientists viewed this approach with great optimism and felt that it will have great application in the future in developing high-quality protein materials meeting producer, consumer, and industrial acceptance. The genetic modifiers are complex in their inheritance, the additive genetic effects being more important than the dominance effects. Also their expression varies with the genetic background of the material in regard to kernel modification, density, and biochemical composition of the grain. Our own experience also demonstrates that in some genotypes, protein quality may suffer a decline. Monitoring of protein quality thus becomes an important component in accumulating modifiers not affecting quality. The new strategy thus brought successes, but also new challenges unknown previously. The progress was slow and frustrating in the beginning as the starting point was zero or so-called soft *opaque-2* materials. Modifiers also exert maternal influence thus complicating the selection process. Negative influence on protein quality in some genotypes was disturbing as protein quality had to be monitored continuously and slowed down progress in agronomic performance. Conversion program remained no simpler. In fact, it became lengthy, cumbersome, and difficult. The segregating generations need to be handled much more carefully to avoid mistakes in selection. The breeding work also had to depend on analyses provided by the laboratory.

2.6 Development of QPM Stocks

Moving from soft opaques to hard endosperm was highly critical if such materials were to be accepted in developing countries. Any approach under consideration should therefore involve a change in kernel phenotype. Of the three options at hand at that time, a combination of *opaque-2* gene and genetic modifiers was considered most appropriate to rectify phenotypic appearance and other agronomic problems affecting this maize. CIMMYT's choice of this approach has proved to be a successful and a viable strategy. No other option(s) used by other institutes has demonstrated success of a similar magnitude. Shift in strategy from soft to hard endosperm was thus a major change but at the same time an equally challenging task. There was a lot of routine hard work, frustration in encountering modified kernel variation, lack of stability and reversion to soft texture in the ensuing generation, and more disturbingly a decline in protein quality in some instances. To deploy this strategy on a massive scale required development of white- and yellow-seeded QPM donor stocks. Genetic variation for kernel modification was encountered in several *opaque-2* conversions and *opaque-2* populations under improvement. Frequencies of modified kernels, however, varied considerably among different genotypes. Higher frequencies were observed in flint Cuban and Caribbean germplasm as well as in Thai *opaque-2* composite and composite K. Modified *opaque-2* ears were selected independently from several genetic backgrounds during the conversion process and during maintenance and multiplication of *opaque-2* materials. Selected kernels were planted each generation separately from each ear for several generations. This effort led to the development of a large number of white and yellow hard endosperm *opaque-2* selfed families. Later, white and yellow families were recombined separately to give rise to white and yellow hard endosperm *opaque-2* composites, respectively. This constituted the first approach in developing QPM donor stocks. Genetic mixing coupled with selection for modified ears and modified kernels of good protein quality was practiced for 3–4 cycles.

The second approach involved intrapopulation selection for modifiers in some populations exhibiting a higher frequency of modified kernels. Four tropical and one highland population which met this criteria were chosen for this purpose. These populations were composite K, Ver.181-Ant.gpo.2 × Venezuela-1 opaco-2, Thai *opaque-2* composite, PD (MS6) H.E.o2, and composite I. The breeding procedure used was controlled full-sib pollinations initially followed by modified ear-to-row system suggested by Lonnquist (1964). Between- and within-ear selection for modification was practiced at all stages.

Some unexpected chance events inspired confidence and aided in a big way in deploying two genetic systems strategy on a grand scale. This was particularly true in a population cross of two *opaque-2* versions originating independently in Thailand and Venezuela which exhibited a very high frequency of mosaic modified kernels.

QPM donor stocks developed using above mentioned approaches improved considerably in the frequency of modifiers by mid-1970s. At this point, it was decided to use these materials as donor stocks as well as QPM populations for further improvement using appropriate schemes.

2.7 Expanded QPM Germplasm Development Efforts

Choosing carefully a single approach/strategy was critical in contrast to several independent ones in tackling problems. Also any practical strategy in use must address the problem of yield penalty and phenotypic appearance of the kernel. The strategy combined use of *opaque-2* gene and genetic modifiers was most appropriate in this respect.

QPM germplasm development efforts were not limited to a few genetic backgrounds. In addition, several different approaches and strategies were put into action to develop a wide array of germplasm products meeting the requirements

Table 2.1 Across-location performance of normal materials and corresponding tropical QPM germplasm, 1987

	Grain yield (kg/ha)		
Material	Normal	QPM	QPM (% of normal)
Pool 23	5,405	5,330	98.6
Pool 24	5,706	5,457	95.6
Tropical high oil	5,733	5,170	90.2
Population 62	5,347	5,484	102.6
Population 65	5,255	5,369	102.2
Population 63	5,705	6,236	109.3

Source: CIMMYT

Table 2.2 Mean grain yield of four highest yielding QPM varieties, normal reference variety, and best local checks at 11 locations, CIMMYT EVT 15B, 1988

Variety	Grain yield (t/ha)	Grain yield (% of normal ref. entry)
Across 8563 QPM	5.68	103.2
Capinopolis 8563 QPM	5.48	99.7
Iboperenda 8563 QPM	5.47	99.4
Tarapoto 8563 QPM	5.37	99.7
Suwan 8222 NRE	5.50	100.0
Best local check	5.36	97.5

Source: CIMMYT

for different agroecologies. The tactical strategy was to go in a big way to develop huge germplasm volume followed by merging and reorganization of germplasm considering adaptation, maturity, and kernel colors. This would help reducing germplasm to a manageable level and permit systematic handling. We are harnessing benefits of this strategy, without which QPM may have found only limited use in some countries.

In developing huge volume of QPM germplasm, the thrust was on two principal approaches. The first was to convert most of the advanced unit populations representing tropical, subtropical, and highland adaptation involving different maturities, grain color, and texture. A backcross-cum-recurrent selection procedure was used for this purpose (Vasal et al. 1984b). During the conversion process, emphasis was placed on yield, kernel modification and appearance, reduced ear rot incidence, rapid drying, and other agronomic attributes. Compared to normal counterparts, some of the QPM versions have performed quite similar in yield and agronomic traits (Vasal et al. 1984a, b, 1993).

The second approach involved formation and improvement of QPM gene pools. These pools were developed using different procedures but had homozygous *opaque-2* background to facilitate selection and accumulation of genetic modifiers. A modified half-sib system was used for the improvement of these pools. A total of seven tropical and six subtropical QPM gene pools were developed and improved continuously for varying numbers of cycles.

There was proof of continuing progress in improving germplasm which provided clear-cut evidence for others to see and appreciate (Bjarnason and Vasal 1992; Bjarnason et al. 1998; Vasal 2000, 2002; Vasal et al. 1994). Improvements were quite evident in grain yield (Tables 2.1, 2.2, 2.3, 2.4, 2.5, and 2.6) and in improving kernel modification (Tables 2.5, 2.6, 2.7, and 2.8) in several different genetic backgrounds. The protein quality as well was maintained during the selection process (Tables 2.9 and 2.10).

2.8 Reorganization and New Thrusts in QPM Germplasm Management Strategy

Beginning mid-1970s and extending next 5–6 years, a huge volume of QPM germplasm was developed that could meet germplasm needs of several production environments in the tropics and subtropics. At this point, it was realized that continuing conversion program will serve

Table 2.3 Superior white QPM hybrids tested across fifteen locations at El Salvador, Guatemala, and Mexico, 1998

Pedigree	Yield (t/ha)	Ear rot (%)	Tryptophan (%)	Ear modification	Silking (days)	Plant height (cm)
CML142 × CML146	6.48	3.7	0.096	2.0	55	242
CML159 × CML144	6.39	4.3	0.100	1.6	56	230
(CLQ6203 × CML150) CML176	6.28	5.7	0.088	2.1	55	239
CML145 × CML144	5.81	5.8	0.840	2.0	54	241
CML158 × CML144	5.59	7.1	0.103	1.3	55	228
CML146 × CML150	5.48	8.1	0.084	3.6	56	222
POZA RICA 8763 TLWD	5.34	12.0	0.095	2.8	54	230
Normal hybrid check	5.58	9.5	0.070	2.0	56	228

Local checks: HB-83, CB-HS-5G, H-59, XM7712, GUAYOPE

Table 2.4 Performance of superior tropical QPM hybrids across 41 locations in Latin America and Asia, 1999–2000

Pedigree	Grain yield (ton/ha)	Ear rot (%)	Silking (50 %)	Endosperm[a] hardness	Tryptophan in sample
(CML-141 × CML-144)	6.40	5.5	55	1.6	0.088
(CML-141 × CML-144) CML-142	6.39	6.2	55	1.7	0.081
(CML-142 × CML-146)	6.28	6.3	55	2.2	0.100
(CML-142 × CML-150)	6.20	7.8	55	2.0	0.089
(CML-142 × CML-150) CML-176	6.08	7.5	55	2.0	0.086
(CLQ-6203 × CML-150)	5.80	7.2	55	2.3	0.090
(CML-144 × CML-159) CML-176	5.64	6.0	56	1.7	0.094
(CML-144 × CML-159) (RE)	5.93	5.9	56	1.9	0.093
Local Check-1	5.95	7.6	55	1.9	0.050

[a]Rating scale 1–5: 1 – completely hard, 5 – completely soft

Table 2.5 Comparison of original and latest cycle of selection in two QPM gene pools

Material	Cycle	Yield (kg/ha)	Days to flower	Plant height (cm)	Soft cars (%)	Ear aspect[a]	Endosperm[b] modification
Temperate × tropical QPM (flint)	C_0	4,437	59	212	66	4.0	4.2
	C_{12}	4,920	55	192	4	2.2	1.6
Temperate white QPM	C_0	3,445	55	204	34	3.5	3.7
	C_5	3,669	55	192	10	2.3	1.9

[a]Rating scale 1–5: 1 – excellent, 5 – poor
[b]Rating scale 1–5: 1 – completely modified, 5 – completely soft

no further useful purpose. Instead, perhaps it will be more appropriate to accelerate progress by switching over to working in homozygous *opaque-2* genetic backgrounds. Merging, consolidation, and reorganization of QPM germplasm were done to reduce the number of QPM gene pools and populations to a level so that these could be handled in a more systematic way and have some correspondence between pools and populations. From the available QPM

Table 2.6 Agronomic characters of first and last cycles of selection in six QPM gene pool and populations

Pool/population	Cycles of selection	Grain yield (kg/ha)	Days to silk[a]	Plant height (cm)	Endosperm modification
Population 62 QPM	C0	4572	57.7	239	3.4
	C5	4825	56.7**	225**	2.0**
Population 65 QPM	C0	4277	57.5	235	3.1
	C5	4406	55.3**	228	2.0**
Pool 23 QPM	C1	4919	60.3	251	3.9
	C19	5146	57.4**	227**	1.8**
Pool 24 QPM	C1	5007	60.9	243	3.9
	C19	5450**	58.6**	234*	2.4**
Pool 31 QPM	C0	5834	65.9	211	3.0
	C16	5880	65.0**	195**	1.6**
Pool 32 QPM	C0	5883	66.3	210	3.1
	C16	6172**	65.2**	200**	2.0**

Source: CIMMYT (Bjarnason, Short, Vasal, and Villegas)
*, **Significant at 5 % and 1 % probability levels respectively
[a]Rating scale 1–5: 1 – completely modified, 5 – completely soft

Table 2.7 Improvement in kernel modification during different cycles of selection

	Kernel modification[a]			
Material	C0	C4	C9	L.S.D (.05)
Temperate × Trop. H.E.o_2 (flint)	3.1	2.6	2.1	0.26
Temperate × Trop. H.E.o_2 (dent)	3.0	2.5	2.2	2.20
Blanco Cristalino H.E.o_2	3.7	2.8	2.3	0.20
Amarillo Dentado H.E.o_2	3.2	2.6	2.4	0.43

[a]Rating scale 1–5: 1 – completely vitreous, 5 – completely soft

Table 2.8 Mean kernel modification score, standard deviation, range, and Weinberg constant values of different cycles of selection in yellow QPM grown in 1978

Cycle	$\bar{x}$ kernel modification score[a]	Standard deviation	*Range*	Weinberg constant
C_1	3.7	1.07	2.9–4.8	1.60
C_4	2.9	0.92	2.3–3.7	1.57
C_7	2.3	0.81	1.6–3.2	1.29
C_9	2.2	0.91	1.2–3.5	1.23

[a]Rating scale 1–5: 1 – completely modified, 5 – completely soft

germplasm, seven tropical and six subtropical gene pools were formed and these are listed in Table 2.11. In addition, six tropical and four subtropical populations were developed (Table 2.12). The handling of QPM gene pools and QPM populations has been discussed in earlier publications (Vasal et al. 1979, 1984a, b, 1997).

2.9 QPM Hybrid Development and Testing

There have been several turning points in QPM research at CIMMYT. This led to changing emphasis in the program more than once in

Table 2.9 Percent protein, tryptophan, and lysine in the whole grain of QPM pools

QPM gene pool	Protein (%)	Tryptophan in protein (%)	Lysine in protein (%)	Quality index
Pool 15 QPM	9.1	0.94	4.2	4.6
Pool 17 QPM	8.9	1.04	4.5	4.5
Pool 18 QPM	9.9	0.93	4.0	4.6
Pool 23 QPM	9.1	1.03	3.8	4.2
Pool 24 QPM	9.4	0.92	3.8	4.0
Pool 25 QPM	9.8	0.94	4.0	4.0
Pool 26 QPM	9.5	0.90	4.1	4.3
Pool 27 QPM	9.5	1.05	4.2	4.8
Pool 29 QPM	9.2	1.06	4.3	4.8
Pool 31 QPM	10.2	0.96	4.1	4.5
Pool 32 QPM	8.9	1.04	4.2	4.5
Pool 33 QPM	9.3	1.05	–	4.2
Pool 34 QPM	9.1	1.10	4.1	4.5

Table 2.10 Protein quality in different cycles of selection (endosperm analysis)

Material	Cycle	Protein (%)	Tryptophan (%)	Lysine (%)
CIMMYT H.E. o2	C0	8.0	0.78	2.90
	C4	8.6	0.77	2.90
PD (MS) 6 H.E. o2	C0	8.5	0.78	2.50
	C4	8.8	0.78	2.60

Table 2.11 QPM gene pools and their characteristics

QPM pool number	Adaptation	Maturity	Seed color	Seed texture
Pool 15 QPM	Tropical	Early	White	Flint-dent
Pool 17 QPM	Tropical	Early	Yellow	Flint
Pool 18 QPM	Tropical	Early	Yellow	Dent
Pool 23 QPM	Tropical	Late	White	Flint
Pool 24 QPM	Tropical	Late	White	Dent
Pool 25 QPM	Tropical	Late	Yellow	Flint
Pool 26 QPM	Tropical	Late	Yellow	Dent
Pool 27 QPM	Subtropical	Early	White	Flint-dent
Pool 29 QPM	Subtropical	Early	Yellow	Flint-dent
Pool 31 QPM	Subtropical	Intermediate	White	Flint
Pool 32 QPM	Subtropical	Intermediate	White	Dent
Pool 33 QPM	Subtropical	Intermediate	Yellow	Flint
Pool 34 QPM	Subtropical	Intermediate	Yellow	Dent

different years and at different stages of germplasm development efforts. As discussed earlier, some of these changes related to shifts in the use of mutants or combined use of more than one genetic system, changing emphasis from soft to hard endosperm opaques, breeding approaches and strategies, huge germplasm development efforts initially and later consolidating into a more definite number to satisfy germplasm needs and permitting handling in a more

Table 2.12 QPM populations and their characteristics

Population number	Name	Adaptation	Maturity	Seed color	Seed texture
61	Early yellow flint QPM	Tropical	Early	Yellow	Flint
62	White flint QPM	Tropical	Late	White	Flint
63	Blanco Dentado-1 QPM	Tropical	Late	White	Dent
64	Blanco Dentado-2 QPM	Tropical	Late	White	Dent
65	Yellow flint QPM	Tropical	Late	Yellow	Flint
66	Yellow dent QPM	Tropical	Late	Yellow	Dent
67	Templado Blanco Cristalino QPM	Subtropical	Intermediate	White	Flint
68	Templado Blanco Dentado QPM	Subtropical	Intermediate	White	Dent
69	Templado Amarillo QPM	Subtropical	Intermediate	Yellow	Flint
70	Templado Amarillo Dent QPM	Subtropical	Intermediate	Yellow	Dent

systematic manner, and more importantly working in homozygous *opaque-2* genetic backgrounds. These constant changes and adjustments were made to achieve rapid progress in overcoming problems affecting QPM materials and to emphasize competitive agronomic performance of these materials to similar or comparable normal counterparts.

Perhaps the next turning point was most important and significant in QPM research efforts. This was a new initiative related to introduction of QPM hybrid research and development at CIMMYT in 1984–1985 (Vasal 1986, 1987, 1994, 2002). This decision was particularly important for QPM research as the use of inbred-hybrid approach offered several advantages, including seed purity, enhanced kernel modification and added uniformity and stability, ease in monitoring and maintaining protein quality, realizing enhanced yield potential, addressing and circumventing problems more effectively and efficiently, greater market acceptability, and more importantly spurring seed industry growth. With the initiation of hybrid development activities, combining ability information was generated, and heterotic patterns of important classes of QPM germplasm were established (Vasal et al. 1986; Vasal et al. 1993a, b). Inbreeding was introduced more vigorously in population improvement activities to improve inbreeding tolerance and to increase the frequency of extracting superior inbreds from such populations. Other aspects of hybrid development including inbred development, inbred evaluation, and hybrid formation were initiated on a modest scale. Inbred and hybrid performance also permitted formation of synthetics based on combining ability information. Even with a few hybrids formed and tested, the results were very encouraging. Several QPM hybrids had performance levels quite similar or superior to better performing hybrids (Tables 2.3 and 2.4). The past 5 years or so have witnessed much greater emphasis on QPM hybrid formation and testing at CIMMYT. The international testing of QPM hybrids has increased substantially and many national programs are finding these hybrids quite satisfactory in performance to the available commercial check hybrids. Several countries have released hybrids based on CIMMYT QPM lines while others are on the verge of releasing soon. CIMMYT maize program has also released 59 QPM lines in two separate announcements in 1992 and 2003 (CIMMYT 1998, 2004).

Hybrid initiative turned out to be exceedingly important for QPM efforts and success. Without hybrid development efforts, it is likely that QPM OPVs may not have made the same impact and success stories. We are all aware that good science is not free from difficulties, frustration, and criticism. This should be viewed to generate creativity, revisiting different approaches and activities, and making constant adjustments for efficient use of resources at all levels. Periodic

reviews help to de-emphasize some aspects of the program while expanding others if necessary and introducing new initiatives which were not already in place. One good example will be hybrid initiative in mid-1980s, which proved extremely beneficial for QPM research and promotional efforts. Without this initiative we perhaps may not have realized full potential and benefits of QPM. It is encouraging to state that we are already harnessing the fruits of this important decision. Many countries in recent years have released directly CIMMYT-developed hybrids and in a few cases are using CIMMYT lines in combination with their own lines. The outlook of QPM appears promising, and many countries in the developing world are becoming increasingly interested in QPM efforts.

2.10 Interdisciplinary Cooperation and Some Important Lessons Learned

During QPM development over the years, a number of important lessons were learned which have a bearing and relevance to similar plant breeding situations in maize and other crops especially where major gene(s) is used to introduce some important attributes into the elite germplasm. Invariably major genes with drastic effects are associated with negative and pleiotropic effects. To achieve success and desired results will require accumulation of favorable modifying alleles and will necessitate the need to work in homozygous genetic backgrounds contrary to commonly used backcross programs.

New initiatives are important and could make a big difference and in some instances result in breakthroughs. This is particularly true for those breeding objectives which are important yet controversial. Selection of breeding options and strategies needs to be based on convincing evidence and progress as was the case with the use of combined *opaque-2* and genetic modifiers approach.

Choosing carefully a single strategy/approach is critical in contrast to several independent ones in tackling multiple problems. The approach used at CIMMYT in QPM research is an excellent example to prove this point. Deploying the right strategy of *o2* and genetic modifiers, all problems plaguing these materials were addressed and good progress made on all fronts.

In problematic situations as was the case with high-lysine mutants, the germplasm efforts should not be limited to a few genetic backgrounds. Also several different approaches and strategies need to be put into action to develop a whole array of germplasm products meeting requirements for different agroecologies. The tactical strategy in QPM research was to develop a huge volume of germplasm followed by merging and reorganization of total germplasm into a definite numbers of pools and populations that were required for different ecologies. This helped reducing germplasm volume to a manageable level and also permitted more systematic handling through appropriate breeding schemes. We are harnessing benefits of this strategy, without which QPM may have found its use in only limited environments and perhaps in only a few countries.

QPM development required continuous monitoring of protein quality and thus needed a strong support from biochemical laboratory (Villegas et al. 1992). Simple analytical techniques were developed and used to analyze large number of samples in a rapid and efficient manner to provide results to the breeders in a timely fashion to make right decision at right time. This resulted in saving of resources and manpower use. Collaboration between biochemists and breeders was excellent, and each group was aware of the needs, urgencies, and work priorities of the other. Interdisciplinary cooperation in QPM work at CIMMYT is perhaps exemplary, and without the strong support of laboratory, this work may not have attained success that we are witnessing today. The roles of other disciplines also cannot be underestimated especially pathology, entomology, and physiology which rendered services

Table 2.13 QPM releases involving CIMMYT germplasm

Name	Institutions/country	CIMMYT germplasm involved
SHAKTIMAN – 1	DMR, India	(CML 142, CML 150)
SHAKTIMAN – 2	DMR, India	(CML 176, CML 186)
HQ 2000	NMRI, Vietnam	(CML 161, CML 165)
YunYao 19	Yunnan, China	(CML 140)
Yun You 167	Yunnan, China	(CML 194)
Qian 2609	Guizhou, China	(CML 171)
Lu Dan 206	Shandong, China	(P70)
Lu Dan 207	Shandong, China	(P70)
Lu Dan 807	Shandong, China	(P70)
Hybrid 2075	Sichuan, China	(CIMMYT QPM populations)
Zhongdan 9409	CAAS, China	(Pool 33 QPM)

Table 2.14 QPM releases in some countries

	No. of releases		
Country	QPM OPV	QPM hybrid	Total
Mexico	5	21	26
India	1	2	3
Brazil	3	–	3
Nicaragua	1	1	2
Guatemala	–	1	1
El Salvador	–	1	1
Honduras	–	1	1
China	–	5	5
Colombia	–	1	1
Mozambique	1	–	1
Mali	1	–	1
Uganda	1	–	1
Benin	1	–	1
Burkina Faso	1	–	1
Guinea	1	–	1
South Africa	–	1	1
Peru	–	1	1
Venezuela	–	1	1
Vietnam	–	1	1
Total	16	37	53

and support in evaluating QPM germplasm under different stresses.

QPM has been an excellent training ground for maize researchers, especially postdoctoral fellows and visiting scientists. Researchers could be exposed to a whole array of activities going on in the maize program in addition to QPM. Many current CIMMYT staff had the privilege of working in QPM subprogram in the initial stages of their careers.

2.11 Renewed Interest and Emphasis on QPM Research

Quality protein maize is a scientific breakthrough and a great success story. Maize scientists feel proud of QPM research at CIMMYT and elsewhere. CIMMYT maize staffs have gained confidence and strength in QPM research efforts and are aspiring to do more to spread the benefits of this maize to more developing countries. Recent releases and field days have been impressive (Tables 2.13, 2.14, 2.15, and 2.16). Expectations of all of us are to have more area under this maize in the developing countries in the future. Sasakawa Global 2000 under the leadership of Dr. Norman E. Borlaug and several other staff of this organization has played a vital role in promoting QPM in several countries of Africa. The success has revitalized QPM research at CIMMYT and in many national programs around the world.

The past few years have witnessed lots of QPM excitement. Hybrid performance of CIMMYT-developed QPM hybrids has been very encouraging (Tables 2.3 and 2.4). It is time to think of plans for the future if widespread benefits of this research are to be realized. There is a need to further strengthen QPM research at CIMMYT and in some of its regional programs. It is time that some of the strong NARs should also revive QPM research. Currently, maximum use of CIMMYT germplasm is made, but this germplasm needs to be diversified and broadened at national and international level.

Table 2.15 Recent QPM releases in some countries

Name	Type	Pedigree	Country
HQ INTA-993	Hybrid	(CML144 × CML159) CML176	Nicaragua
NB-Nutrinta	Open pollinated	Poza Rica 8763	Nicaragua
HB-PROTICTA	Hybrid	(CML144 × CML159) CML176	Guatemala
HQ-61	Hybrid	(CML144 × CML159) CML176	El Salvador
HQ-31	Hybrid	(CML144 × CML159) CML176	Honduras
Zhongdan 9409	Hybrid	Pool 33 × Temp QPM	China
Zhongdan 3850	Hybrid		China
QUIAN2609	Hybrid	Tai 19/02 × CML171	China
Yun Yao 19	Hybrid	(CML140)	China
Yun You 167	Hybrid	(CML194)	China
Lu Dan 206	Hybrid	(P70)	China
Lu Dan 207	Hybrid	(P70)	China
Lu Dan 807	Hybrid	(P70)	China
Hybrid 2075	Hybrid	(CIMMYT QPM Populations)	China
ICA-	Hybrid	(CML144 × CML159) CML176	Colombia
Susuma[a]	Open pollinated	Across 8363SR	Mozambique
Obatampa[a]	Open pollinated	Across 8363SR	Mali
Obangaina[a]	Open pollinated	Across 8663SR	Uganda
Obatampa[a]	Open pollinated	Across 8363SR	Benin
BR-473	Open pollinated		Brazil
BR-451	Open pollinated		Brazil
AssumPreto	Open pollinated		Brazil
Obatampa[a]	Open pollinated	Across 8363SR	Burkina Faso
Obatampa[a]	Open pollinated	Across 8363SR	Guinea
QS-7705[a]	Hybrid		South Africa
GH-132-28[a]	Hybrid	P62, P63	China
INIA-	Hybrid	CML161 × CML165	Peru
FONAIAP	Hybrid	(CML144 × CML159) CML176	Venezuela
HQ-2000	Hybrid	CML161 × CML165	Vietnam
SHAKTIMAN-1	Hybrid	(CML142 × CML150) CML176	India
SHAKTIMAN-1	Hybrid	CML176 × CML186	India
In Mexico, 21 hybrids and 5 open pollinated varieties including…			
44IC	Hybrid	CML142 × CML116	Mexico
H-551C	Hybrid	CML142 × CML150	Mexico
H-553C	Hybrid	(CML142 × CML150) CML176	Mexico
H-519C	Hybrid	(CML144 × CML159) CML170	Mexico
H-368EC	Hybrid	CML186 × CML149	Mexico
H-369EC	Hybrid	CML176 × CML186	Mexico
VS-537 C	Open pollinated	POZA RICA 8763	Mexico
VS-538 C	Open pollinated	ACROSS 8762	Mexico

Table 2.16 Some prominent QPM varieties and hybrids released in different countries

S. no.	QPM varieties/hybrids	Countries
1	(CML144 × 159) × CML176	Nicaragua, Guatemala, El Salvador, Honduras, Mexico, Venezuela, Colombia
2	CML176 × CML186	India, Mexico
3	CML161 × CML165	Peru, Vietnam, Guangxi (China)
4	(CML142 × CML150) × CML176	India, Mexico
5	Across 83635R	Mozambique, Mali, Uganda, Benin, Burkina Faso, Guinea
6	Poza Rica 8763	Nicaragua, Mexico

More researchers need to be trained who can deploy QPM skills. Resources should be mobilized for more QPM work, and future strategies should make increasing use of modern technologies and tools. QPM dissemination should accompany extensive on-farm research and demonstration of superior QPM varieties and hybrids on farmer's fields.

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Maize Protein Quality and Its Improvement: Development of Quality Protein Maize in India

3

M.L. Lodha

Abstract

The quality of maize protein is poor due to the deficiencies of two essential amino acids, lysine and tryptophan, and excess of leucine. However, the discovery of association of high lysine and tryptophan with *opaque-2* maize endosperm in 1964 opened up new vistas in improving maize protein quality. Consequently, many countries started developing maize varieties incorporating *opaque-2* gene, and in the Indian Maize Programme, three *opaque-2* composite varieties were developed and released for commercial production in the year 1970. Though the nutritional superiority of *opaque-2* maize over normal maize was very well established, the newly developed high-lysine varieties could not become popular because of agronomic and acceptability problems associated with their soft chalky endosperm. To circumvent this problem, efforts were initiated in 1971 itself towards developing hard-endosperm *opaque-2* maize lines/strains through the continuous process of vigorous selection for kernel vitreosity and monitoring tryptophan/lysine for maintaining protein quality. The modified *opaque-2* maize with hard endosperm and having high lysine and tryptophan is known as quality protein maize (QPM). At present, in India we have one QPM composite and more than nine QPM hybrid varieties released, and many more are in pipelines. However, what is required is the popularization of these varieties with farmers as well as consumers highlighting their nutritional significance, especially to the vulnerable group, i.e. infants and preschool children and pregnant and lactating mothers.

3.1 Introduction

In India, maize is the third most important food crop after rice and wheat. Its production has reached 19.77 million tons, out of which about 25 % is used as human food, 12 % as animal feed and 49 % as poultry feed (Dass et al. 2008). Thus,

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maize plays an important role in food and nutritional security. Besides being the principal source of carbohydrates and energy, maize like other cereals is also the largest single source of protein in the diet of the people for whom it is a staple food. However, the nutritional quality of maize protein is poor because of imbalanced amino acid composition due to deficiencies of two main essential amino acids, lysine and tryptophan, and excess of leucine. Alcohol-soluble protein fraction zein (prolamine), which is extremely deficient in lysine and tryptophan, is the most abundant protein (40–60 %) in maize endosperm. In whole grain of maize varieties, the protein content ranges from 8.5 % to 13.6 %, whereas lysine from 2.5 % to 3.6 % and tryptophan from 0.37 % to 0.67% of protein. In mature maize kernel, endosperm accounts for 80–85 % and contributes as much as 80 % of total kernel protein. Embryo (germ) accounts for 8–10 % of total weight and contributes 15–20 % of total protein. The niacin/tryptophan deficiency disease, pellagra, caused by high concentration of leucine or high leucine–isoleucine ratio, is commonly associated with maize-eating people. The deficiency is recognized by 3 Ds: dermatitis, diarrhoea and dementia.

The discovery of association of high lysine and tryptophan with *opaque-2* maize endosperm by Mertz et al. (1964) opened up new vistas in improving the protein quality of maize. The *opaque-2 (o2)* single recessive gene, which is located on chromosome 7, encodes a transcriptional activator that regulates the expression of alpha-zein genes (Schmidt et al. 1990). In the presence of *opaque-2* gene, the synthesis of 22 kD alpha-zein, which is extremely deficient in lysine and tryptophan, is reduced and the synthesis of non-zein protein fractions, particularly glutelin, is increased in the endosperm resulting in overall increase in the concentrations of lysine and tryptophan in endosperm protein. In addition, there exists a highly positive correlation between lysine concentration and protein synthesis factor EF-1alpha, which is a lysine-rich abundant protein and can be used as a biochemical marker in the assay of lysine-containing proteins (Habben et al. 1995; Moro et al. 1996).

3.2 Essential Amino Acids and Protein Quality

Proteins are an important constituent of our diet and are required for (i) foetal development in pregnancy and milk output during lactation, (ii) growth in infants and children and (iii) maintenance (replacing the wear and tear in tissues) in adults. Amino acids are the building blocks of the proteins. Food and tissue proteins contain 20 amino acids, of which eight (nine for infants) are designated as 'essential amino acids', since they cannot be synthesized in the body. The rest of the amino acids are called 'non-essential' as they can be formed in the body. The proteins needed by our body are supplied through the diet we consume. The dietary proteins are broken down into amino acids, absorbed as such and used by the body to synthesize the proteins needed for various functions like tissue building, replacement of proteins depleted and synthesis of various functional molecules.

The quality of dietary proteins depends on the pattern of essential amino acids it supplies. The best quality protein is one which provides essential amino acid pattern very close to the pattern of the tissue proteins. The minimum amount of essential amino acids required by infants is also taken as reference pattern for defining the quality of proteins. Milk and egg proteins, which are of superior quality, also serve as reference protein. As per the recommendations of the Joint FAO/WHO Expert Consultation (1991), the essential amino acid composition (mg/g protein) of reference protein for 2–5-year-old children is lysine 58, threonine 34, tryptophan 11 and methionine + cysteine 25. As such, the first limiting amino acid in cereal proteins is lysine, while the second limiting amino acid is threonine/tryptophan. Pulse proteins are rich in lysine but deficient in sulphur-amino acids, mainly methionine. Cereal or pulse proteins individually are, therefore, incomplete proteins. However, when cereals and pulses are consumed together, they have a mutually supplementary effect; deficiency of an amino acid in one can be made good by an adequate level in another. A mixed cereal-based

diet can meet the protein requirements of adults and older children provided they consume enough of the diet to meet their energy needs. However, it is the vulnerable group of infants and preschool children who need protein of superior quality, and cereal-based diet or a mixed diet of plant proteins cannot meet their protein requirements adequately. For this group, the requirement of protein per unit body weight is also higher, the safe level of reference high-quality protein for preschool children being 1.1 g/kg and for adults 0.75 g/kg body weight.

The nutritive value of food grain proteins, depends upon their essential amino acid make up as well as digestibility. For chemical evaluation of protein quality, first and/or second limiting amino acid in respective food grains is determined. For example, in maize protein, lysine is the first limiting amino acid and tryptophan is the second. The overall quality, i.e. the nutritive value of food grain proteins is determined in a nitrogen-balance study with laboratory animals like growing albino rats. From this study, biological value (BV) of proteins, which is a percentage of N-retained by the experimental animals out of N-absorbed from the diet, is obtained. This test also gives an estimate of true digestibility (TD) of proteins which indicates the per cent N-absorbed from the diet. Net protein utilization (NPU) value, a multiplication product of TD and BV, gives a measure of per cent N-retained out of total N-consumed. Utilizable protein (UP) value, a multiplication product of NPU and per cent protein in the test food grain, is a measure of both protein quality as well as quantity. Another simple measure of the nutritive value of proteins could be protein efficiency ratio (PER), which indicates the gain in weight of young animals per unit weight of protein consumed. For this type of study, usually weanling albino rats are used.

3.3 Development of *Opaque-2* Maize Varieties and Improvement in Protein Quality

Soon after the discovery that *opaque-2* gene improves the contents of lysine and tryptophan in maize endosperm, maize breeders all around the world started incorporating this gene into their elite maize varieties. In India also the work started in 1966, and three *opaque-2* maize composite varieties, namely, Shakti, Rattan and Protina, were developed under the auspices of All India Coordinated Maize Improvement Project and released for commercial production during 1970. In the endosperm of these varieties, lysine and tryptophan contents increased by about 100 % and 160 %, respectively, while leucine content decreased by about 35 % as compared to normal maize hybrid Ganga-5 (Table 3.1).

The *opaque-2* gene apparently acts as zein suppressant in the developing endosperm. In normal maize endosperm, zein accounts for more than 50 % and glutelin for about 35 % of the total protein. In *opaque-2* endosperm, however, glutelin becomes the major fraction and zein content is reduced drastically. In addition, there are relative increases in albumin and globulin fractions (Table 3.2), which are rich in lysine and tryptophan.

The nutritional quality of normal and *opaque-2* maize protein has been evaluated using albino rats, swine, children as well as adults.

Table 3.1 Amino acid composition of three *opaque-2* composites and normal maize hybrid endosperm protein

		Opaque-2		
Amino acid (g/100 g endosperm protein)	Ganga-5 (normal)	Shakti	Rattan	Protina
Lysine	1.88	4.07	3.76	3.68
Tryptophan	0.35	0.92	0.93	0.90
Leucine	14.76	9.19	9.72	9.26
Protein (%)	8.5	9.20	9.0	6.1

Source: Singh and Koshy (1974)

Table 3.2 Protein fractionation of normal, soft opaque and hard-endosperm *opaque-2* versions of Tuxpeno-1 maize

Fraction	Normal	Soft *opaque-2*	Hard-endosperm *opaque-2*
I (Albumin + Globulin)	6.2	20.6	15.5
II (Zein)	39.2	8.1	10.4
III (Zein-like)	19.7	10.7	16.2
IV(Glutelin-like)	13.6	18.5	21.4
V (Glutelins)	22.4	42.5	36.6

Source: Vasal (1994)

Table 3.3 Nutritive value of proteins of normal and *opaque-2* maize

Protein value expressed as %	Casein	Vijay (composite)	Ganga-5 (hybrid)	Bassi (local)	Shakti (*opaque-2* composite)
TD	100	92.1	92.8	93.7	95.2
BV	84.1	56.4	59.8	56.0	76.0
NPU	84.2	52.2	55.6	52.6	72.3
UP	71.38	5.75	6.56	6.94	8.44
Protein (%)	84.81	11.00	11.81	13.19	11.69

Source: Lodha et al. (1976b)
TD true digestibility, *BV* biological value, *NPU* net protein utilization, *UP* utilizable protein

3.4 Rat-Feeding Experiments

The nutritional superiority of *opaque-2* maize over normal maize has been well established in feeding trials with albino rats as experimental animals. In a nitrogen-balance study, the BV of *opaque-2* maize (Shakti) protein was found to be 90 % of milk protein casein, while that of normal maize (Vijay, Ganga-5, Bassi) protein was less than 70 % of casein (Lodha et al. 1976b). The NPU and UP values were also higher for *opaque-2* maize (Table 3.3). The protein efficiency ratio (PER) value for *opaque-2* maize has been found to be 62–110 % superior to that of ordinary maize, the PER for *opaque-2* maize being 2.3–2.8 and that for normal maize 1.3–1.6. Further, 1 g of dietary *opaque-2* maize resulted in 0.226 g weight gain in albino rats as against only 0.131 g with normal maize.

3.5 Child-Feeding Experiments

Opaque-2 maize has been proved to be a good supplement to the home diet of weanling and young children in terms of their weight and height gain and gain in mid-arm and chest circumference. The experiments were carried out under the auspices of All India Coordinated Maize Improvement Project, IARI, for a period of 6 months (November 1975–May 1976) with 18–30-month-old children from low-income families. The children were fed on isocalorie (405 Kcal) and comparable protein (about 10 g) diets as a supplementary midday meal, to meet out one-third of the recommended daily allowances. The children fed on *opaque-2* maize diet gained in weight 8.3 % and 25 % more as compared to those fed on skim milk and normal maize-based diets, respectively. As regards height, children fed on *opaque-2* maize diet gained 29.4 % more compared to normal maize. Similarly, children of the milk group showed maximum gains in chest and arm circumference, followed by *opaque-2* maize group and least gains were observed in normal maize group (Table 3.4).

3.6 Therapeutic Uses of *Opaque-2* Maize

Studies carried out at Universidad del Valle Hospital in Cali, Colombia, and at the National Institute of Nutrition (NIN), Hyderabad, have shown that children severely ill with ‘kwashiorkor’

Table 3.4 Average gain in weight, height, chest and mid-arm circumference of children after 6 months of supplementary feeding

	Gain in			
Feeding group	Weight (kg)	Height (cm)	Chest circumference (cm)	Mid-arm circumference (cm)
Control[a]	0.72	2.86	1.94	0.23
Milk	1.20	4.22	3.12	0.74
Normal maize	1.04	2.99	1.90	0.16
Opaque-2 maize	1.30	3.87	2.97	0.47

Source: Singh et al. (1980)
[a]For control group, the data were recorded after 5.5 months

Table 3.5 Nutritive value of proteins of developing kernels of normal and *opaque-2* maize

	Ganga-5 (normal)			Shakti (*opaque-2*)		
Protein value expressed as %	25 days	35 days	Mature	25 days	35 days	Mature
TD	76.3	81.9	92.8	80.0	84.4	95.2
BV	76.3	70.8	59.9	86.5	78.0	76.0
NPU	58.2	58.0	55.6	69.2	65.7	72.4
UP	7.8	7.3	6.6	8.8	7.7	8.4
Protein (%)	13.4	12.5	11.8	12.7	11.7	11.7

Source: Gupta et al. (1978)

can be easily cured from the protein deficiency disease if fed regularly on *opaque-2* maize diet. In addition, another study carried out at NIN with dogs suggested that if the population having maize as a staple food regularly consumes *opaque-2* maize diet, then the incidence of pellagra could be prevented.

3.7 Protein Quality of Immature Kernels

In addition to their direct use after roasting or boiling, immature kernels from green ears are used in preparing many recipes. With the development of the kernels, the TD of the protein is improved; however, the BV decreases in both normal and *opaque-2* maize. The BV values being equal (76.3 and 76.0), immature normal maize kernels harvested at the early-dough stage (25 days post-pollination) were found to be as good as mature *opaque-2* maize kernels from their BV point of view (Table 3.5).

3.8 Problems Confronting Chalky *Opaque-2* Maize and Development of Modified *Opaque-2* Maize

Though the nutritional superiority of *opaque-2* maize over normal maize has been very well established, the *opaque-2* maize varieties could not become popular all over the world due to the following agronomic and acceptability problems associated with soft and chalky endosperm: 10–15 % lower grain yield, poor kernel appearance, high susceptibility to ear rot and stored grain pests (Singh et al. 1974; Vasal 2000).

Fortunately, during the process of converting normal maize populations to their *opaque-2* versions, partially hard endosperm (i.e. vitreous) or 'modified' grains had been observed by many researchers including breeders at CIMMYT in Mexico. Separation of such grains began as early as in 1969 (Prasanna et al. 2001). At CIMMYT, under the dynamic leadership of Dr. S.K. Vasal, and at the University of Natal, various endosperm

modifier genes were identified that could favourably alter the grain characteristics, thereby overcoming an important obstacle in the popularization of high-lysine *opaque-2* maize (Sofi et al. 2009). The mechanism by which the endosperm modifiers change the grain structure from chalky to vitreous in modified *opaque-2*, is not clearly understood. The modified *opaque-2* maize with hard endosperm is known as quality protein maize (QPM). Thus, the term QPM now refers to maize homozygous for the *o2* allele, with increased lysine and tryptophan contents but without the negative secondary effects of a soft and chalky endosperm. The QPM essentially has about twice the levels of lysine and tryptophan than normal maize and also increased levels of histidine, arginine, aspartic acid and glycine, but reduced level of leucine.

In the Indian Maize Programme, though the initial emphasis was on the development of soft-endosperm *opaque-2* maize varieties, gradually the emphasis shifted to developing modified *opaque-2* maize strains with hard endosperm. The work was initiated during *kharif* (rainy season) of 1971, and a large number of inbred lines of varying vitreosity were selected from the three *opaque-2* composites, Shakti, Rattan and Protina. These lines were subjected to vigorous selection for kernel vitreosity by growing them in Delhi during *kharif* (rainy season) and during *rabi* (winter season) in Hyderabad for 3 years (1972–1974). After each harvest, the selection was made based on kernel vitreosity and tryptophan content. In each cycle of selection, semi-opaque and semi-normal kernels were sown separately. Based on light transparency, the kernels were classified as opaque (O), 100 % opaque with no light transmission; semi-opaque (SO), partial light transmission; semi-normal (SN), over two-third of the kernel from the crown is vitreous; and normal (N), nearly vitreous kernels with slight spotting or cloudiness. The development of hard-endosperm inbred lines of *opaque-2* maize over five cycles of selection and their analyses showed that protein content, 100-kernel weight and kernel density increased with the increase in kernel vitreosity; however, tryptophan (as % of endosperm protein) decreased slightly. There was variability in tryptophan content suggesting a possibility of selecting hard-endosperm *opaque-2* strains with high tryptophan (more than 0.7 % in endosperm protein) and nearly normal-looking vitreous type of kernels (Lodha et al. 1976a; Singh et al. 1974, 1985). Utilizing the selected hard-endosperm *opaque-2* inbred lines, a SO/SN composite was developed which was subjected to extensive biochemical and nutritional studies. Later on, some of these lines were used by the Directorate of Maize Research (DMR) in developing a QPM composite, Shakti-1 (0.92 % tryptophan), which was released in 1997.

The major emphasis was, however, given on the development of QPM hybrids by practically all the breeders. CIMMYT-developed QPM lines along with their own lines were mostly involved in two-parent QPM hybrids. At present, in India we have one QPM composite (Shakti-1) and more than nine QPM hybrid varieties, including Shaktiman and HQPM series and Vivek QPM 9. The QPM version of extra-early Vivek Hybrid 9, Vivek QPM 9, was developed by following marker-assisted selection (MAS) and had been released for commercial cultivation in 2008. Phenotypically, the kernels of the QPM hybrid are as vitreous as those of its normal maize counterpart (Fig. 3.1). This QPM hybrid contains 4.19 % lysine and 0.83 % tryptophan in its endosperm protein as against 3.25 % lysine and 0.59 % tryptophan in its normal counterpart (Gupta et al. 2009).

The QPM hybrids, Shaktiman-1, Shaktiman-2, Shaktiman-3 and Shaktiman-4, respectively, contain 1.01 %, 1.04 %, 0.73 % and 0.93 % tryptophan in their endosperm proteins. The protein in the endosperms of these QPM hybrids ranges from 9.3 % to 9.9 %. Similarly, in the QPM hybrids, HQPM-1, HQPM-5 and HQPM-7, which respectively contain 0.94 %, 0.76 % and 0.72 % tryptophan, protein in the endosperms ranges from 9.36 % to 9.80 %.

3.9 Chemical and Biological Evaluation of Modified *Opaque-2* Maize Protein Quality

As a result of selection for modifiers, several changes occur in physical and biochemical characteristics of the modified *opaque-2* maize

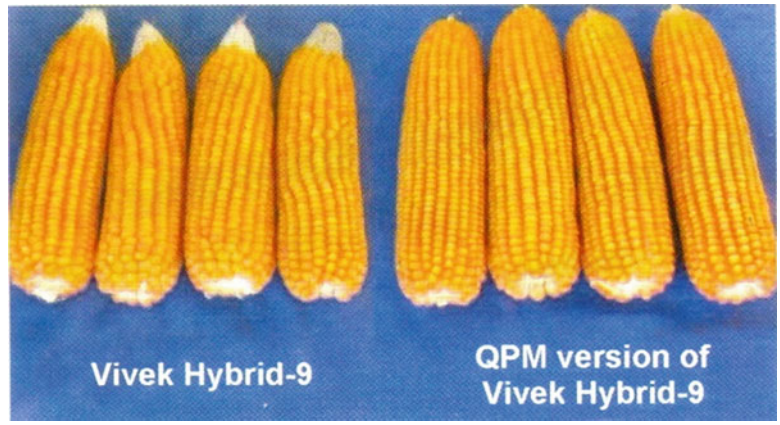

Fig. 3.1 Normal and QPM version of Vivek hybrid 9 (Courtesy: Dr. H.S. Gupta, IARI, New Delhi)

Table 3.6 Nutritive value of proteins of different categories of modified *opaque-2*, chalky *opaque-2* and normal maize

Protein value expressed as %	Vijay (normal)	SO/SN composite kernel categories (modified *opaque-2*)			Shakti (*opaque-2*)
		25 % opaque	50 % opaque	75 % opaque	
TD	92.7	97.1	95.5	94.5	94.4
BV	60.6	72.2	73.1	73.3	78.1
NPU	56.2	70.1	69.8	69.2	73.7
UP	5.41	8.28	8.20	8.00	7.97
Protein (%)	9.63	11.81	11.75	11.56	10.81

Source: Gupta et al. (1979b)

kernels. Based on light transparency, modified *opaque-2* maize kernels are classified into the following categories: vitreous (nearly normal), 25 % opaque–75 % vitreous, 50 % opaque–50 % vitreous, 75 % *opaque*–25 % vitreous and completely opaque. The vitreous fraction of endosperm contains more protein but less lysine and tryptophan than the opaque fraction (Lodha et al. 1976a). With the increase in kernel vitreosity, protein and leucine contents increase, while lysine and tryptophan contents decrease in the endosperm. This is because albumin, globulin and glutelin fractions decrease, while the zein fraction increases in the endosperm with the increase in kernel vitreosity (Gupta et al. 1979a). Although chemical analysis showed differences in endosperm protein quality of various categories of modified *opaque-2*, rat-feeding experiments showed more or less similar nutritional quality of the grain protein based on UP values, as described below.

Nitrogen-balance study carried out with albino rats utilizing three kernel categories of modified *opaque-2* with hard endosperm (SO/SN composite), viz. 25 % opaque, 50 % opaque and 75 % opaque; normal composite Vijay and Shakti *opaque-2* showed about 20 % higher BV for different categories of modified *opaque-2* as compared with normal maize (Table 3.6). Although the BV of Shakti *opaque-2* was slightly better (6.6–8.2 %) than that of various categories of modified *opaque-2*, the UP values of Shakti and three categories of modified *opaque-2* were not significantly different from one another. The change was mainly because of higher protein in different kernel categories of modified *opaque-2* composite in comparison to Shakti composite, which has got chalky endosperm (Table 3.6). Chalky *opaque-2* and modified *opaque-2* maize also supported better liver growth compared to normal maize in albino rats. A similar trend was also observed for protein per cent in liver.

Table 3.7 Protein distribution in the endosperm of normal, chalky *opaque-2* and modified *opaque-2* maize

	Per cent of total recovered protein		
Protein fraction	Vijay (normal)	Shakti (chalky *opaque-2*)	SO/SN composite (modified *opaque-2*)
Albumin	3.56	13.92	14.58
Globulin	4.75	10.22	6.95
Prolamine (zein)	43.33	10.22	18.06
Glutelin	43.04	51.68	52.79
Residue	5.32	13.96	7.62

Source: Gupta et al. (1980)

So far as distribution of protein fractions in the endosperm is concerned, albumin content was more or less equal in chalky *opaque-2* - composite (Shakti) and modified *opaque-2* with hard endosperm (SO/SN composite), and it was fourfold higher than in normal maize Vijay. The content of globulin was also twofold higher in chalky and 1.5-fold higher in modified *opaque-2* compared to normal maize. However, the content of zein was drastically reduced in *opaque-2* types, being 76 % lower in chalky *opaque-2* and 58 % lower in modified *opaque-2*. The content of glutelin was about 20 % higher in opaque types compared to normal maize (Table 3.7).

3.10 Child-Feeding Experiments

Nutritional superiority of QPM over normal maize was also demonstrated in a child-feeding experiment. In a 6-month study carried out with preschool children at Rajendra Agricultural University, Pusa, gains in weight and arm circumference were, respectively, about 30 % and 100 % higher for Shakti-1 (QPM composite)-fed group compared to the group fed with normal maize (DMR 2001).

3.11 Genetic Systems and Need for Monitoring Protein Quality of QPM

There are three distinct genetic systems which are involved in the development of QPM: (i) recessive homozygous allele of the *opaque-2* gene, (ii) modifiers for endosperm hardness and (iii) amino acid modifiers which affect the relative levels of lysine and tryptophan in the endosperm (Gupta et al. 2009).

The recessive mutant allele of the *opaque-2* gene as described earlier is the first and most important component. The second distinct genetic system is comprised of the alleles of endosperm hardness modifier genes which convert the soft/opaque mutant endosperm to a hard/vitreous endosperm with little loss of protein quality. It has been shown that increased levels of gamma-zein contribute to the recovery of a hard-endosperm phenotype as the modified *opaque-2* (QPM) grains have approximately double the amount of gamma-zein in the endosperm relative to the *o2*-only mutants. These modifiers along with the *o2* mutant allele can be initially selected by using a rapid and low-cost method of selection, whereby light is projected through the vitreous grains or blocked by the opaque grains, respectively. Thus, when the kernels are screened against light, varying degrees of modification, ranging from completely vitreous to fully opaque, may be observed. As shown in Fig. 3.2, varying degrees of kernel modification may also be observed in QPM ears.

Two genetic loci which affect the modification of the endosperm hardness in *o2o2* backgrounds have been mapped to the long arm of chromosome 7, and interestingly one endosperm modifier locus maps near a gamma-zein gene '*gzr*1'. The third genetic system critical to a QPM breeding programme is comprised of a distinct set of amino acid modifier genes which affect the relative levels of lysine and tryptophan content in the grain endosperm. The lysine level in normal and QPM maize average 2.0 % and 4.0 % of total protein in whole grain flour,

Fig. 3.2 Varying degree of kernel modification in QPM ears, ranging from completely modified to very low level of modification (from *left* to *right*) (Courtesy: Dr. B.M. Prasanna, CIMMYT, Kenya)

respectively, but range across genetic backgrounds from 1.6 to 2.6 in normal maize and 2.7–4.5 % in their *o2*-converted counterparts (Moro et al. 1996). Since QPM looks like normal maize (Fig. 3.1), there is a need to monitor lysine or tryptophan levels in its endosperm continuously during the breeding process.

After initial screening of QPM materials against light, the endosperm samples of selected materials having vitreous kernels are subjected to chemical analysis for lysine/tryptophan and protein. Because of the existence of a relationship of approximately 1–4 in tryptophan and lysine in maize endosperm protein and also because of simplicity of its estimation, initially the tryptophan content may be used as a single parameter for protein quality evaluation. Because of its simplicity and rapidity, a colorimetric method in which a single-step papain hydrolysis is utilized for protein solubilization is used more extensively for screening maize germplasm for tryptophan content (Villegas and Mertz 1971). With this method, it is possible to analyse up to 75 samples each day. Subsequently, the lysine determination is performed only on those samples selected as having high tryptophan values or when lysine value in addition to tryptophan is desired. Because of its simplicity and rapidity, a colorimetric method modified by Villegas (CIMMYT), which also utilizes a single-step papain hydrolysis for protein solubilization, is followed extensively for screening maize germplasm for lysine content (Villegas and Mertz 1971). By this method up to eight samples can be processed simultaneously, and it gives an estimate of available lysine. In addition, since the concentration of lysine in the maize endosperm is highly correlated with the content of a single non-zein, protein synthesis factor EF-1 alpha, assay of its content by ELISA also provides a reliable index of lysine in endosperm (Habben et al. 1995; Prasanna et al. 2001). Once a QPM variety reaches a final stage of its release for commercial production, it is advisable to analyse it for complete amino acid profile and subject it to animal-feeding experiments for biological evaluation of its nutritive value.

3.12 Improving Nutritive Value of Normal Maize and QPM by Amino Acids and Pulse Supplementation

The protein quality of ordinary maize and even of hard-endosperm modified *opaque-2* maize can be improved by supplementation with limiting essential amino acids or pulses because of the complementary nature of essential acids present in

pulses and cereals. The UP value, which takes into account both protein quality as well as quantity, showed substantial improvement with amino acid supplementation as well as by addition of pulse to normal and modified *opaque-2* diet. Studies carried out by Gupta et al. (1979b) with albino rats showed that supplementation with lysine alone in case of modified *opaque-2* and with lysine + tryptophan in ordinary maize improved substantially the nutritive value. When modified *opaque-2* was supplemented with 0.22 % L-lysine HCl, its UP value increased from 6.6 % to 8.3 %, showing thereby about 25 % improvement in nutritive value of protein. In the case of Vijay normal maize, the UP value increased from 6.1 % to 8.2 % (about 34 % improvement) when it was supplemented with 0.45 % L-lysine HCl + 0.06 % DL-tryptophan. At the same time, synergistic effect to the order of 25.0 % and 22.0 % has been observed in the case of normal maize and modified *opaque-2* maize, respectively, when these were supplemented with chickpea pulse (7:3 on protein basis).

3.13 Future Strategies

The nutritional superiority of Quality Protein Maize (QPM) over normal maize is well established. QPM can become a boon to young children, especially to those suffering from protein malnutrition. More so, because its consumption does not require any change in traditional food habits. However, there is a need to popularize QPM varieties with farmers as well as consumers, highlighting its nutritional importance so that its cultivation gains the required momentum. To achieve this objective, it is also important that farmers get remunerative price for their produce. There is also a need to highlight that QPM is a product of conventional breeding, and no genetic engineering is involved in its development. The produce may be utilized in the midday meal programme of school children. In addition, value addition is also required and efforts are needed to popularize already developed QPM products, for example, infant food, health food, snacks and savoury items, convenience food and specialty food (Singh 2001). This is going to benefit specially the vulnerable groups, i.e. infants, preschool children, pregnant and lactating mothers and elderly people. From the poultry industry point of view, concerted efforts are required towards developing maize rich in amino acid methionine.

The lysine content of presently available QPM varieties is below 5 mg/100 mg protein, the FAO/WHO recommended minimum level for human diets. Therefore, by exploiting variability for lysine content in QPM germplasm, it may be possible to select economically viable genotypes with lysine content exceeding the recommended level. Moreover, it is also important to establish as to how the modification of *opaque-2* locus by modifier genes is affected at biochemical and molecular level. This should help understand the mechanism of action of modifier genes, the expression of which is affected by genetic and environmental factors.

3.14 Conclusion

An important goal of plant breeding programmes has been improvement in nutritive value, in particular amino acid profile, of food grains. The biggest success was achieved when high-lysine *opaque-2* mutant of maize was identified, and subsequently QPM developed by exploiting genetic modifiers of *opaque-2* locus. QPMs are the mutants that have a hard, vitreous endosperm; a normal yield; and a high nutritional quality. Since, there exist a lot of variability for lysine/tryptophan content in the germplasm, and breeding and development of QPM varieties by accumulating the modifiers being a multistep process, monitoring of tryptophan/lysine level at each step is required as QPM kernels cannot be easily distinguished visually from normal maize kernels, and chemical analysis is the only way. Thus, establishment of a few well-equipped biochemical laboratories to provide rapid and reliable analyses would be able to give a desired boost to the ongoing QPM programme.

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Molecular Interventions for Enhancing the Protein Quality of Maize

4

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Abstract

Maize, one of the three most popular cereal crops of the world, globally contributes 15 % of the protein and 20 % of the calories derived from food crops in the world's diet. However, cereals do not provide a nutritionally balanced source of protein. For nutritional security, it is necessary to adopt a genetic enhancement strategy in which essential amino acids are either incorporated or increased in grain protein to alleviate hunger, increase income, and improve livelihood. Quality protein maize (QPM) is having high nutritive value of endosperm protein with *opaque2* (*o2*) mutation leading to 60–100 % increased content of lysine and tryptophan. The lysine value of *o2* maize is 2.5–4.0 g/100 g of endosperm protein, which is more than twice that of the normal maize (1.3 g lysine/100 g protein). International Maize and Wheat Improvement Center (CIMMYT), Mexico, played a significant role in the development of QPM maize. The breeding of QPM involves three genetic systems: (i) the recessive mutant allele of the *o2* gene, (ii) the endosperm hardness modifier genes, and (iii) the amino acid modifier genes influencing free amino acid content in the endosperm. Due to recessive nature of the *o2* gene, complex action of modifier genes, and presence of amino acid enhancer genes, the use of DNA marker-assisted selection (MAS) accelerated the selection efficiency and expedited the development of new QPM cultivars. Using a combination of MAS and phenotypic selection techniques, a single cross, short duration Vivek QPM 9 hybrid was developed and released in 2008 by Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora, India. Alternatively, manipulating the plant lysine metabolic pathway provides possible enzyme targets for genetic engineering to increase free lysine content in corn grain. Furthermore, RNA interference

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(RNAi) has been used to specifically suppress α-zein production in transgenic corn, resulting in a doubling of the lysine content of corn grain. QPM is likely to gain wider acceptance if QTLs for kernel modification, and enhancers for amino acids are fine mapped to develop markers to follow MAS for vitreous kernels and high levels of lysine. However, the major constraints in adoption of QPM hybrids are contamination with normal maize pollen in field, resulting in erosion of the trait in farmer-saved seed system. It is essential to give training on good seed production practices to the local communities and development of linkage between the seed producers, farmers, and the industry for sustainable higher nutritional benefits of QPM in the long term.

4.1 Introduction

Maize (*Zea mays* L.) is one of the world's three most popular cereal crops. It is a major cereal crop for both livestock feed and human nutrition worldwide and of great economic importance not only as a grain and fodder crop but also as an industrial raw material. Maize ranks next to rice and wheat with respect to area and grain production. India is the fifth largest producer of maize in the world contributing about 3 % of the global production. In India, maize was grown on an area of 8.33 million hectare with productivity of 2.4 tonnes/hectare in the year 2010–2011. The demand for cereal grains will continue to increase as a consequence of the expanding human population, which could add >1.5 billion people by the year 2025 (Lutz 2001). However, cereals do not provide a nutritionally balanced source of protein. Protein-containing foods are necessary for the rapid growth of children (Millward and Rivers 1989), and in some countries, maize is a primary weaning food for babies.

From nutritional perspective, the protein of normal maize is deficient in the essential amino acids, lysine and tryptophan (Bhatia and Rabson 1987). Protein deficiency, especially in children, causes kwashiorkor, a potentially fatal syndrome characterized by initial growth failure, irritability, skin lesions, edema, and fatty liver. Nutritional deficiency is of concern, particularly for people with high protein requirements, e.g., young children, pregnant and lactating women, and patients. For nutritional security, it is necessary to adopt a genetic enhancement strategy in which essential amino acids are either incorporated or increased in grain protein to alleviate hunger, increase incomes, and improve livelihoods.

4.2 Maize Protein

Maize kernel consists of endosperm, embryo, and the outer seed coat. Both the embryo and endosperm contain proteins but differ substantially in the content and the quality of the protein. The endosperm accounts for 80–85 % and the embryo 8–10 % of the total kernel dry weight. The endosperm of maize has two district regions. The aleurone layer, the outermost layers, is rich in hydrolytic enzymes. The starch-rich endosperm is present within the aleurone layer, which is vitreous (Fig. 4.1). The protein fraction constitutes only 8–10 % of the endosperm, while starch accounts for about 70 % of the kernel (Lawton and Wilson 1987; Prasanna et al. 2001). The various fractions of endosperm protein are albumin 3 %, globulin 3 %, zein (prolamine) 60 %, and glutelin 34 %, whereas the embryo protein largely consists of albumins (60 %).

The zein proteins found in vitreous region form insoluble accretions called protein bodies in the lumen of rough endoplasmic reticulum and toward maturation are densely packed between starch grains (Gibbon and Larkins 2005). Based on their solubility, genetic properties, and the

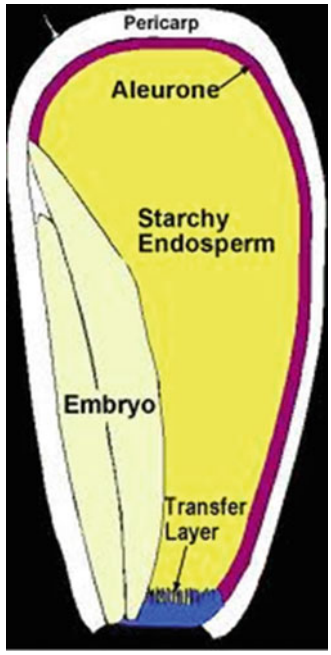

Fig. 4.1 Structure of maize kernel (Source:http://www.public.iastate.edu/~becraft/Endosperm.htm)

apparent molecular masses, zeins have been classified into α-zein (22 and 19 kDa), the most abundant; β-zein (14 kDa); γ-zein (27 and 16 kDa); and δ-zein (10 kDa) (Wilson et al. 1981). The zein fraction α is rich in cysteine, while β and γ fractions are rich in methionine. The zein fraction in normal maize generally contains a higher proportion of leucine (18.7 %), phenylalanine (5.2 %), isoleucine (3.8 %), valine (3.6 %), and tyrosine (3.5 %) but smaller amounts of other essential amino acids such as threonine (3 %), histidine and cysteine (1 %), methionine (0.9 %), and lysine (0.1 %) and is devoid of tryptophan. The non-zein protein fraction is balanced and rich in lysine and tryptophan. The high proportion of zeins in the endosperm is the primary reason for the poor protein quality of maize (Vasal 2000). Protein supplementation to correct such deficiencies is costly and wasteful of energy in animal nutrition and is not feasible for most developing countries, which rely on cereal for human consumption. Therefore, increasing the amounts of protein-bound essential amino acids depends on improving cereal storage proteins.

4.3 High-Lysine Mutants in Maize

Several mutations, both spontaneous and induced, have been recognized that affect the amino acid composition of maize endosperm. In the early 1920s, *opaque2 (o2)* mutation was first described by Jones and Singleton; but later on in 1963, researchers at Purdue University, USA, discovered that *opaque2* (*o2*) increases grain proteins nearly twice as those of normal maize proteins (Mertz et al. 1964). In fact, maize homozygous for the recessive *o2* allele has substantially higher lysine (>69 %) and tryptophan content compared to normal maize. Nelson et al. (1965) discovered another mutation, the *floury2* gene, which in homozygous form also increases lysine and tryptophan levels in maize. Later on, a large number of mutants were discovered, which fall into three categories: the recessive mutants *opaque1* through *opaque17* (*o1*, *o2*, *o5*, *o7*, *o9–o11*, and *o13–o17*), the semidominant floury mutants (*fl1*, *fl2*, and *fl3*), the dominant mutants *Mucronate* (*Mc*), and *Defective endosperm B30* (*De-B30*). The position of these mutations and zein genes on maize genetic map is shown in Fig. 4.2. The *o2* and *De-B30* mutants are located on chromosome 7, *fl2* on chromosome 4, *fl3* on chromosome 8, and *o7* on chromosome 10. Chromosome 2 carries *o4*, *o8*, *fl1*, and *Mc* mutants.

It has been reported that recessive mutations affect regulatory genes, whereas semidominant and dominant mutations affect the storage proteins. All these mutations are associated with increase in non-zein protein content and decrease in the level of zein subunits. However, premature death of the mutant seedlings and the complex genetics behind lysine trait hinder utilization of these mutant genes except for *o2* in corn breeding and production (McWhirter 1971; Nelson 1979, 1981). Epistatic interactions have also been reported among various regulatory mutants; *o2* and *o7* are epistatic over *fl2*, whereas *o2* and *Mc* have synergistic effect (Prasanna and Sarkar 1991).

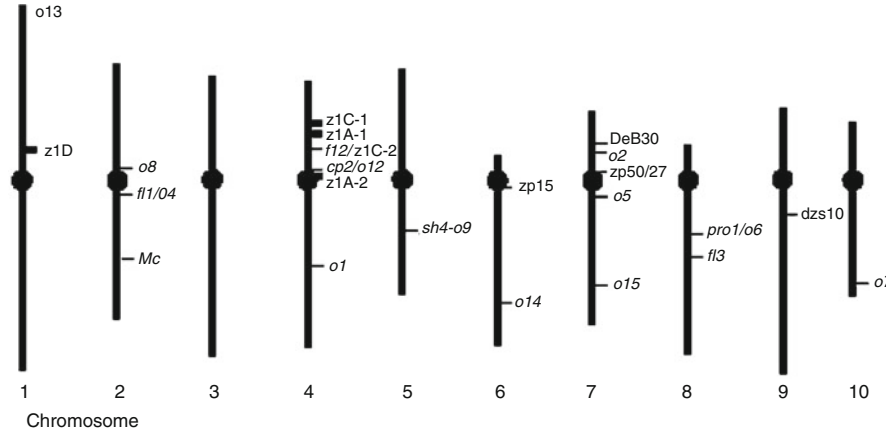

Fig. 4.2 Map position of zein genes and mutants (Source: Gibbon and Larkin 2005)

4.4 Beginning of Genetic Manipulation of Protein Quality

The development of quality maize begins initially with the manipulation of *o2* and *fl2* mutants singly or in combination. Later on, it was realized that *fl2* conversion programs were difficult to handle as the kernel expression and quality depended on dosage effect. On the other hand, *o2* mutants have simple recessive inheritance, no dosage effect, and easily scorable phenotypic expression as opaque kernels help in identification of the presence/absence of *o2* gene. The intensive work for utilization of *o2* mutation in breeding programs was continued in 1969 at CIMMYT, Mexico. All potentially important maize material was converted to *opaque2* with varying degrees of backcrosses. India was among the first few countries in the world to focus on improvement of maize quality and a research program initiated in 1966 under the All India Coordinated Maize Improvement Project (AICMIP). Three *o2* composites, namely, Shakti, Rattan, and Protina, were developed and commercially released in 1970 (Dhillon and Prasanna 2001; Prasanna et al. 2001). The *o2* composites and hybrids were experimentally tested and grown commercially in Brazil, Colombia, India, the USA, South Africa, Yugoslavia, and Hungary during the late 1960s and early 1970s. The *o2* maize had some pleiotropic effects such as reduced grain yield, chalky and dull kernel appearance, susceptibility to ear rots and stored grain pests, and slower drying of grain following physiological maturity. Due to these limitations, the *o2* maize did not become popular with farmers as well as with consumers, despite its nutritional superiority. The kernel became soft and more prone to mechanical damage that decreased the yield by 8–15 %, and the plants were more susceptible to fungi and insects (Lambert et al. 1969; Salamini et al. 1970). The search then continued by exploiting other mutants in breeding programs such as *o6*, *fl3*, and *Mc*, but none of them showed any additional advantage over *o2* and declined the efforts for enhancing the nutritional value of maize kernel. Double-mutant combination of *o2su2* had vitreous and small kernels and protein quality at par to *o2* but reduced grain yield by 15–25 %, whereas *o2fl2* double-mutant combination resulted in vitreous kernels only in few genetic backgrounds. Despite the discouraging results, researchers at CIMMYT, Mexico; the University of Natal, South Africa; and Crow's Hybrid seed company at Milford, IL, continued their efforts for improving the protein quality of maize. The only viable strategy that came out was to combine *o2* with genetic modifiers of *o2* for rewarding results.

4.5 Development of Quality Protein Maize (QPM)

Maize breeders began to identify modifier genes (*mo2*) that alter the soft, starchy texture of the endosperm, giving it a normal appearance while maintaining the increased essential amino acid content of *o2* germplasm. During the process of converting normal maize to *o2* version, partially hard endosperms were observed by Paez et al. (1969). The plant breeders at CIMMYT, Mexico (Vasal et al. 1980), and Pietermaritzburg, South Africa (Gevers and Lake 1992), developed hard endosperm o*2* mutants, designated as quality protein maize (QPM). QPM looks and performs like normal maize, except that their nutritional value gets improved. Since the mid-1990s, QPM was tested at multiple research stations all over the world, with encouraging results. Data from 32 locations across Africa, Asia, and Latin America showed that QPM hybrids were capable of outperforming the normal in some of the poorest parts of countries such as China, Mexico, Ghana, and Peru. In India, it led to the development of a modified, nutritionally superior *o2* composite in 1997, designated as "Shakti-I." The QPM research gained further momentum by the launch of National Agricultural Technology Project (NATP) on QPM in 1998 by the Indian Council of Agricultural Research (ICAR). In this project, a multidisciplinary team of multi-institutes involving Directorate Maize Research (DMR), New Delhi; Punjab Agricultural University (PAU), Ludhiana; Chaudhary Charan Singh Haryana Agricultural University (CCSHAU), Karnal Center; Acharya NG Ranga Agricultural University (ANGRAU), Hyderabad; and Rajendra Agricultural University (RAU), Pusa, wherein the QPM germplasm received from CIMMYT was acclimatized to suit the local agroclimatic conditions in India. The lines were evaluated for their productivity and deployed in combination breeding, which led to release of first QPM three-way cross hybrid, Shaktiman-1, in 2001 followed by the release of the first QPM single-cross hybrid, Shaktiman-2, in 2004. Another QPM single-cross hybrid, HQPM-1, is the first yellow grain QPM single-cross hybrid released for its cultivation across the country in 2005. Later in the series of QPM, Shaktiman-3, Shaktiman-4, HQPM-5, HQPM-7, and Vivek QPM-9 were released. Vivek QPM-9 has a unique distinction of the first molecular-marker-assisted (MAS) converted product of normal hybrid Vivek-9. The cultivation of QPM hybrids will ensure higher income to farmers as well as nutritionally superior food to the consumers. Therefore, the QPM can be a strong support to the mission of food and nutritional security of the country particularly in underprivileged and tribal regions, where maize is consumed as a staple food. QPM will also ensure quality feed for poultry and animal sector, which are the largest consumers of maize in India.

4.6 Molecular Basis of *o2* and Modifier Gene Action

The breeding of QPM involves three genetic systems: (i) the recessive mutant allele of the *o2* gene, (ii) the endosperm hardness modifier genes, and (iii) the amino acid modifier genes influencing free amino acid content in the endosperm. Isolation and characterization of *o2* gene revealed that it encodes a transcriptional factor that regulates the expression of zein gene and a gene encoding a ribosomal inactivating protein (Schmidt et al. 1990; Lohmer et al. 1991; Bass et al. 1992). The *o2* mutation typically reduces the level of 22-kDa α-zein and enhances the synthesis of a number of non-zein proteins, particularly EF-1α, that contain relatively higher levels of lysine and tryptophan (Damerval and Devienee 1993; Habben et al. 1993; Gaziola et al. 1999). In higher eukaryotes, EF-1α is encoded by a multigene family. In maize, there are 10–15 genes, five of which are expressed in endosperm (Carneiro et al. 1999). Wu et al. (2002) identified two significant QTLs (on chromosomes 2S and 4S) and two suggestive QTLs (on chromosomes 5S and 6S) for EF-1α. The o*paque2* gene is also known to be involved in the synthesis of the enzyme lysine-ketoglutarate reductase that is associated with free lysine degradation. As a consequence, in the grain with *o2* mutation, a dramatic reduction in this enzyme leads to a corresponding increase in free lysine

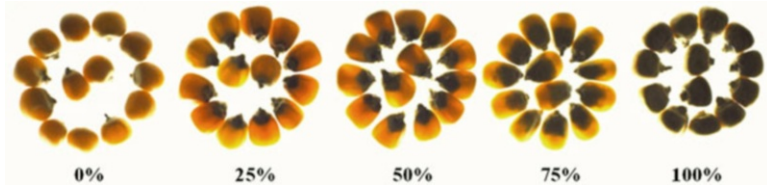

Fig. 4.3 Kernels on a light table showing varying degrees of endosperm modifications

in the endosperm (Brochetto-Braga et al. 1992). The *o2* allele is inherited in a simple recessive manner, and the presence of *o2* allele in homozygous state (*o2o2*) is the prerequisite for the entire process of obtaining high-lysine/high-tryptophan maize.

The genetic modifiers are complex in their inheritance and they are additive in nature. The expression of modifiers varies with respect to kernel modification, density, and biochemical composition of the grain of the genetic background. Genetic analysis has shown that there are two major modifier genes: one modifier locus is tightly linked with γ-zein (27 KDa) encoding sequence near the centromere of chromosome 7, while the other is near the telomere of chromosome 7L (Lopes et al. 1995). Using two independently developed QPM lines, Holding et al. (2008) mapped several major *o2* modifiers (*Opm*) QTLs to chromosomes 1, 7, and 9. In addition, five other QTLs are also present: one each on chromosomes 5S, 7L, and 10S and two on chromosome 1L. So far, 10 gene modifiers have been identified. A microarray hybridization and real-time PCR performed with RNA from true breeding *o2* progeny with vitreous and opaque kernel phenotypes identified several candidate genes that were upregulated and mapped at or near the *Opm* QTLs, suggesting linkage of *o2* endosperm modification with these differentially expressed genes. One of the QTLs is linked to the 27-kDa γ-zein locus on chromosome 7S. Moreover, QPM lines have 2–3-fold higher levels of the 27-kDa γ-zein, but the physiological significance of this increase is not known. Because the genes encoding 27- and 16-kDa γ-zein are highly conserved in DNA sequence, Wu et al. (2010) introduced a dominant RNAi transgene into a QPM line (CM105Mo2) to get rid of their expression. Elimination of γ-zeins disrupts endosperm modification by *o2* modifiers, signifying their hypostatic action to γ-zeins. Abnormalities in protein body structure and their interaction with starch granules in the F_1 with Mo2/+; *o2*/*o2*; γRNAi/+ genotype suggest that γ-zeins are essential for restoring protein body density and starch grain interaction in QPM. RNAi is to eliminate pleiotropic effects caused by *o2*, resulting in protein bodies formation such as honeycomb-like structures that clearly demonstrate that γ-zeins play a mechanistic role in QPM molecular breeding. Proteomic analysis revealed that altered starch structure was associated with endosperm modification in QPM as granule-bound starch synthase I (GBSS I) level was increased in modified kernels, which alters the amylopectin branching in QPM. Compared with normal and soft *o2* genotypes, amylopectin in QPM had reduced levels of intermediate length α-1, 4-linked glucose chains. As a consequence, the QPM starch swelled more than the normal and formed tight contacts between starch granules in mature endosperm, resulting in vitreous kernel phenotype. However, the mechanism by which modifier genes create a vitreous phenotype is yet to be understood. The beneficial alleles of the modifying loci that control γ-zein production can be selected using a rapid, low-cost, light table method. Due to segregation of genes for endosperm hardness (or softness), varying degrees of softness/hardness are expressed in the endosperm of segregating generations (i.e., varying levels of opaqueness are observed on a light table (Fig. 4.3). Gradation in the opaqueness is scored on a 1–5 scale for easy description of the various classes. Score 1 is given for fully modified kernels (0 % opaqueness), scores 2, 3, and 4 for 25 %, 50 %, and 75 % opaqueness, respectively, whereas score 5 for soft, chalky, and 100 % opaque kernels.

The modified *o2* version maize lines have varying levels of lysine and tryptophan content. This indicates presence of amino acid enhancer genes (minor modifying genes), which necessitate systematic biochemical evaluation of lysine and tryptophan levels in each segregating generation. At least three genes associated with lysine level have been mapped to locations on chromosomes 2, 4, and 7 (Wang et al. 2001). Ten significant and one suggestive QTL for free amino acid (FAA) content were identified on all 10 chromosomes (Wu et al. 2002). The QTL on chromosome 2L is coincident with genes encoding two aspartate kinase enzymes, which control important steps in amino acid biosynthesis and lysine degradation pathways. A negative correlation was observed between endosperm texture trait and amino acid content (Gutierrez-Rojas et al. 2008). Therefore, their regular monitoring at each step is essential as one could end up with a maize cultivar having the *o2o2* genotype with lysine and tryptophan levels equivalent to those in normal maize (Krivanek et al. 2007).

4.7 Molecular Interventions for Enhancing the Protein Quality

The expression of the *o2* allele is specific to seeds, and recessive, conventional introgression approaches require the inclusion of a selfing progeny test to monitor the introgression of the *o2* allele within each backcross (BC) population, and also higher plant population, enormous time duration, labor, and spatial resources are required. Numerous advantages such as reduced time and population size are known to accrue to the breeder by use of marker-facilitated genotype selection rather than classical phenotypic selection or by genetic engineering for endosperm-specific expression of high lysine and tryptophan content.

4.7.1 Molecular Breeding for QPM

Mapping and tagging of agriculturally important genes have been greatly accelerated by an array of molecular markers in crop plants. The use of DNA markers in backcrossing has greatly increased the efficiency of selection. The practical application of molecular markers in crop improvement is in marker-assisted selection (MAS), which is of great importance as it helps in improving the efficiency of plant breeding through specific transfer of genomic regions of interest (foreground selection) and accelerating the recovery of the recurrent parent genome (background selection). MAS have been more extensively employed for simply inherited traits. In 2001, the primer sequence of three SSR markers (phi112, umc1066, and phi057), all at the *o2* locus, were released at the website www.agron.missouri.edu, which facilitated the study and application of the *o2* gene. Phi057 is located between G box and 3 upstream ORF in the leader sequence of the *o2* gene, and its mutation can affect transcription of the *o2* gene. The umc1066 and phi057 are located in exon 1 and exon 6, respectively; these are the two largest exons among the six exons within the *o2* gene. To follow MAS, there are three main steps:

1. Validation of markers, i.e., the markers for the target gene should give polymorphism between recipient and the donor parent.
2. Foreground selection of the target gene with linked molecular markers and recombinant selection for recurrent parent allele at markers flanking the target allele. Flanking markers are used to select rare individuals that are the result of recombination near the target gene, thus minimizing the effects of linkage drag (Ibitoye et al. 2010). Using very close markers is the only way to reduce linkage drag, substantially.
3. Marker-assisted background selection for noncarrier chromosomes. Using markers, it can be achieved by BC_2 generation (Visscher et al. 1996; Hospital and Charcosset 1997, Frisch et al. 1999a, b), thus saving four BC generations accelerating the rate of recurrent parent genome recovery.

The utility of three maize SSR markers present within the *o2* gene in molecular-marker-assisted selection for *o2* has been successfully used for conversion of non-QPM to QPM lines

in many parts of the world including CIMMYT, Mexico; VPKAS, Almora; and PAU, Ludhiana, in India and elsewhere in Asia and Africa (Babu et al. 2005; Manna et al. 2005; Jompuk et al. 2006; Gupta et al. 2009; Agrawal and Gupta 2010; Zhang et al. 2010). At PAU, Ludhiana, three microsatellite primers, viz., phi112, umc1066, and phi057, within the gene *o2* have been surveyed among non-QPM lines (LM5, LM6, LM11, LM12, LM13, and LM14), CML lines (161, 163, 170, 189, 193, and 165), DMR lines (7, 11, 56–1, 56–4, 56–8, 72, and 74), and derived QPM lines (in different generations, S1–S4) for their validation. With phi057, three alleles were detected, and this primer clearly distinguished between QPM and normal lines. With primer phi112, null allele was observed in most of the QPM lines except CML 189, DMR 7, DMR 56–8, and few derived QPM lines. With primer umc1066, only two of the non-QPM lines, viz., LM11 and LM13, were distinguished from QPM lines (unpublished data). Xu and Crouch (2008) also reported that umc1066 is easily visualized on agarose gels but is commonly not polymorphic in CIMMYT breeding populations, while phi112 is a dominant marker and hence cannot be used in the identification of heterozygotes in F_2/BC populations. It is best to use three markers together in MAS for high-lysine maize materials (Babu et al. 2005; Danson et al. 2006; Mboogoi et al. 2006). A rapid line conversion strategy using MAS program is given in Fig. 4.4. Using combination of MAS and phenotypic selection techniques, linkage drag can be reduced by selecting for flanking markers of recipient allele type (selection of either single or double recombinants) and recovery of the maximum amount of recurrent parent genome (90–95 %) coupled with higher lysine and tryptophan content within a short span of 3 years, which would otherwise require 10 generations by conventional backcrossing method.

Danson et al. (2006) used a modifier marker, umc 1216, to select modifiers for the *o2* phenotype showing two peaks for the non-QPM at 112 bp and 115 bp, while only one peak for the QPM donor at 115 bp. However, Micic-Ignjatovic et al. (2008) reported this marker as monomorphic. So, this marker could not be employed in MAS for selection of modified endosperm. Effective markers associated with modifying loci for both endosperm hardness and amino acid levels need to be identified. Besides, phenotypic selection is applied for grain hardness and amino acid content; the rapid line conversion strategy using MAS was followed to develop QPM hybrid Vivek QPM 9 in India at VPKAS, Almora (Gupta et al. 2009), which was released in 2008 At PAU, Ludhiana, non-QPM lines, viz., LM11, LM12, LM13, and LM14, have been converted into QPM versions, which are parental lines of two important hybrids, Buland (LM11 × LM12) and PMH1 (LM13 × LM14).

Another high-lysine mutant *opaque16* (*o16*) has been isolated from the Robertson Corn Mutant library in Guizhou Institute of Upland Food Crops, China. It has been mapped on long arm of chromosome 8 and umc1141 marker is linked to this gene. Double-recessive mutant effect of the *o16* with *o2* was evaluated on lysine content and the double-recessive hybrid showed 6 % higher lysine content than both the parents (Yang et al. 2005; Zhang et al. 2010). This information may be useful for marker-assisted selection and gene pyramiding in high-lysine maize breeding programs.

4.7.2 Transgenics for QPM

Efforts in genetic transformation are focused on developing a dominant *opaque2* trait in maize. RNA interference (RNAi) has been used to specifically suppress α-zein production in transgenic corn, resulting in doubling of the lysine content of corn grain. Segal et al. (2003) used RNAi technology to engineer transgenic maize for 22-KD α-zein gene, producing a dominant opaque phenotype. The phenotype segregated in a normal Mendelian fashion and eliminated 22-kD zein without affecting the accumulation of other zein proteins. It was also found that the dominant phenotypes generated were correlated with increased lysine content. Similarly in another experiment, 19-kD alpha-zeins got reduced by using antisense transformation constructs (Huang et al. 2004, 2005). In

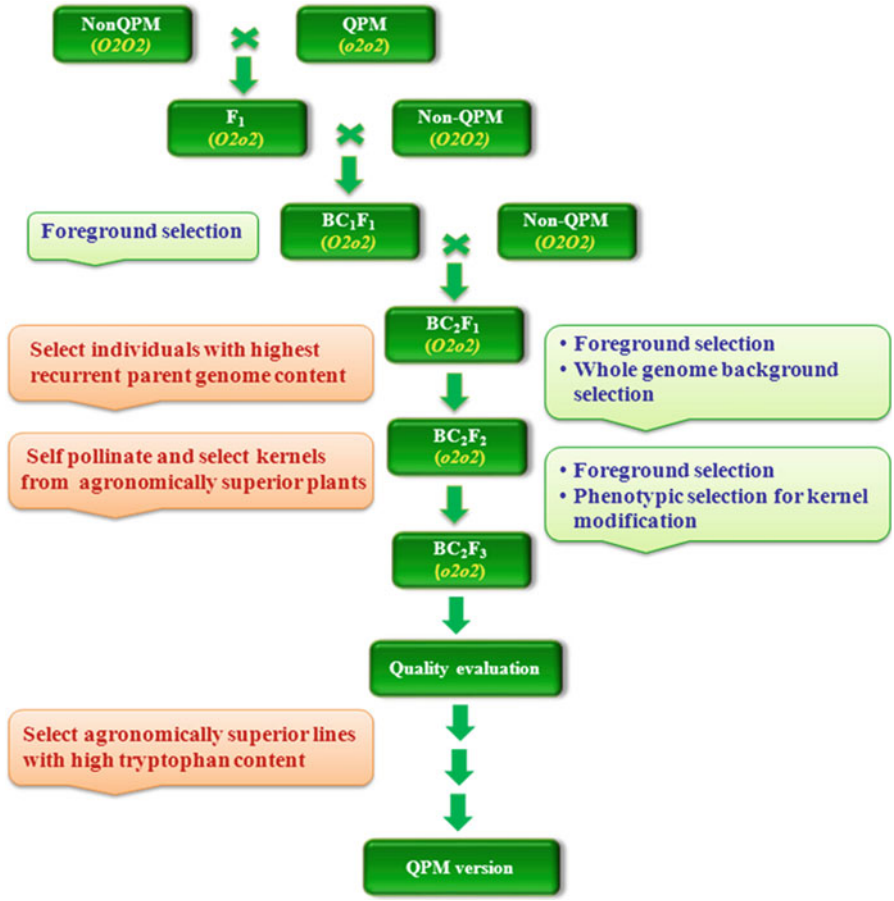

Fig. 4.4 Marker-assisted foreground and background selection scheme for quality protein maize (Source: Babu et al. 2005)

both the studies, the increase of lysine was far below than is reported in *o2o2* maize. Huang et al. (2006) changed the gene construct and used a double-stranded RNA (ds RNA) to suppress the 19-kD and 22-kD α-zein gene families. Thus they could achieve the lysine 5.62 % and tryptophan 1.22 %, which is higher that is achievable with *opaque2*. This approach of using ds RNA looks promising. While the dominant nature of the antisense transgene is a definite advantage as compared to recessive allele of *o2*, the opaque endosperm still needs to be modified by endosperm modifier genes whose epistasis with the transgene has not yet been tested.

Alternatively, the plant lysine metabolic pathway provides possible enzyme targets for genetic engineering to increase free lysine content in corn grain. As shown in Fig. 4.5, lysine, along with methionine, threonine, and isoleucine, is derived from aspartate; dihydrodipicolinate synthase (DHDPS) catalyzes the first committed step of lysine biosynthesis. A bifunctional enzyme, lysine-ketoglutarate reductase/saccharopine dehydrogenase (LKR/SDH), is responsible for lysine catabolism (Azevedo et al. 2006; Stepansky et al. 2006).

The free lysine level in plant cells is thought to be regulated by lysine feedback inhibition of DHDPS and feed-forward activation of LKR/SDH. Indeed, the expression of a lysine feedback-insensitive DHDPS from *Corynebacterium glutamicum*, CordapA, as well as the

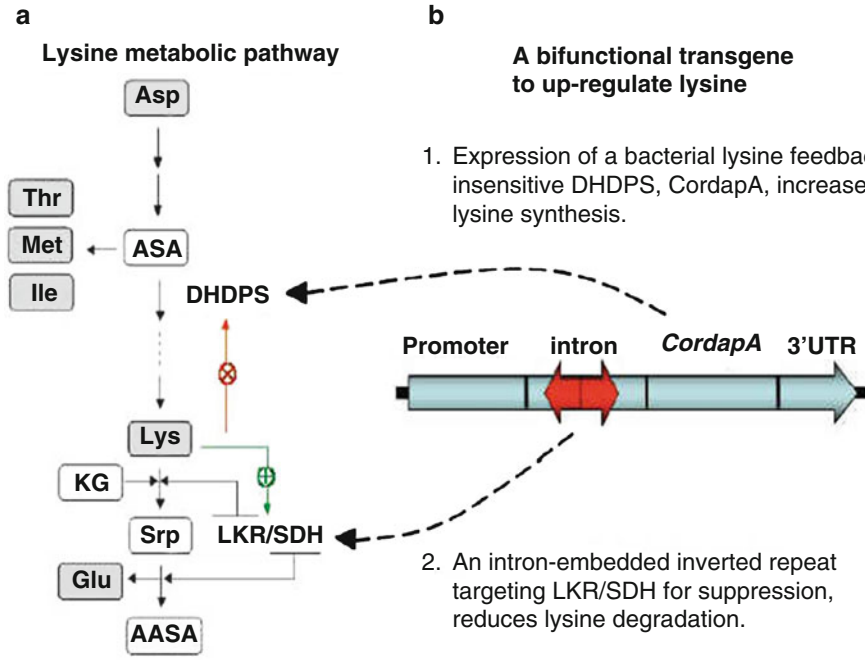

Fig. 4.5 (**a**) The lysine metabolic pathway in plants. In the biosynthesis of aspartate (*Asp*)-derived amino acids, dihydrodipicolinate synthase (*DHDPS*) catalyzes the committed step to (*Lys*) production and is sensitive to feedback inhibition by Lys (*red line*). The bifunctional lysine catabolic enzyme lysine-ketoglutarate reductase/saccharopine dehydrogenase (*LKRSDH*) is activated by excess lysine (*green line*). Other amino acids (*shaded box*) and metabolites (*open box*) shown in the pathway are threonine (*Thr*), methionine (*Met*), isoleucine (*Ile*), glutamate (*Glu*), 3 aspartate semialdehyde (*ASA*), and α-aminoadipic-8-semialdehyde (*AASA*). (**b**) The design of a bifunctional transgene cassette that simultaneously deregulates lysine biosynthesis and reduces lysine degradation (Source: Huang et al. 2008)

suppression of LKR/SDH have resulted in transgenic corn with higher levels of free lysine (Huang et al. 2005; Houmard et al. 2007). To overcome lysine catabolism in the endosperm, maize plants were transformed with a single endosperm-specific bifunctional expression/silencing transgene, which encodes a bacterial feedback-insensitive DHDPS with a LKR/SDH RNAi sequence in an intron (Frizzi et al. 2008). The suppression of LKR/SDH in the endosperm tissue increases free lysine to 1,324 ppm (~30-fold increase). The combination of CordapA expression and LKR/SDH suppression in a single transgene produces over 4,000 ppm free lysine (~100-fold increase), the highest ever reported in corn kernels. This construct resulted in a significant elevation in the seed lysine level, proving that maize endosperm possesses enzymatic activity responsible for both lysine biosynthesis and catabolism. GM crops with higher levels of other important amino acids, namely, methionine, tryptophan, and threonine, are also expected. The opportunities for and the impacts of GM crops with enhanced nutritional quality depend on public acceptance.

4.8 Nutritional Superiority and Biological Value of QPM

Maize, being a potential crop in India, occupies an important place as a source of human food (25 %), animal feed (12 %), poultry feed (49 %), industrial products mainly as starch (12 %), and 1 % each in brewery and seed. The maize grain accounts for about 15–56 % of the total daily calories in diets of people in about 25 developing countries, particularly in Africa and Latin America, where animal

protein is scarce and expensive and consequently unavailable to a vast sector of the population (Prasanna et al. 2001). Research suggests that QPM can help reduce protein deficiencies, particularly in young children, where maize dominates in the diets (Vasal 2000). In QPM varieties, leucine/isoleucine ratio was improved and became better balanced which in turn is considered beneficial as it helps to liberate more tryptophan for more niacin biosynthesis, thus helping to combat pellagra (Prasanna et al. 2001). The other nutritional benefits of QPM include higher calcium and carbohydrate (Bressani 1995) and carotene utilization (De Bosque et al. 1988).

Because of the 60–100 % increase in these two essential amino acids, increased digestibility, and increased nitrogen uptake relative to normal-endosperm maize, the biological value of QPM is about 80 %, whereas that of normal maize is 40–57 % (Bressani 1992). The lysine content of *o2* maize is 2.5–4.0 g/100 g of protein, which is more than twice that of an endosperm from the normal maize (1.3 g lysine/100 g protein). Only 37 % of common maize protein intake is utilized compared to 74 % of the same amount of *o2* maize protein. A minimum daily intake of approximately 125 g of *o2* maize might guarantee nitrogen equilibrium. This could not be obtained by using even twice the amount of normal maize. The nitrogen balance index for skim milk and *o2* maize protein is 0.80 and 0.72, respectively, which indicates that the protein quality of QPM is 90 % of that of milk. Besides, around 24 g of normal maize per kg of body weight is required for nitrogen equilibrium, compared to only around 8 g for QPM (Bressani 1995; Graham et al. 1980). This nutritional value of the QPM genotypes meets the requirements of preschool children for their protein needs (Mertz et al. 1964; Vasal 2000).

The decreased level of zein (5–27 %) in *o2* maize in contrast to normal endosperm (54–59 %) considerably improves its nutritional quality. Another important application of QPM is as animal feed, especially for monogastric animals such as pigs and poultry, as it results in improved growth. Small-holding farmers (who typically cannot afford balanced feeds) and commercial farmers find this extremely remunerative. Therefore, the development and adoption of improved QPM cultivars have significant potential to alleviate hunger, increase incomes, and improve livelihoods.

4.9 Conclusion

Malnutrition is one of the important issues in India and the world. Therefore, development of QPM hybrids, which are widely grown in specific locations will provide food and nutritional security and thereby help in reducing poverty. QPM is likely to gain wider acceptance if QTLs for kernel modification and enhancers for amino acids are fine mapped to develop markers to follow MAS for vitreous kernels and high levels of lysine. It also provides an ideal platform for stacking of other nutritional genes for enhanced Fe and Zn content and low phytate content for multiple benefits. However, the major constraint in adoption of QPM hybrids is contamination with normal maize pollen in field, resulting in erosion of the trait in farmer-saved seed system. It is essential to give training on good seed production practices to the local communities and development of linkage between the seed producers, farmers, and the industry for sustainable higher nutritional benefits of QPM in the long term.

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Part III

Nutritional Quality of Maize

Maize: Grain Structure, Composition, Milling, and Starch Characteristics

5

Narpinder Singh, Amritpal Kaur, and Khetan Shevkani

Abstract

Maize is the third important food grain after wheat and rice, and its demand is increasing because of its increased use for biofuel production. Starch is the main component of maize, which is produced by wet milling process. Maize starch functionality varies with the starch structure and composition, which vary with genotypes and cultural practices. The average size of maize starch granules ranges between 1 and 7 μm and 15 and 20 μm, respectively, for small- and large-sized granules. Maize starches exhibit a typical A-type pattern, in which double helices comprising the crystallites are densely packed. Sugary maize starch has lower crystallinity, while waxy maize starch has greater crystallinity as compared to normal maize starches. The sugary maize starch has lower gelatinization temperature and enthalpy. The maize starch with long-branch chain length amylopectin and higher crystallinity has higher gelatinization temperature and enthalpy. The maize products (canned, frozen, and boiled sweet maize) have lower glycemic index (GI) than white rice and wheat flour bread. Waxy maize starch is more rapidly digested and have high GI than high-amylose starches. Thermal treatments such as autoclaving, baking, steam cooking, and parboiling processes affect starch digestibility and consequently the GI of maize-based products. Maize also contains various bioactive constituents, such as carotenoids, anthocyanins, and phenolic compounds, which vary with maize type. Maize has a higher antioxidant capacity compared to wheat, oat, and rice.

5.1 Introduction

The global maize (corn) production during 2010 was 844 million tons (mt) from an area of about 162 million hectares (mha). The USA, China, Brazil, Mexico, Argentina, Indonesia, India, France, South Africa, and Ukraine are the top ten

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Table 5.1 Production of maize during 2000–2010[a] (metric tons)

Country	2010	2005	2000	1995	1990
USA	316,165,000	282,261,000	251,852,000	187,969,000	201,532,000
China	177,540,788	139,498,473	106,178,315	112,361,571	97,213,875
Brazil	56,060,400	35,113,300	31,879,400	36,267,000	21,347,800
Mexico	23,301,900	19,338,700	17,556,900	18,352,900	14,635,400
Argentina	22,676,900	20,482,600	16,780,700	11,404,000	5,400,000
Indonesia	18,364,400	12,523,900	12,043,200	8,245,900	6,734,030
India	14,060,000	14,709,900	12,043,200	9,534,000	8,961,700
France	13,975,000	13,687,700	16,018,400	12,739,600	9,400,500
South Africa	12,815,000	11,715,900	11,431,200	4,866,000	9,180,000
Ukraine	11,953,000	7,166,600	3,848,100	3,391,800	–

Source: [a]FAOSTAT (2012)

maize-producing countries in the world (FAOSTAT 2012). The USA is the largest producer and consumer of maize in the world, contributing around 316 mt to the total global maize production during 2010 (FAOSTAT 2012). China is the second largest producer of maize with a production of 177 mt. The maize production in Brazil and India was 56 and 21.72 mt, respectively, in 2010. The maize production of major maize-producing countries is shown in Table 5.1. The production of maize in the USA, China, Brazil, and India was merely 201, 97, 21, and 8.9 mt, respectively, in 1995. Japan, Republic of Korea, Mexico, China, Spain, and Egypt are the main maize-importing countries. Japan imported 3.7 mt of maize during 2009. The USA, France, Argentina, Brazil, Ukraine, and Hungary are the main maize-exporting countries. The USA, Argentina, and Brazil exported 47.8, 8.5, and 7.7 mt, respectively, of maize during 2009. Different maize classes and cultivars are grown in different countries. There are five general classes of maize – flint, pop, flour, dent, and sweet corn – which differ significantly in physicochemical characteristics and end-use quality. The variations in maize characteristics and end-use quality are attributed by hereditary and environmental factors. On the basis of starch composition, maize is categorized into three classes: (1) waxy maize, which contains almost 100 % amylopectin; (2) high-amylose maize, with starch containing amylose content between 40 and 70 %; and (3) sugary maize, with lower starch content and higher level of sucrose (Nelson and Pan 1995). The dull (*du*) and sugary (*su2*) genes have been associated with higher amylose content (Inouchi et al. 1984; Boyer and Liu 1985). The color of maize ranges from white to yellow, red, and purple. Blue-, purple-, and red-pigmented maize kernels are rich in anthocyanins with well-established antioxidant and bioactive properties (Adom and Liu 2002). The pigmented maize is rich in anthocyanins, carotenoids, and phenolic compounds, which have antioxidant and bioactive properties.

In India, maize has become the third important food grain after wheat and rice. The demand for maize is growing throughout the world because of its increased demand for biofuel production. The biofuel production is also being supported by government policies in many countries. This will further contribute to the increasing demand of maize in the future. This may cause an increase in the price of maize in the international market. Maize is dry milled into grit used in the production of breakfast cereals, snacks, etc. Wet milling of maize is done to produce starch, which is the raw material for the production of dextrins, glucose, fructose syrups, sorbitol, etc. The present chapter focuses on the grain structure, composition, milling, and starch characteristics of maize.

5.2 Grain Structure and Composition

Maize grain is composed of endosperm (82–83 %), germ (10–11 %), pericarp (5–6 %), and tip cap (0.8–1.0 %). The pericarp is the

Table 5.2 Total phenolics, anthocyanin, carotenoid, and ferulic acid contents of different maize types

Maize types	Total phenolics[a] (mg/100 g of dry wt)	Anthocyanin content[b] (mg/100 g of dry wt)	Carotenoids		Ferulic acid (mg/100 g dry wt)
			Lutein	β-carotene	
White[c]	260.7 ± 6.1	1.33 ± 0.02	5.73 ± 0.18	4.92 ± 0.18	120
Yellow[c]	285.8 ± 14.0	0.57 ± 0.01	406.2 ± 4.9	33.6 ± 1.2	102
Yellow[d]	551 ± 3.8	70.2 ± 0.9	–	–	140
Red[c]	243.8 ± 4.6	9.75 ± 0.44	121.7 ± 12.1	20.2 ± 1.9	130
Red[d]	465 ± 4.4	85.2 ± 2.2	–	–	153
Blue[c]	266.2 ± 0.7	36.87 ± 0.71	5.17 ± 0.49	23.1 ± 2.1	130
Blue[d]	343 ± 8.6	99.5 ± 1.8	–	–	152
Orange[d]	215 ± 5.1	30.6 ± 0.9	–	–	164
Purple[d]	465 ± 9.8	93.2 ± 1.1	–	–	154
Black[d]	457 ± 7.4	76.2 ± 2.2	–	–	151
High carotenoid[c]	320.1 ± 7.6	4.63 ± 0.06	245.6 ± 9.4	45.8 ± 3.9	153

[a]Expressed as gallic acid
[b]Expressed as cyanidin-3-glucoside
[c]de la Parra et al. (2007)
[d]Lopez-Martinez et al. (2009)

outermost layer that is characterized by high crude fiber content, mainly consisting of hemicelluloses, cellulose, and lignin. Hemicellulose is present in the highest concentration in the crude fiber. Pericarp thickness varies in different maize types and extends to the base of the kernel joining the tip cap. The pericarp and tip cap constitute a small portion of total kernel lipids. The endosperm is composed of a large number of cells, each packed with starch granules embedded in a continuous matrix of protein. The cell wall consists of non-starchy polysaccharides (β-glucan and arabinoxylan), proteins, and phenolic acids. Maize grain has two types of endosperm – floury and horny. Floury endosperm contains loosely packed starch granules surrounding the central fissure, while horny endosperm has tightly packed, smaller starch granules toward the periphery. The grains with higher proportion of harder endosperm are preferred for the dry milling. The storage proteins of endosperm are located within subcellular bodies, simply known as protein bodies, and comprise the protein matrix. Protein bodies are composed almost entirely of a prolamine-rich protein fraction known as zein, which is extremely low in lysine. Whole maize grain has protein, starch, and fat content between 8 and 11.5, 68 and 74, and 4.0 and 5.5 %, respectively. The endosperm contains around 85 % starch and 8.5 % protein content. Fat content of the endosperm is relatively low (1 %). The lipids present in endosperm contain more saturated fatty acids compared to germ lipids. The germ portion is characterized by a high fat (≈33 %) and protein (≈18 %) content. Germ has low starch content (≈8 %), and its oil is high in polyunsaturated fatty acids. Germ oil is relatively stable due to the presence of high levels of natural antioxidants and considered good for health because of its fatty acid composition, mainly consisting of oleic and linoleic acids.

Maize contains various bioactive constituents, such as carotenoids, anthocyanins, and phenolic compounds that have many health-promoting and disease-preventing properties. Maize has a higher antioxidant capacity compared to wheat, oat, and rice (Adom et al. 2005). Lutein and zeaxanthin are main carotenoids in maize, while α- and β-cryptoxanthin as well as α- and β-carotene are present to lesser extent. These bioactive compounds vary in different maize types (Singh et al. 2011). Total phenolic and anthocyanin contents in maize vary with the color (Table 5.2). The anthocyanins present in blue maize come from cyanidin and malvidin (mainly from derivatives of the former), whereas those in red maize come from pelargonidin, cyanidin, and malvidin. Yellow maize has more carotenoids

than floury maize. Blue and white maize are low in lutein and zeaxanthin content, whereas yellow and high-carotenoid maize have a higher content (de la Parra et al. 2007). Bioactivities of purple-, blue-, and red-pigmented maize are associated with the presence of anthocyanins. Purple maize is an important source of anthocyanins with potential application as natural food colorants and antioxidants (Aoki et al. 2002). High-carotenoid genotypes have the best overall phytochemical profile, followed by yellow, blue, and red maize. White maize genotypes have lower antioxidant activity due to lower amounts of anthocyanins and carotenoids. Maize is also known to contain a wide range of phenolic acids. Ferulic acid is an important phytochemical in maize, and its concentration vary in different maize types. The high-carotenoid maize contains higher amount of total ferulic acid compared to white, yellow, red, and blue maize. Most of the ferulic acid in maize is present in bound form (Adom and Liu 2002). The bulk of phenolics (phenolic acids, flavonoids, and conjugated amines) are concentrated in the pericarp and aleurone layers as well as the germ, with traces in the endosperm (Sen et al. 1994). The highest concentration of anthocyanin pigments in maize is present in the pericarp portion, whereas the aleurone layer contains small amounts (Moreno et al. 2005).

5.3 Milling of Maize

5.3.1 Dry Milling

Maize is dry milled to grit, meal, and flour, which are used in preparation of flakes, extruded snacks, breakfast cereals, tortillas, etc. In India, traditionally, the maize is dry milled in mechanized stone mills to get whole meal or "atta" that is used in the preparation of unleavened flat bread or "roti." The objective of dry milling of maize into grit is to get maximum recovery of grit with minimum contamination of hull, germ, and tip cap. Flow diagram of dry milling process of maize is illustrated in Fig. 5.1. The grains are cleaned to remove impurities, conditioned with cold/hot water/steamed, and then tempered for varying time to equilibrate the moisture. The conditioning makes the hull and germ tough and endosperm mellow. The grains are milled by either Beall de-germinator or fluted roller mills to separate germ and hull. The Beall de-germinator consists of a cast iron cone rotating within a perforated conical housing with protrusions on the inner surface. These protrusions rub off the germ and hull. The roller mills for de-germination consist of a set of fluted rolls that rotate at differential speeds (1.25:1–1.5:1) in the opposite direction. The gap between the rolls is adjustable depending upon the size of grain and grit. The de-germination using roller mill is comparatively simple; however, the separation of germ and hull is relatively inefficient. After de-germination, the material is passed through 3–5 pairs of rolls and graded on purifier to get fractions of different particle sizes, ranging from large hominy grits to fine flour. The products obtained from dry milling include flaking grits; coarse, medium, and fine grits; coarse or granulated meal; and fine meal. The separated germ is used for oil extraction either by solvent extraction or screw pressing.

5.3.2 Wet Milling

Throughout the world, large quantities of maize are wet milled to produce starch and other valuable by-products such as gluten, germ, and bran. Flow diagram of wet milling process of maize is illustrated in Fig. 5.2. Wet milling of maize involves steeping of maize in water for 30–40 h at 50 °C in the presence of SO_2 (0.02 %) to soften the kernels. SO_2 prevents fermentation and facilitates the separation of starch from protein. After steeping, the steep water is drained, and grains are coarsely ground in Fuss mill to free the germ from endosperm and hull. The Fuss mill consists of two upright, toothed metal plates within a bronze-lined chamber. One plate of the mill rotates while other remains stationary. The soaked grains are fed between these plates which cracks open the grains and free the germ. The

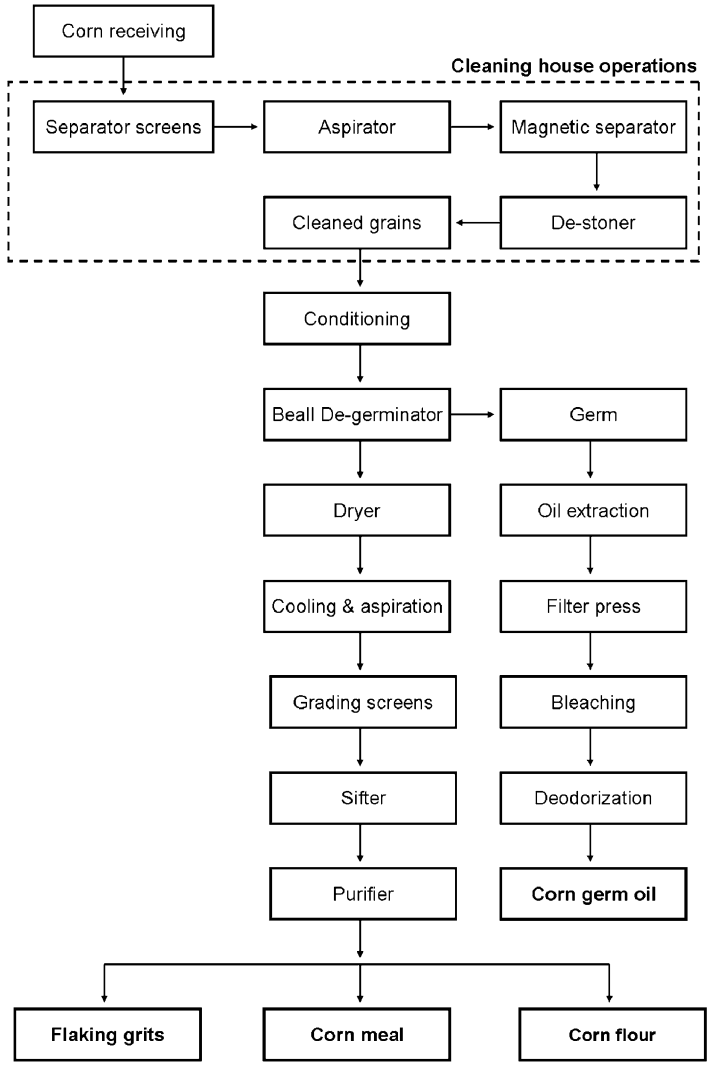

Fig. 5.1 Flow diagram of dry milling process of maize

germ portion is then separated using flotation tanks, washed, dried, and expelled for oil extraction. The de-germed suspension consisted of fiber, starch, and protein is then fine milled in attrition or impact mill. Fiber is separated by screening and centrifugation. The fiber is washed and dewatered by pressing and finally dried. The suspension consisted of starch and protein is sent to batteries of hydro-cyclones where they are separated on the basis of differences in their densities. Both the starch and protein (gluten) are dried separately. The starch is utilized for production of a number of value-added products, such as dextrose, fructose syrups, dextrins, modified starches, and sorbitol, by chemical and/or enzymatic processes. The gluten is dried and commonly used as animal feed.

5.4 Starch Characteristics

5.4.1 Granule Morphology

Morphological characteristics of starches from different maize types vary with the genotype and cultural practices. The morphology of starch granules depends on the biochemistry of the chloroplast or amyloplast as well as physiology of the

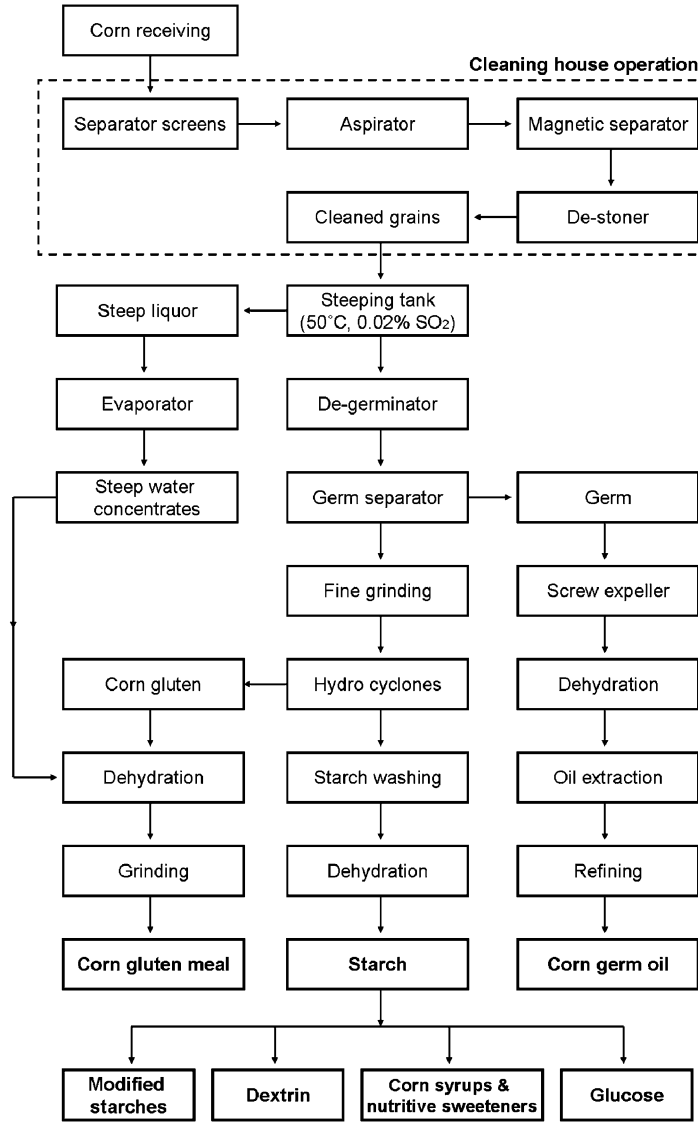

Fig. 5.2 Flow diagram of wet milling process of maize

plant (Badenhuizen 1969). Scanning electron micrographs of the starch granules from different maize types are illustrated in Fig. 5.3. The average size of small- and large-sized granules ranges between 1 and 7 μm and 15 and 20 μm, respectively. Normal maize and waxy maize starch granules displayed spherical or angular shape, while sugary maize starch displayed irregular-shaped granules consisting of lobes (Sandhu et al. 2007). Normal and waxy maize starch granules had the smooth surface, whereas sugary maize starch granules displayed rough surface (Perera et al. 2001; Sandhu et al. 2007).

5.4.2 Structure

Maize starch, like starches from other cereals, consists of amylose and amylopectin. Amylose is essentially a linear polymer of glucose, while amylopectin is branched. Normal starch contains 25 % amylose and 75 % amylopectin. Maize with

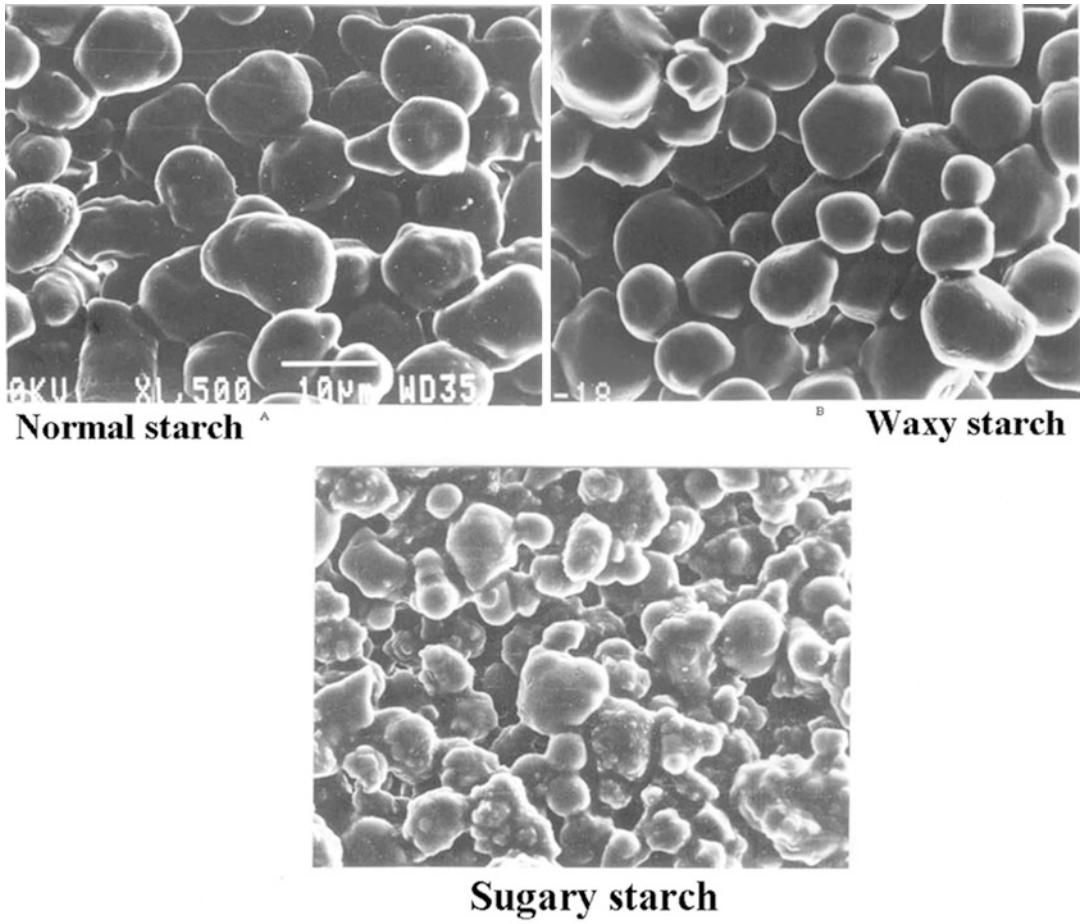

Fig. 5.3 Scanning electron micrograms (SEM) of starch granules from different types of maize (Source: Sandhu et al. 2007)

amylose as high as 85 % (amylomaize) is also reported. Waxy maize starch mainly consists of amylopectin (99 %) and contains negligible amylose (<1 %). Amylose and amylopectin packing in the granules vary among the starches from different species. The branches of the amylopectin molecules form double helices that are arranged in crystalline regions. X-ray diffractometry is used to reveal the presence and characteristics of the crystalline structure of the starch granules. The "A-," "B-," and "C-type" patterns are different polymeric forms of the starch determined by X-ray diffractogram. These types differ in the packing of the amylopectin double helices. Maize and other cereal starches exhibit a typical A-type pattern, in which double helices comprising the crystallites are densely packed with low water content. Tuber starches show the B-type pattern, in which crystallites are less densely packed and have more open structure containing a hydrated helical core (Tester et al. 2004). Sugary maize starch showed a weak A-type pattern with lower crystallinity as compared to other maize starches. Sugary maize starch showed larger amount of amylose–lipid complex as compared to normal and waxy starch (Singh et al. 2006). Waxy maize starches were reported to have greater crystallinity. Among the waxy maize starches, differences in crystallinity have been observed and were related to the amount of longer amylopectin chains. Waxy mutant starch has been reported to be more crystalline than regular cereal starches (Singh et al. 2003; Vandeputte et al. 2003).

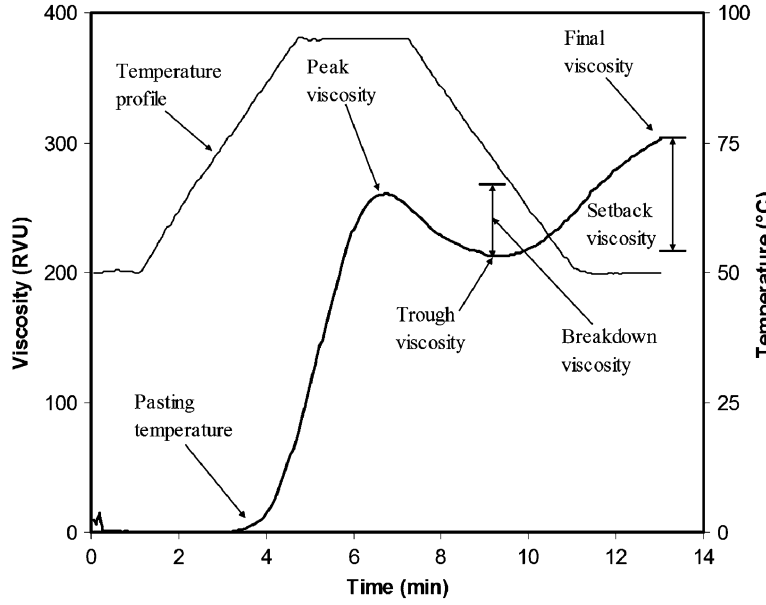

Fig. 5.4 Typical pasting/RVA profile of maize starch

5.4.3 Pasting Properties

The pasting profile represents change in the viscosity of starch suspension during heating and cooling under constant stirring conditions. The Brabender Viscoamylograph and Rapid Visco Analyser (RVA) are extensively used for measuring pasting profile of different starches. The typical pasting curve of maize starch is shown in Fig. 5.4. The pasting curve shows a steep increase at a certain point (pasting temperature), representing minimum temperature required for initiation of gelatinization. Waxy starches have lower pasting temperature than the normal starches (Liu et al. 1997; Sandhu et al. 2007). The peak viscosity reflects the point of maximum swelling of the granules. The starches from normal and waxy maize show sharp peak, whereas that of sugary maize show the flatter peak (Li and Corke 1999; Perera et al. 2001; Sandhu et al. 2007). Waxy starches show higher peak viscosity than normal and sugary starches. The higher peak viscosity of waxy starches is attributed to the absence of amylose–lipid complex. The lipids and phospholipids have been reported to form complex with the amylose and long-branch chains of amylopectin in the cereal starches, consequently severely retarding swelling and inhibiting amylose leaching (Larson 1980). Waxy cereal starches are reported to have negligible lipid contents, swell rapidly, and achieve higher peak viscosity (McPherson and Jane 1999). Normal starches have much higher amylose content, which restricts swelling and limits the increase in viscosity by developing aggregated structure (Becker et al. 1981). Further heating of starch suspensions at elevated temperatures resulted into breakdown in viscosity. The breakdown viscosity reflects the shear thinning of the starch gels. Waxy starches showed greater breakdown and higher shear thinning of the gels due to the absence of amylose (Bahnassey and Breene 1994). Low viscosity and almost negligible breakdown for sugary maize have been observed by Li and Corke (1999), and Singh et al. (2006). Sugary maize starches having less breakdown may be due to the loosely branched amylopectin structure (Campbell et al. 1994). Upon cooling, the gelatinized starch molecules partially reassociate to form gel (retrogradation), and this leads to an increase in the viscosity. The rise in viscosity during cooling varies in different maize starches, and these differences are attributed to the variation in amylose.

5.4.4 Thermal Properties

Starch undergoes irreversible changes characterized as gelatinization when heated with excess of water. Gelatinization is the disruption of molecular order within the starch granule. It results in granular swelling, crystallite melting, loss of birefringence, viscosity development, and solubilization. Differential scanning calorimeter (DSC) has been widely employed to study starch gelatinization. DSC endotherms provide information on gelatinization behavior of starches as onset temperature (To), peak temperature (Tp), conclusion temperature (Tc), and enthalpy of gelatinization (ΔHgel). To, Tp, and Tc of normal maize starches range between 63.5 °C and 68.4 °C, 67.8 °C and 71.6 °C, and 73.2 °C and 76.3 °C, respectively, while the waxy maize starches have a range of 64.4–69.1 °C, 70.4–73.5 °C, and 80.5–81.3 °C, respectively (Singh et al. 2006). Sugary maize starch shows lower transition temperatures and ΔHgel attributed to the presence of lower crystallinity. Higher degree of crystallinity provides structural stability to the granules and makes them more resistant to gelatinization and consequently results into higher transition temperatures (Barichello et al. 1990). The starches with long-branch chain length amylopectin show higher gelatinization enthalpy, indicating that more energy is required to gelatinize the crystallites of long chain length. The difference in gelatinization range ΔT {2(Tp–To)} among the starches from different maize types also differ may be due to the presence of crystalline regions of different strengths in the granules (Banks and Greenwood 1975). The variability in ΔT values in starches is due to the difference in the degree of heterogeneous crystallites, which have slightly different crystal strengths (Vasanthan and Bhatty 1996; Gunaratne and Hoover 2002). The ΔHgel followed the order: waxy maize starch > normal maize starch > sugary maize starch (Perera et al. 2001; Sandhu et al. 2007). Higher ΔHgel for waxy maize starches has been attributed to the presence of greater amount of crystallinity than that present in normal maize and sugary maize starches (Singh et al. 2006). The high ΔHgel of waxy maize starches suggested that the double helices (formed by the outer branches of adjacent amylopectin chains) that unravel and melt during gelatinization are strongly associated within the native granule. The ΔHgel for sugary maize starch (su2) was reported to be lower as compared to normal maize starch (Inouchi et al. 1984). Higher ΔHgel value of the waxy maize as compared to the normal maize has been observed by Liu et al. (1997). Perera et al. (2001) reported the lower value of transition temperatures of sugary maize as compared to normal and waxy maize starches. The gelatinization enthalpy values of starches has been reported to be affected by factors such as granule shape, percentage of large and small granules, and the presence of phosphate esters (Stevens and Elton 1971; Yuan et al. 1993). The lower ΔHgel of baby maize starch may be attributed to its small granule size and low amylose content.

5.4.5 Starch Digestibility

In humans, the starch is digested in the small intestine. However, a part of it is not digested in the small intestine and reaches the large intestine where it is fermented by the gut microflora that produces short chain fatty acids as end products, which are known to promote the optimal function of the viscera (Topping and Clifton 2001). This indigestible portion of starch is known as resistant starch. During heating, starch is gelatinized and the semicrystalline structure of starch disintegrates. The gelatinized starch tends to reassociate (recrystallization) to form an ordered gel-like structure during cooling known as retrogradation. Retrogradation process during the storage of processed starchy foods (noodles, bread, and cooked rice) affects the digestibility and often consumer acceptability of such foods. The retrogradation rate and its extent in starch foods vary with starch properties (molecular and crystalline structure) and storage conditions (temperature, duration, water content, etc.). Foods containing starches with higher

Table 5.3 Glycemic index (GI) of maize, rice, and wheat products

Food	GI
Maize meal porridge	109
Corn muffin, low amylose	102
Corn flakes	84
Maize chapatti	59–64
Sweet corn, boiled	60
Whole sweet corn (canned)	46
Whole sweet corn (frozen)	47
Corn tortilla (Mexican)	52
Corn muffin, high amylose	49
Sweet corn on the cob (boiled, 20 min)	48
White rice	64
Brown rice	55
Parboiled rice	47
Bulgur wheat	48
Puffed wheat	74
Wheat bread	70
Wholemeal bread	69

Source: Foster-Powell et al. (2002). Reference food is glucose

amylose content have higher retrogradation rates (Singh et al. 2006). The sugary maize starch with higher amylose has a higher retrogradation rate than normal maize starch (Singh et al. 2006). The retrogradation makes the starch resistant toward breakdown by digestive enzymes and consequently reduces the glycemic index (GI) (Fredriksson et al. 2000). GI characterizes the carbohydrates consumed in the form of different foods on the basis of the postprandial level of blood glucose (Jenkins 2007). Carbohydrates that are quickly digested and release glucose rapidly into the bloodstream are considered to have a high GI. GI of maize, rice, and wheat products is shown in Table 5.3. Canned, frozen, and boiled sweet maize has lower GI (46–47) as compared to white rice (64) and wheat bread (70). Long-term intake of foods with a high GI is associated with obesity, diabetes, and cardiovascular diseases. Foods with low GI are recommended for the prevention of diseases that are associated with metabolic disorders (Englyst et al. 1999; Jenkins et al. 1981; Ludwig 2000). Starch is classified into three groups depending upon the rate of release and absorption of glucose in the gastrointestinal tract: rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). RDS is the group of starches that can be rapidly hydrolyzed by digestive enzymes, SDS is the group that is digested at a relatively slow rate (Englyst et al. 1992), and RS is not digested by digestive enzymes and consequently is transferred into the colon. Waxy maize starch is more rapidly digested than high-amylose starch, attributed to more surface area per molecule of the amylopectin than amylose. Many health benefits such as improved cholesterol metabolism and reduced risk of type II diabetes and colon cancer have been associated with the consumption of RS (Hoebler et al. 1999). Amylolysis susceptibility of normal maize starch is more, as compared to high-amylose maize starch, may be due to the presence of surface pores and channels that facilitate enzymatic diffusion (Zhang et al. 2006). The association between amylose chains and their potential for amylose–lipid complex formation (Morita et al. 2007), higher crystalline lamella thickness, and a thicker peripheral layer (Jenkins and Donald 1995) are the factors that make the high-amylose maize starch granules resistant to amylolysis. Thermal treatments such as autoclaving, baking, steam cooking, and parboiling processes affect the gelatinization and retrogradation processes and, consequently, the formation of RS in foods. Hi-maize® is rich in amylose (80 %) and RS (42 %), which is a commercial source of RS that is widely used in various baked goods. The chemical modifications of starches are carried out to change the functional properties that also change the susceptibility toward the action of enzymes, e.g., esterification, etherification, and cross-linking of starch make it resistant to α-amylase and reduce digestibility (Hood and Arneson 1976).

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Oil Improvement in Maize: Potential and Prospects

6

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Abstract

High-quality maize oil, having low level of saturated fatty acids, is highly suitable for human consumption. It is considered to be better than most of other edible oils due to its fatty acid composition and stability during storage and cooking. There is about 3–4 % oil content in maize kernel. However, more than 6–7 % oil is reported in high-oil corn genotypes. High-oil lines, in general, have reduced yields. Large numbers of genes/QTLs were reported to control this trait, thus, making it difficult to improve. A combination of conventional breeding methods, marker-assisted selection and transgenic approach would help in developing high-yielding genotypes with enhanced oil content in maize.

6.1 Introduction

Maize is an annual crop with high productivity and exceptional geographic adaptability that has helped its cultivation to spread throughout the world. It is one of the three most important food grain crops in the world. In India, it occupies the third position among food grains with respect to production and productivity and ranks fifth for the area under cultivation. It is used for human food, livestock and poultry feed, industrial raw material and as fodder. In addition to food and feed, it has wide range of industrial applications from food processing to manufacturing of ethanol.

Maize is one of the oldest human-domesticated plants. Its origins are believed to date back to at least 7,000 years ago when it was grown in the form of a wild grass called *teosinte* in Central Mexico. Recognizing its early potential as a major food crop, over time, the *Mesoamerican* natives managed to improve the crop, by systematically selecting certain varieties for their desired traits. This process led to the gradual transformation of *teosinte* to its present day form known as maize, a name which is a likely derivative of *mahis*, meaning "source of life" for *Taino* people. Maize is also known as *corn* in the United States, the world's largest producer, consumer and exporter of maize. This reference to corn, rather than maize, in the United States can be traced to the arrival of the

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early European settlers in the New World when maize was referred to as *Indian corn*. Indian corn is a type of maize known for its colourful kernel. The term corn most likely originates from the Germanic *korn*, which referred to any edible grass. Maize occupies a dominant place in the world economy and trade as an industrial grain crop (White and Johnson 2003).

Plant breeding has been extremely successful in improving the yield of maize. Advances made by breeders in quality improvement resulted in maize kernels with a wide range of structures and compositions. By exploiting genetic variation, the composition of the kernel was altered for both the quantity and quality of starch, proteins and oil. The great economical and nutritional value of the maize kernel is mainly due to its high starch content that represents 75 % of the mature seed weight, along with the protein complement (10 % of the mature seed weight), mainly found in the form of zeins (storage proteins) and oil (4.6 %). The intensive use of the maize kernel is due not only to its high starch content but also to the oil stored in the embryo. Oil, in fact, is the most valuable coproduct from industrial processing of maize grain, and it represents a source of high-quality oil for humans (Hartings et al. 2012).

6.2 Status of Corn Oil in India

Cultivation of a large number of oilseed crops in India ensures availability of a vast range of edible oils; therefore, edible purpose corn oil is not very popular for domestic uses. In the United States, corn oil constitutes 4.3 % of vegetable oil production and about 7.2 % of domestic oil utilization. In India, however, it is not an ingredient in the traditional food recipes, and this is the main reason for less popularity of corn oil for cooking purpose. High price and lack of public awareness of corn oil further restrict its popularization in the country. Almost 2 mt of maize is used in the starch industry, yielding about 70,000 tonnes of corn oil as a by product, annually. This figure is expected to rise up to 8 mt by 2050, which can produce about 0.3 mt tonnes of oil (Hegde 2012). Local factories are extracting 3.0–3.5 % oil from corn, which otherwise contain at least 5–6 % of oil. Thus, in India, there is a huge scope in increasing the production of corn oil by improving the efficiency of oil extraction from corn germs. Furthermore, efforts are needed to develop maize hybrids with higher oil content in the germ, which in turn will help in obtaining higher oil yield from maize processing.

6.3 Quality of Corn Oil

Corn oil is valued for its high energy content. Energy value of 100 g corn oil is 884 kcal as against 86 kcal in case of equal amount of corn meal. Good quality of cooking oil is usually associated with elevated proportion of unsaturated to saturated fatty acids. Maize oil is highly regarded for its low levels of saturated fatty acids, with an average 11 % palmitic acid and 2 % stearic acid, against relatively high levels of polyunsaturated fatty acids such as linoleic acid (24 %). Maize oil is comparatively stable since it contains only small amounts of linolenic acid (0.7 %) and high levels of natural antioxidants (Val et al. 2009). Furthermore, presence of significant amount of phytosterols (968 mg/100 g) in corn oil improves its quality significantly by reducing the amount of cholesterol absorption from food. Corn oil also provides a good source of vitamin E (21.11 mg ATE/100 g) and tocopherol (14.3 mg/100 g). These are responsible for increased shelf life of corn oil. Four isomers of tocopherols are found in corn, viz., α, β, γ and δ, and out of these, α-tocopherol is the most common. It has been found that genotype and environmental factors affect fatty acid composition of corn oil. The fatty acid composition of corn oil is affected by position of kernels on the ear. Palmitic and linoleic acid content of the oil increases for kernels from the base to the tip of the ear. On the other hand, oleic acid content of the kernels decreases from base to tip. Sampling of kernels in the central portion of the ear is recommended for the analysis of fatty acid composition (Zheng et al. 2008).

6.4 Importance and Merits of Corn Oil

Most common use of corn oil is as salad oil, cooking oil and in margarine. Preference of corn oil over other vegetable oils is mainly due to its stability during storage and cooking that saves the consumers from the additional synthetic antioxidants. The presence of tocopherols in corn oil acts as natural antioxidants by protecting double chemical bonds from oxidation. Tocopherols along with low levels of linoleic acid (18:3) are responsible for stability of corn oil. In addition, its blend flavour and high smoke point (>200 °C) are mainly responsible for its popularity. Phytosterol in corn oil is responsible for lower cholesterol absorption in the body from cholesterol-containing food materials, and it increases the percentage of high-density lipoproteins (HDL) in blood. Large numbers of cosmetic products principally based on corn oil are available in the markets. Crude corn oil can also be used as surfactant. There are reports in which corn oil has been used to increase the efficiencies of fungal biocontrol agents.

6.5 High-Oil Corn

It is estimated that 80–84 % of the total oil is located in the germ, 12 % in the aleurone layer and 5 % in the endosperm. Normal corn is rich in starch and provides around 4 % oil, whereas high-oil corn (HOC) contains more than 6 % oil (Lambert 2001). There is a 3–4 % increase of oil in high-oil maize over normal corn. Additionally, protein quality and quantity are increased to some extent in high-oil corn due to larger germ (including embryo and scutellum) size. High-oil corn differs from normal corn with respect to kernel anatomy and composition. The germ in HOC has about 1 % higher total protein and it also contains higher levels of essential amino acids like lysine, threonine and methionine, therefore, making it highly suitable for human and animal consumption (White et al. 2007).

High-oil maize shows a greater feed efficiency than normal maize in animal feed trials because the oil imparts 2.25 times higher caloric content than that of starch on dry weight basis and also because of its fatty acid composition, mainly oleic and linoleic acids (Gross and Kerr 1992). It has been observed that when oil content is higher (more than 6 %) in feed rations, the animals usually observe a significant feed response over normal rations. It has been reported from feeding experiment that lambs on high-oil and high-protein rations gained 7 % more weight, retained more nitrogen and required 6 % less feed per pound of grain than the normal diet (http://expert.iasri.res.in/agridaksh/html_file/maize/Introduction_highoilcorn.htm). High-oil corn diets fed to cows during the last 30 days of gestation resulted in significantly greater body weight gains and higher fat levels in the colostrum than normal diets. Feeding trials on laying hens showed that diet of 17 % protein with high-oil corn leads to better egg to feed ratio than normal ration and egg production and yield increased with the high-protein high-oil diets. Skin and plasma pigmentation was also improved in chicks fed with high-oil diets. Feeding trials with lactating dairy cattle showed 12 % greater intake of dry matter for high-oil diet. On an average, feeding trials suggested an advantage of 7 % or more with high-oil corn rations in chicken, swine, dairy cattle and sheep over normal rations. High-oil corn, therefore, plays an important role in feed rations due to its nutritional advantage and greater energy per unit of feed.

Protein concentration in high-oil hybrids is higher and protein quality is enhanced because of the larger scutellum size compared with normal corn. Amino acid lysine increased from 0.244 % (normal corn) to 0.297 % (high-oil corn). High-oil corn hybrids usually have higher proportion of yellow pigments (carotenoids, xanthophylls, etc.), which are beneficial in poultry feed and play important biological functions in mankind.

Improvement in the oil content of the hybrids is most important for the growth of corn oil industry and production of low-cost oil. This, in turn, will help in acceptance of this oil by the consumers.

6.6 Genetic Resources

Knowledge of genes involved in determining quality-related traits (structure and chemical diversity) of starch, proteins, oil and other compounds is important for improving the industrial, nutritional and food-making properties of the maize grains. Corn kernel oil content ranges between 1.2 and 20.0 on weight basis. In maize, kernel oil content is considered a quantitative trait controlled by many genes with small effects (Dudley 1977). The number of genes controlling this trait estimated by quantitative genetic and biochemical methods was found to be up to 69 (Dudley and Lambert 1992). Genetic mapping of oil traits indicates that multiple (>50) QTLs are involved in lipid accumulation (Laurie et al. 2004), thus making it difficult to improve.

Oil and starch are accumulated in different compartments of kernel. Eighty-five percent of the oil is stored in the embryo, whereas 98 % of the starch is located in the endosperm. Studies on the biosynthesis of oil indicate that plant oil is synthesized from glycerol-3-phosphate and fatty acyl-CoA in the endoplasmic reticulum as triacylglycerols (TAGs—esters of fatty acids and glycerol) (Baud and Lepiniec 2010, Barthole et al. 2012). Fatty acids are synthesized from acetyl-CoA in plastid and then transported to the cytoplasm in the form of fatty acyl-CoA where they are used for the acylation of the glycerol-3-phosphate. Resulting TAGs are stored in specialized structures called oil bodies. The primary determinant of lipid content in maize kernel is the genetic makeup (Lambert and Hallauer 2001). In spite of a good understanding of the oil biosynthetic pathway in plants and identification of the genes involved, the molecular basis for oil quantitative trait loci (QTLs) is largely unknown. However, Zheng et al. (2008) found an oil QTL (*qHO6*) affecting maize seed oil and oleic acid content. This QTL encodes an acyl-CoA:diacylglycerol acyltransferase (DGAT1-2), which catalyses the final step of oil synthesis. Evidence has been obtained that genetic variation also exists for the fatty acid composition of the kernel (Lambert 2001). Studies indicate that the inheritance of oleic, linoleic, palmitic and stearic acid content is complex and under polygenic control. Molecular characterization of *fatty acid desaturase-2* (*fad2*) and *fatty acid desaturase-6* (*fad6*) reveals that *fad2* and *fad6* are not associated with QTLs for the ratio of oleic/linoleic acid. This suggests that some of the QTLs for the oleic/linoleic acid ratio involve other genes that may influence the flux via enzymes encoded by *fad2* or *fad6* (Motto et al. 2011).

Application of new technologies, such as transcription profiling, metabolic profiling and flux analysis, should prove valuable to more precisely identify the genes and enzymes, determining the composition of maize oil (Hartings et al. 2012). In addition, identification of transcription factors or other regulatory proteins that control oil biosynthesis or embryo development will be particularly useful for biotechnological approaches in the future. Arabidopsis orthologs in maize that trigger genes involved in TAG metabolism *(LEAFY COTYLEDON1-2*, i.e., *ZmLEC1*), and those necessary to mediate the regulators towards late glycolytic and oil metabolism of the transcription factor *WRINKLED1* (i.e. *ZmWRI1a* and *ZmWRI1b*), have been identified (Pouvreau et al. 2011). In maize, both genes are preferentially expressed in the embryo and exhibit a peak of expression at kernel maturity. *ZmWRI1a* is induced by *ZmLEC1* (Shen et al. 2010). Furthermore, transcriptomic analysis of ZmWRI1a overexpressing lines led to the identification of putative target genes of ZmWRI1a involved in late glycolysis and fatty acid or oil metabolism.

6.7 Breeding for Oil Yield

Efforts directed toward increasing oil content of maize kernels through breeding have been successful, but unfortunately high-oil lines have significantly reduced yield (Moose et al. 2004) as, compared to starch, synthesis of oil is an energy-consuming process. In general, high-oil corn hybrids yield 5–10 % lower than normal corn; therefore, attempts are being made to enhance oil content without compromising yield potential. Continuous genetic selection

has steadily improved agronomic performance and yield of corn hybrids with higher oil content, thus, narrowed the yield gap between high-oil corn and normal corn hybrids. Alexender (1988) started the breeding programme of high-oil maize using recurrent selection with the genetic diverse synthetic, and after 27 selection cycles, the oil concentration of the improved population reached 21.2 %. Song et al. (2004) developed high-oil maize from synthetic variety Zhongzong No. 2 through a succession of 18 recurrent selections and increased the oil content from 4.71 % to 15.55 %.

The genotype of the sporophyte greatly determines the amount of oil produced by high-oil hybrids so that these hybrids may have a more efficient energy-trapping system. It has been observed that the female parent had the largest influence on oil percentage of the kernel as compared to the male parent. Even then, xenia effects existing in corn for total oil content possibly resulted from an increase in grain size and increased oil content in the germ as high-oil content of the pollen source increases the proportion of germ in the kernel. Similar relationship exists for oil per cent in the germ. Under open pollinated conditions, the oil levels of the lower-oil hybrids may be increased, and the oil levels of the higher-oil hybrids may be reduced because of xenia effects.

Genotype × environment interactions and crop management practices such as dosage of fertilizers has been reported to influence oil content in high-oil genotypes. However, effect of genotype on oil values seems to be greater. Variation in total oil content is not associated with total protein content but is positively correlated with per cent germ protein and relative concentration of tryptophan in the kernel. Association of increased oil content with reduced ear length, smaller ear diameter, lighter kernel weight, reduced plant height and ear height and early flowering has been reported. Successful breeding for high oil content in the Illinois high-oil strains has mainly been achieved through an increase in embryo size (Moose et al. 2004). To enhance the oil content in maize kernels, the relative proportion of the oil-rich embryonic tissue within the grain needs to be increased. However, the molecular mechanism responsible for this has not been understood well.

Recombinant DNA technology is being utilized to enhance the quality and quantity of maize oil. In the transgenic maize lines, expressing the wheat Purindoline a and b (*PINA* and *PINB*) genes, the total oil content of the kernel was increased by 25 % (Zhang et al. 2010). An examination of carbon metabolism in maize embryos suggested that the flux of carbon through NADP-ME may constitute a metabolic bottleneck (Alonso et al. 2010). The oil content of the kernel is, thus, positively correlated with malic enzyme activities in maturing embryos (Doehlert and Lambert 1991), which makes NADP-ME an attractive target for engineering high-oil trait in maize. Numerous biotechnological approaches have been devised to maximize the flow of C into oil by overexpression of enzymes of the TAG assembling network in oilseed crops. In maize, several attempts have been made to overexpress diacylglycerol acyltransferases (DGAT). The embryo-specific overexpression both of maize DGAT1–2 and of fungal DGAT2 (Zheng et al. 2008; Oakes et al. 2011) resulted in significant increase in kernel oil content. These studies provide insights into the molecular basis of natural variation of oil and oleic acid contents in plants.

Seed-specific expression of ZmWRI1 enhanced oil accumulation in transgenic maize without any associated abnormalities. It was also found that ZmWRI1 not only increases the fatty acid content of the mature maize grain but also of certain amino acids (Lys, Glu, Phe, Ala and Val) and of several compounds involved in amino acid biosynthesis (Pouvreau et al. 2011). However, expression of ZmLEC1 under similar conditions severely affected growth and development of the transgenic plants (Shen et al. 2010).

A maize breeding programme for oil content in kernels should be designed to circumvent the decrease in grain yield. Understanding the nature of quantitative genetic variation for oil content in maize is essential to develop a suitable breeding programme. Development of divergent high-oil parental lines and their use in hybrid development is the most important option for development of high-oil maize. Favourable

alleles for high oil content can be assembled through practising selection generation after generation accompanied by intermating of the selected individuals or families. Though, this will help in improving the genetic ceiling continuously, but is a slow and time-taking process. Efforts to accumulate the favourable QTLs in the parental lines through marker-assisted recurrent selection (MARS) or development of nested populations are needed. Transgenes like *PINA, PINB, ZmWRI1*, and *ZmLEC1* would be helpful in further improving the oil content in the maize inbreds and hence in hybrids. These different approaches, if used in combination, may help in speedy improvement of oil content in maize populations or inbreds.

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Maize Carotenoid Composition and Biofortification for Provitamin A Activity

7

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Abstract

Carotenoids are fat-soluble antioxidant vitamin compounds derived from the isoprenoid biosynthetic pathway. These natural pigments are secondary metabolites and can be divided into two classes—carotenes and xanthophylls—which play diverse biological roles in plants and animals. Carotenoids with unsubstituted β-ring end groups become more important because of their provitamin A activity. Maize is the third most staple food worldwide and also contains appreciable amount of provitamin A carotenoids with wide range of genetic variability. This makes it a good candidate crop for biofortification of provitamin A carotenoids. The quantity of provitamin A carotenoids needed to alleviate vitamin A deficiency (VAD) through biofortification depends upon its bioavailability, which is influenced by a number of factors in an individual. The bioavailability of biofortified maize can be known through determining vitamin A equivalence. Recent advances have shown that β-carotene in biofortified maize has good bioavailability as a plant source of vitamin A. So, a quantity of 15 μg provitamin A g^{-1} dry weight of kernel was targeted for biofortification. This chapter also includes the carotenoid biosynthetic pathway, biofortification strategies, recent advancements made toward biofortification of provitamin A, and future perspectives.

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7.1 Introduction

Carotenoids are natural pigments derived from the isoprenoid biosynthetic pathway and produced by most photosynthetic organisms that have been shown to be beneficial to both plants and animals. Carotenoids represent a diverse group of more than 750 structures found in

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bacteria, fungi, algae, and plants (Britton et al. 2004). Of the carotenoids found in nature, 20–50 are common in the human diet and about 20 are found in human blood and tissues (Johnson 2004). Biochemically, these secondary metabolites are terpenoids and constitute a class of fat-soluble antioxidant vitamin compounds containing polyisoprenic structure. There are two distinct classes of carotenoids—carotenes, which contain only carbon and hydrogen, and xanthophylls, which contain oxygen groups (Van den Berg et al. 2000). Carotenoids are lipophilic and are found in hydrophobic areas of cells in close proximity to proteins and lipids (Britton 1995; Van den Berg et al. 2000). Carotenoids play various biological roles in plants and animals. Some recent studies suggesting that greater intakes of carotenoid-containing foods result in reduced risks for several chronic diseases have stimulated greater interest in carotenoids (Canene-Adams et al. 2005). The exact chemical structure of individual carotenoids is decisive for their biological properties because it determines how they interact with other molecules and integrates into membranes (Rouseff et al. 1996).

Carotenoids can be divided into two groups, provitamin A and non-provitamin A carotenoids, depending upon their ability to release vitamin A. Vitamin A deficiency has emerged as a serious global health concern. A sustainable solution to eliminate VAD is increasing the provitamin A carotenoids in the major staple food crops, i.e., through biofortification. As maize is a staple food worldwide and also rich in natural genetic diversity for provitamin A carotenoids, qualify as a suitable candidate crop for biofortification (Wurtzel 2004). Recent advancements in areas of genetics, biotechnology, biochemistry, and analytical tools have led to the identification of QTLs, genes/alleles, or allozymes controlling the regulatory steps of carotenoid biosynthetic and utilizing pathways. Utilizing the available natural genetic variability for provitamin A carotenoids, maize breeders have succeeded in developing biofortified maize lines containing $\leq$15 μg β-carotene/g dry kernel weight (Yan et al. 2010). So, this chapter elaborates the importance of carotenoids, various findings related to metabolic pathway, developments, and future strategies toward biofortification of maize.

7.2 Biosynthetic Pathway

The biosynthesis of carotenoids in plants occurs on membranes of plastids with the regulating enzymes encoded in the nuclear genes and targeted to these plastids (Gallagher et al. 2004). Carotenoids are derived from the isoprenoid biosynthetic pathway and are precursors of the plant hormone abscisic acid and of other apocarotenoids. The first committed step of this pathway involves the condensation of two geranylgeranyl pyrophosphate (GGPP) to form 15-cis-phytoene, a colorless C40 compound. This constitutes the key regulatory step of the pathway and is catalyzed by phytoene synthase (PSY). Phytoene is converted to all-trans lycopene (a red pigment) through a series of reactions mediated by phytoene desaturase (PDS), ζ-carotene isomerase (Z-ISO), ζ-carotene desaturase (ZDS), and carotene isomerase (CRTISO). The first branch point of this pathway occurs at cyclization of lycopene cyclized by lycopene ε-cyclase (LCYE) and/or lycopene β-cyclase (LCYB) to generate α- and β-carotenes, respectively. Relative activities of LCYB and LCYE are hypothesized to regulate the proportion of carotenes directed to each branch of this pathway. α- and β-carotenes are subsequently hydroxylated and modified to form the various xanthophylls. The carotenoid biosynthetic pathway in plants is shown in Fig. 7.1.

7.3 Biological Roles

In plants and animals, these pigments play diverse biological roles as mentioned below:

As natural pigments, carotenoids are responsible for different colors present in plant parts, which is determined by the number and location of the double bonds present within the structure (Watson 1962). As accessory pigments in the photosynthetic apparatus, they play various roles

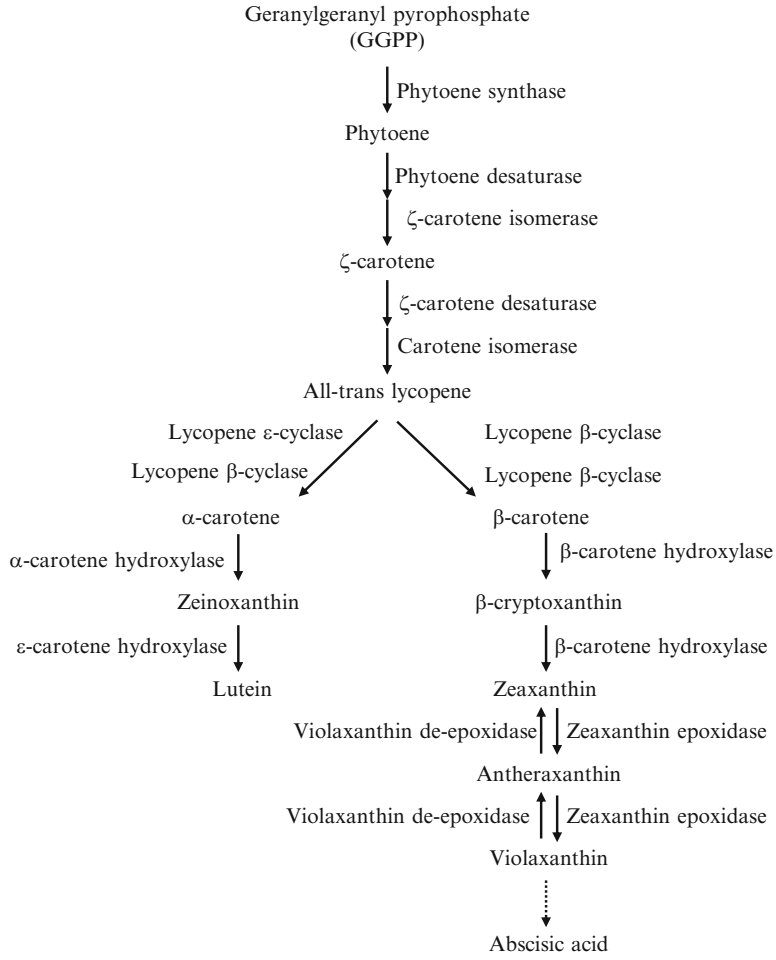

Fig. 7.1 Carotenoid biosynthetic pathway in plants

such as in light harvesting and photoprotection, as attractants for seed dispersal and pollination, and as precursors of some scents as antioxidants (Cunningham 2002; Fraser and Bramley 2004; Howitt and Pogson 2006). In spite of various biological functions, their role as antioxidants appears to be ubiquitous, which is best understood in chloroplasts, where desaturated carotenoids quench triplet chlorophyll and singlet oxygen, preventing the formation of reactive oxygen species and photo-oxidation of the contents of the organelle (Niyogi 1999). Carotenoids also act as precursors to various cleavage products, such as the apocarotenoid abscisic acid (ABA), which regulates plant growth, embryo development, dormancy, and stress responses (Nambara and Marion-Poll 2005), and additional apocarotenoid products, such as strigolactone and others, whose functions are still not known but are considered essential elements that may affect plant yield (Booker et al. 2004; Akiyama and Hayashi 2006). Carotenoid antioxidants protect membranes from lipid peroxidation under heat and light stress conditions (Havaux et al. 2007; Johnson et al. 2007).

Provitamin A activity is the classical biological function of carotenoids in mammalian systems (USDA 2008). Carotenoids such as α-carotene, β-carotene, and β-cryptoxanthin have provitamin A activity as these contains unsubstituted β-ring end groups. Among these, β-carotene contains two provitamin A structures, i.e., two non-hydroxylated β-ionone rings, in comparison to β-cryptoxanthin and α-carotene,

which contains single non-hydroxylated β-ionone ring, so it has twice the activity of the others. In case of mammals, provitamin A carotenoids are cleaved in the intestinal lumen to produce retinal (vitamin A). In addition, xanthophylls such as lutein and zeaxanthin are the essential components of the macular pigment of the eye (Beatty et al. 1999). Macular pigment protects retinal rods and cones by filtering harmful UV/blue light wavelengths (Mares-Perlman and Klein 1999; Kopsell and Kopsell 2006). Apart from these activities, several other essential biological functions of carotenoids, particularly in human health, include reducing the risk of degenerative diseases such as cardiovascular, cancers, cataract, and muscular degeneration via their role as antioxidants and/or as regulators of the immune system (Fraser and Bramley 2004; Johnson 2004; Tang et al. 2005).

Economic benefits to animal production systems are also associated with carotenoids. Diets of chickens with high levels of carotenoid pigments have also been associated with a desirable color of the egg yolks and of the skin of broilers and fryers (Blessin et al. 1963; Perez-Vendrell et al. 2001). β-carotene levels may work synergistically with other fat-soluble vitamins in the protection of broiler leg meat from oxidation (Ruiz et al. 1999). This coloration of yolks and skin is perceived as associated with good health and quality by some consumers (Hadden et al. 1999). The industrial use of carotenoids involves their application for nutrient supplementation, pharmaceutical purposes and as food colorants and animal feeds (Weber 1987).

7.4 Implications of Vitamin A Deficiency

Provitamin A activity is the classical biological function of carotenoids in mammalian systems as only its deficiency in the body may result into causal factor for numerous other diseases. It is well known that adequate vitamin A intake is important for vision, growth, cellular differentiation and proliferation, reproduction, and the integrity of the immune system (Sommer 1982). Globally, approximately one-third of preschool-age children and 15 % of pregnant women are estimated to be vitamin A deficient (WHO 2009). The problem becomes more severe particularly in the developing countries whose poor populations rely on a single staple crop for their sustenance, e.g., Africa and Southeast Asia have the highest burden of vitamin A deficiency (WHO 2009). The consequences of VAD include blindness, reduced growth in children, and increased morbidity and mortality (Sommer and West 1996; Shankar et al. 1999; Rice et al. 2004; Maida et al. 2008).

7.5 Maize Carotenoid Composition

Maize exhibits considerable natural variation for kernel carotenoids, with some genotypes accumulating as high as 66.0 μg/g (Harjes et al. 2008). Yellow maize kernel carotenoids are present in different isoforms, including two carotenes, α- and β-carotene, and three xanthophylls, β-cryptoxanthin, zeaxanthin, and lutein (Watson 1962; Weber 1987). The predominant carotenoids in maize kernels, in decreasing order of concentration, are lutein, zeaxanthin, β-carotene, β-cryptoxanthin, and α-carotene. Generally, provitamin A carotenoids constitute only 10–20 % of total carotenoids in maize, whereas zeaxanthin and lutein each commonly represent 30–50 %. The amounts of provitamin A in traditional yellow maize varieties range from 0.25 μg to 2.5 μg g^{-1} dry weight. In typical maize, concentrations of provitamin A carotenoids, i.e., α-carotene, β-carotene, and β-cryptoxanthin, range from 0 to 1.3, 0.13 to 2.7, and 0.13 to 1.9 nmol/g, respectively (Kurilich and Juvik 1999). Although β-carotene has the highest provitamin A activity, it is present in a relatively low concentration (0.5–1.5 μg/g) in most yellow maize grown and consumed throughout the world (Harjes et al. 2008).

7.6 Biofortification

Biofortification is the development of micronutrient-dense staple crops using the best traditional breeding practices and modern biotechnology. In developing countries, staple food crops predominate in the diets of the poor, so, biofortification inherently targets the most vulnerable populations (Bouis 2003). Biofortification differs from ordinary fortification because it focuses on making plant foods more nutritious as the plants are growing, rather than having nutrients added to the foods when they are being processed. The biofortified seeds can be easily reproduced by poor farmers, and thus the seeds are a sustainable means to target remote rural communities not served by conventional seed markets (Qaim et al. 2007).

To alleviate the vitamin A deficiency from rural areas of developing countries, biofortification of major staple food crops of respective areas or countries with provitamin A carotenoids is the only feasible way, since this will better ensure the targeting and compliance. Furthermore possible economic benefits to animal production systems are also associated with carotenoids, which can be increased through biofortification for carotenoids.

As the third most important cereal staple food crop worldwide (FAPRI 2009), a major cereal staple for African consumers (FAOSTAT 2010), maize has also the advantage of being the only crop that contains appreciable amount of carotenoids (Wurtzel 2004) with wide range of genetic variability. The first solid food given to African infants includes white maize porridges devoid of provitamin A (Faber et al. 2005; Poggensee et al. 2004) and are also widely consumed by older children and adults. The nutritional improvement of maize with provitamin A carotenoids would have a significant impact on the target populations. Utilizing the available natural genetic variability for provitamin A carotenoids, maize breeders have succeeded in developing biofortified maize lines containing $\leq$15 μg β-carotene/g dry kernel weight (Yan et al. 2010).

Before devising a strategy to enhance the provitamin A carotenoids in maize, one question that must be addressed is how much quantity is needed to alleviate VAD. This is related to the bioavailability or the fraction of an ingested nutrient that becomes available to the body for utilization in physiological functions or for storage (Jackson 1997; Fraser and Bramley 2004). There are numerous factors that influence bioavailability, including nutrient status of the host, species of carotenoid, food matrix, and amount of food consumed in the meal. In biofortified maize, the bioefficacy of the β-carotene can be predicted by determining experimentally its vitamin A equivalence. The β-carotene quantity needed to provide vitamin A activity equivalent to 1 μg of retinol is known as vitamin A equivalence. The extent of conversion of β-carotene to vitamin A is highly variable among well-nourished people. A recent study evaluated the effect of genetic polymorphisms in the *BCMO1* gene that encodes β-carotene 15,15′-monooxygenase on the conversion of β-carotene to retinyl palmitate (Leung et al. 2009). Taking these factors into account, nutritionists have estimated that 15 μg provitamin A g^{-1} dry weight of kernel could greatly alleviate vitamin A deficiency (www.harvestplus.org). Li et al. (2010) carried out an experiment on women to quantify the vitamin A equivalence of the β-carotene in β-carotene-biofortified maize based on consumption of a single serving of maize porridge and found that β-carotene in biofortified maize has good bioavailability as a plant source of vitamin A.

So, improving the micronutrient balance of staple crops such as maize through biofortification is therefore an economically and socially sound way to address micronutrient malnutrition, including VAD, on a global scale (Tanumihardjo et al. 2008).

7.7 Strategies for Biofortification of Maize

To increase the provitamin A potential of maize, there is a need to maximize biosynthetic flux toward carotenoid synthesis, limit the

carotenoids degradation, inhibit branching points leading to non-provitamin A carotenoids and inhibit enzymes leading to the conversion of provitamin A to non-provitamin A carotenoids. This can be achieved by regulating the rate-limiting steps of the pathway or identifying the alleles contributing toward synthesis of provitamin A carotenoids as mentioned below.

The enzyme phytoene synthase catalyzes the first rate-limiting step of the carotenoid biosynthetic pathway and is the most important as its activity controls the carbon flux toward carotenoid biosynthesis. The first branch point of this pathway occurs at cyclization of lycopene where action of lycopene beta cyclase (LCYB) at both ends of linear lycopene produces a molecule with two β-rings. Alternatively, the coaction of LCYB and lycopene epsilon cyclase (LCYE) generates a β, ε-carotene that is a precursor to lutein. Relative activities of LCYB and LCYE are hypothesized to regulate the proportion of carotenes directed to each branch of this pathway. If somehow flux can be shifted toward more synthesis of β-carotene, it will be a boost for provitamin A synthesis. But as the concentration of provitamin A carotenoids increases, a large amount is hydroxylated to β-cryptoxanthin (β-CX) and zeaxanthin (Z), which have 50 % and 0 % of the provitamin A activity of β-carotene, respectively. So, there is need to downregulate the activities of carotene hydroxylases. Furthermore the inhibition of the steps leading to degradation of the carotenoids or directing toward other metabolic pathways such as synthesis of ABA will also help in increasing the concentration of provitamin A.

7.8 Recent Advancements Related to Metabolic Pathways Toward Increasing Provitamin A Carotenoids

Use of composite interval mapping led to identification of two candidate genes, *yellow 1* and *viviparous 9*, which may be responsible for quantitative variation in carotenoids. The *yellow 1* gene is associated with phytoene synthase, the enzyme catalyzing the first dedicated step, and the *viviparous 9* gene is associated with zeta-carotene desaturase, an enzyme catalyzing an early step, in the carotenoid biosynthetic pathway (Wong et al. 2004). Similarly, Chander et al. (2008) constructed a genetic linkage map using a 233 recombinant inbred lines derived from a cross between By804 and B73 and identified 31 putative QTL in total including 23 for individual and 8 for total carotenoids. Two loci, i.e., *y1* and *y9*, that explained most of the phenotypic variation in carotenoids contents were identified along with a gene-targeted marker (Y1ssr) in the candidate gene phytoene synthase 1 (*psy1*) tightly linked to a major QTL explaining 6.6–27.2 % phenotypic variation for levels of carotenoids. They also emphasize the role of QTL cluster located at *y9* locus for pyramiding favorable alleles controlling contents of carotenoids from diverse maize germplasm. Later on, Chen et al. (2010) found that maize *y9* locus encodes ζ-carotene isomerase (Z-ISO), an enzyme necessary for endosperm carotenogenesis in plants. Another catabolic gene affecting seed carotenogenesis, i.e., *CCD1* from maize, was cloned and found to cleave carotenoids effectively (Sun et al. 2008; Vogel et al. 2008). The position of *ZmCCD1*, chromosome 9.07, was found linked to the dominant *white cap 1* (*wc1*) locus involved in the depletion of endosperm carotenoids through gene dosage effect (Vallabhaneni et al. 2010).

Several haplotypes of the gene encoding lycopene epsilon cyclase (*lcyE*) that substantially increase the ratio of β- to α-carotenoids in maize grain were identified by Harjes et al. (2008). To find out the correlation between carotenoid content and candidate gene transcript levels, a maize germplasm collection was used by Vallabhaneni and Wurtzel (2009), and it was observed that transcript levels of paralogs encoding isoprenoid isopentenyl diphosphate and geranylgeranyl diphosphate-producing enzymes, *DXS3*, *DXR*, *HDR*, and *GGPPS1*, were positively correlated with endosperm carotenoid content. *PSY1* and *CrtISO* transcripts were found to be positively and inversely correlated, respectively, for carotenoid pathway enzymes.

Furthermore, the *ZEP* (zeaxanthin epoxidase) transcript level, the enzyme involved in the depletion of carotenoid pool for its conversion to ABA, was also examined, and carotenoid accumulation was found inversely associated with *ZEP1* and *ZEP2* transcript levels.

Using metabolite sorting on maize diversity core collection, Vallabhaneni et al. (2009) identified the enzyme carotene hydroxylase encoded by the *Hydroxylase3* (*HYD3*) locus, whose transcript levels were negatively correlated with high β-carotene levels and positively correlated with zeaxanthin levels. Using PCR genotyping of 51 maize lines, they also showed that the *HYD3* locus could explain 36 % variation and fourfold difference in β-carotene levels. Yan et al. (2010) demonstrated through association and linkage population studies in maize that the gene encoding β-carotene hydroxylase 1 (*crtRB1*/*HYD3*) underlies a principal quantitative trait locus associated with β-carotene concentration and conversion in maize kernels. *crtRB1* alleles associated with reduced transcript expression were found correlated with higher β-carotene concentrations.

7.9 Progress Made Toward Maize Biofortification for Provitamin A Carotenoids

In the past few years, significant progress has been made toward maize biofortification. Conventional breeding has led to the development of a few high β-carotene maize lines having a maximum of 13.6 μg g^{-1} of provitamin A g^{-1} dry weight kernel that approach the target of 15 μg g^{-1} of provitamin A g^{-1} dry weight kernel (Kurilich and Juvik 1999; Islam 2004; Harjes et al. 2008). Biofortified maize lines containing ≤15 μg β-carotene/g dry kernel weight have been successfully developed by the breeders (Yan et al. 2010). Now these identified source lines are being routinely used as parents for new crosses to obtain new sources with greater provitamin A carotenoids. As breeding programme is cyclic in nature, there is need of continuous screening of germplasm and to exploit the natural variability available through molecular marker-assisted selection (MAS) for biofortification purpose.

Transgenic strategies provide important tools to transfer the desired trait from one species to another. Aluru et al. (2008) show that maize seeds can be metabolically engineered to produce high levels of provitamin A comparable to 50 % EAR values by overexpressing the bacterial *crtB* and *crtI* genes in an endosperm-specific manner, using a modified and highly active c-zein promoter. As maize exhibit considerable natural variation for provitamin A carotenoids, transgenic approaches have not been used for its biofortification.

7.10 Future Perspectives

Maize biofortification for provitamin A carotenoids benefits human health and also adds to commercial value of food as these are natural colorants. National germplasm collections hold untapped potential for maize improvement. Biofortification of maize requires large-scale germplasm screening and utilization of identified high provitamin A carotenoid material into the breeding programme. There is need to identify more QTLs through population development, whereas mutational studies can provide an insight about the regulatory points. Similarly, stage-specific metabolite profiling and its correlation to candidate gene expression will provide important information regarding regulation chemistry and expression patterns. So, an interdisciplinary approach including biochemistry, genetics, plant breeding, and nutrition is required for understanding and identifying more QTLs, the rate-limiting steps of the pathway, gene expression patterns with respect to time, allozymic diversity, etc., which, in turn, might provide important information for deciding futuristic strategies. Furthermore, the developed biofortified maize material should not only contain higher quantities of provitamin A carotenoids but also have all other crucial traits including higher yield, insect-pest and disease resistance, and better nutritional quality.

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Part IV

Biotic and Abiotic Stresses in Maize

Insect-Pests and Their Management: Current Status and Future Need of Research in Quality Maize

8

M.K. Dhillon, V.K. Kalia, and G.T. Gujar

Abstract

Maize (*Zea mays* L.) is an important staple food for millions of people across the world. However, different stress factors mainly the insect-pests, viz., maize stalk borer, pink stem borer, sugarcane leafhopper, shoot bug, armyworm, shoot fly, corn leaf aphid, cob borer, and termites, have constrained the increase in yield potential of the maize genotypes deployed in India. The hybrid initiative turned out to be exceedingly important for Quality Protein Maize (QPM) efforts, and many countries in the developing world including India are becoming increasingly interested in QPM to reduce malnourishment and to sustain nutritional security. However, it is also likely that the increase in concentration and quality of nutritional components particularly the protein in QPM might favor the proliferation of insect-pests as they too prefer quality food for their growth and development and could be major constraints to increasing production and productivity of QPM. Therefore, development and deployment of high-yielding and insect-resistant QPM genotypes under the umbrella of integrated pest management system might help in narrowing down the yield gap by reducing the crop losses caused by insect-pests. Since maize is damaged by an array of insect groups with different feeding habits right from the seedling stage to maturity of the crop, no single strategy is sufficient to manage such complex group of insect-pests. Damage potential of different insect-pests, status of host plant resistance and the mechanisms of resistance involved, and the management of major insect-pests have also been discussed in this chapter.

8.1 Introduction

Maize (*Zea mays* L.) is the third most important food crop after rice and wheat and is an important staple food for millions of people in Asia, Africa, and Latin America. According to recent estimates, the maize is being cultivated over an

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area of 8.12 million hectare (mha) with annual production of 19.77 million tons (mt), contributing 49 % to poultry feed, 25 % to human food, 12 % to animal feed, 12 % to industrial products mainly the starch, and 1 % each in brewery and seed (Dass et al. 2008). Globally, about 200 million children younger than 5 years are undernourished for protein, leading to a number of health problems including stunted growth, weakened resistance to infection, and impaired intellectual development. Several million people, particularly in the developing countries, derive their protein and calorie requirements from maize. The maize grain accounts for about 15 %–56 % of the total daily calories in diets of people in about 25 developing countries, particularly in Africa and Latin America. Although maize has contributed significantly (8 %) to the food basket in India, the nutritional quality of maize protein in the traditional genotypes is poor because of imbalanced amino acid composition due to deficiencies of two main essential amino acids, lysine and tryptophan, and excess of leucine. The discovery of association of high lysine and tryptophan with *opaque-2* (*o-2*) maize endosperm by Mertz and his group in 1964 opened up new vistas in improving the protein quality of maize. Through the combined use of *o-2* gene and genetic modifiers, the QPM gene pools have been improved for yield and associated traits, kernel modification, and appearance and the QPM versions have performed quite similar to normal counterparts in yield and agronomic traits (Vasal et al. 1984, 1994; Vasal 2002). The discovery of another mutation, *floury-2* (*fl2*), also has the ability to alter endosperm nutritional quality (Bjarnason and Vasal 1992). However, *o-2* and *fl2* genes make the QPM endosperm soft and attractive to the stored grain pests. Furthermore, the hybrid initiative turned out to be exceedingly important for QPM efforts and success, and many countries in the developing world, including India, are becoming increasingly interested in it. It is also not surprising to experience increased insect-pest problems under field as well as storage conditions with the deployment of QPM genotypes, as the insects too prefer quality food for their growth and development and could be major constraints to increasing production and productivity of QPM.

The grain yields of traditional maize genotypes in India are quite low, and in spite of sincere efforts, the yield potential of maize genotypes deployed in India has not reached even half to that in the United States. Furthermore, the gap in yield potential of maize genotypes under experimental conditions and that under farmer's fields is huge, due to different stress factors, mainly the insect-pests during vegetative and storage conditions, and needs greater attention to break this yield gap. The maize is attacked by about 139 insect-pests with varying degree of damage under field and storage conditions. Among the field pests, maize stalk borer [*Chilo partellus* (Swinhoe)], pink stem borer [*Sesamia inferens* (Walker)], sugarcane leafhopper [*Pyrilla perpusilla* (Walker)], shoot bug [*Peregrinus maidis* (Ashmead)], armyworm [*Mythimna separata* (Walker)], shoot fly [*Atherigona* spp.], corn leaf aphid [*Rhopalosiphum maidis* (Fitch)], cob borer [*Helicoverpa armigera* (Hubner)], and termites [*Macrotermes* spp. and *Odontotermes* spp.] are some of the major yield-reducing factors of maize right from seedling emergence to harvest. Maize weevils, *Sitophilus oryzae* (L.) and *Sitophilus zeamais* Motschulsky, cause serious grain losses both under field and storage conditions in the tropical countries of the world. The maize stalk borer, *C. partellus* alone, causes 26.7–80.4% yield loss under different agroclimatic conditions in India (Reddy and Zehr 2004). Host plant resistance is one of the effective means of minimizing losses due to insect-pests. However, most of the maize varieties and hybrids released for cultivation are susceptible to *C. partellus* during vegetative stage (Kumar 1997) and maize weevils, *Sitophilus* spp., under field and storage conditions (Arnason et al. 1993; Hossain et al. 2007). Screening of maize germplasm for resistance to spotted stem borer, *C. partellus,* has led to identification of several maize lines with tolerance to this pest (Kanta et al. 1997), and several morphological, anatomical, and biochemical factors have been reported to be associated with resistance/susceptibility to stem borer in maize (Kumar 1997;

Bhanot et al. 2004). However, the success in incorporation of insect resistance in agronomically elite cultivars has been very slow, as it has been difficult to monitor all the traits in breeding populations, and incorporation of yield and quality traits along with insect resistance has not been rewarding in terms of yield advantage. Deployment of high QPM genotypes with resistance to insect-pests and superior yield could be one of the most effective, economic, socially acceptable, and environmentally safe tool for increasing maize productivity. Major insect-pests and their management in maize have also been discussed in this chapter.

8.2 Status of Host Plant Resistance to Insect-Pests in Maize

Considerable genotypic variation in grain protein content has been found in both QPM and non-QPM cultivars; however, protein concentration in the grain decreases with the increase in grain yield (Ortiz-Monasterio et al. 2001; Worku et al. 2007). The zein synthesis can be manipulated by nitrogen fertilization and genetic means, and a positive relationship between the zein content and grain yield needs to be established (Vasal 2001). Thus, it is possible to develop N-efficient cultivars that may combine high yield potential and protein quality at all levels of soil fertility (Worku et al. 2007). Undesirable characteristics such as reduced yield than normal maize, low grain consistence, and a farinaceous endosperm that retains water resulting in a soft, chalky endosperm that upon drying makes it more prone to insect damage and also affects harvest ability (Singh and Venkatesh 2006). The maize weevil, *S. zeamais,* is an important pest of maize in the tropical countries of the world (Arnason et al. 1993), while maize weevil, *S. oryzae,* is major pest of maize under field and storage conditions in India. Several weevil-tolerant QPM lines have been identified, which can be used in breeding for resistance to this pest (Hossain et al. 2007). Stem borers are regarded as a major limiting factor to the production of maize in India (Panwar 2005), of which the spotted stem borer, *C. partellus,* is the most serious and prevalent pest of maize in different agroclimatic regions of the country. Screening of maize germplasm for resistance to spotted stem borer, *C. partellus,* has reported low to moderate levels of resistance to this pest (Kanta et al. 1997; DMR 2007; Rakshit et al. 2008; Sekhar et al. 2008). Several new sources of insect resistance have also been identified and supplemented to the existing resistance sources against corn earworm, *Helicoverpa zea* (Boddie); corn borer, *Ostrinia nubilalis* (Hubner); sugarcane borer, *Diatraea grandiosella* (Dyar); and fall armyworm, *Spodoptera frugiperda* (J.E. Smith) in maize, but most of the maize varieties and hybrids released for cultivation are susceptible to spotted stem borer, *C. partellus* (Kumar 1997). The mechanisms, inheritance, nature of gene action, and application of molecular markers for resistance to several insect-pests such as maize borers, viz., *D. grandiosella, D. saccharalis* (Fabricius), *Sesamia nonagrioides* (Lefebvre), and *O. nubilalis* (Williams et al. 1995, 1998; Kumar and Mihm 1996; Khairallah et al. 1997; Cartea et al. 2001; Butron et al. 2005; Krakowsky et al. 2007); corn earworm, *H. zea* (Widstrom and Snook 2001), and corn leaf aphid, *R. maidis* (Fitch) (Bing and Guthrie 1991), are well understood; however, such information is poorly studied for *C. partellus* in maize. It has been difficult to breed for resistance to stem borers, despite the heavy losses incurred year after year. Moreover, screening for resistance to stem borers in maize has not been used consistently to gauge levels of resistance under controlled greenhouse and field conditions, which is prerequisite for host plant resistance studies. Screening for resistance to stem borers under greenhouse and field conditions using artificial infestation has reported to be the most effective method of characterizing insect-resistant cultivars (Sharma et al. 2007).

8.3 Mechanisms of Resistance in Maize to Insect-Pests

The total biochemical energy in crop plants is fixed, and the changes in biochemical constituents through manipulation of metabolic

pathways happen to be at the cost of change in the concentration of one another. Biochemical mechanism of insect defense in crop plants is mainly governed by constitutive and/or induced plant metabolic compounds. This is in the context that increases in concentration and quality of nutritional compounds particularly the protein in QPM might favor the proliferation of insect-pests, as the insects too prefer quality food for their growth and development and could be major constraints in increasing production and productivity of QPM. Several morphological and anatomical plant characters such as seedling vigor, leaf width, trichome density, plant height, length of internode, tassel ratio, stem thickness and hardness, and lignified vascular bundles have been reported to be responsible for resistance/susceptibility to insect-pests in maize (Durbey and Sarup 1982; Kumar and Saxena 1985; Kumar 1997; Rao and Panwar 2000, 2001; Ashfaq and Farooq-Ahmad 2002; Bhanot et al. 2004). Hairy leaf surface was found to be negatively correlated with oviposition by *C. partellus* (Kumar 1992; van den Berg 2006). In addition, numerous biochemical factors such as nitrogen, phosphorus, potash, silica, iron, sugars, amino acids, protein content, tannins, and polyphenols have also been reported to be associated with resistance/susceptibility to insect-pests in maize (Kumar and Saxena 1985; Kabre and Ghorpade 1997, 1999; Kumar 1997; Bhanot et al. 2004). Biochemical constituents, viz., dotriacontanol, heptadecanol, and nonadecanol, have been reported to be associated with oviposition deterrence by *C. partellus* females on maize (Varshney et al. 2003) and certain chemicals of resistant genotype extractable in hexane, elicited larval repulsion (Varshney et al. 2007). The presence of mixtures of aliphatic aldehydes, ketones, alcohols, esters, as well as terpenoids and other aromatic compounds in the volatile emissions of corn leaves has been reported to be associated with orientation and ultimate recognition of host plants by the insects (Buttery and Ling 1984), and six plant volatiles, viz., octanal, nonanal, linalool, naphthalene, allylanisole, and eugenol, have been reported to mediate host location and oviposition by *C. partellus* females (Khan et al. 2000; Birkett et al. 2006).

Very young corn seedlings govern high level of resistance to the European corn borer (ECB) because of synthesis of high concentrations of 6-methoxybenzoxazolinone (6-MBOA) and 2,4-dihydroxy-7-methoxy-1,4-benzoxazine-3-one (DIMBOA) during this stage (Beck 1965). Later on, Klun and Brindley (1966) found that 6-MBOA is not present in vivo but its precursor—a glucoside—is present when the corn seedling tissue is crushed by insect mandibles, which rapidly hydrolyzed to glucose and aglucon DIMBOA, and the DIMBOA is then converted into 6-MBOA by enzymatic action. Furthermore, Klun et al. (1970) reported that the high concentration of DIMBOA in young corn plants imparts resistance against ECB, and as plant grows older, the DIMBOA starts decreasing in the stalk whorl leaves and get concentrated in roots. DIMBOA has also been found responsible for resistance against corn earworm, *H. zea*. These secondary metabolites are produced independent of the presence of pest in a tissue. In addition to secondary metabolites, ubiquitous phenolic acids, especially ferulic acid, may contribute to insect resistance in maize. The biosynthesis of these defense-related metabolites has a common root in the shikimic acid pathway. Maysin, chlorogenic acid, and phenolic acids originate from the phenylalanine branch of the pathway, and biosynthesis of benzoxazinoids shares intermediates with the tryptophan metabolism (McMullen et al. 2009). Later, it was also found that flavone glycoside and maysin in the maize silk govern antibiosis mechanism of resistance to *H. zea* larvae.

A blend of volatile compounds is being emitted by the herbivore-damaged maize plants, and the natural enemies also use them as chemical cues for spotting the host insects on the damaged plants. In response to foliar damage by lepidopteran larvae in maize, the plant releases a complex mixture of volatiles, which attract females of the parasitic wasp, *Cotesia marginiventris* (Cresson), to the damaged maize plant to oviposit on the insect larvae (Turlings et al. 1990, 1991). The parasitized larvae consume less plant material and will die upon emergence of the parasitoid, which can benefit the plant (Hoballah and Turlings 1999; Hoballah et al. 2004). The

composition of the lepidopteran-induced volatiles varies between different lines of maize and teosinte (Gouinguene et al. 2001; Degen et al. 2004) and is influenced strongly by abiotic factors like temperature, light intensity, and nutritional status of the plant (Gouinguene and Turlings 2002). The phenomenon of tritrophic interactions involving maize, herbivores, and enemies was first demonstrated by Turlings et al. (1990) and afterwards reported in more than 15 plant species (Dicke 1999; Kessler and Baldwin 2002). The attraction of herbivore enemies has been shown to benefit the plants by reducing subsequent herbivory and increasing reproductive fitness (Hoballah and Turlings 1999; Van Loon et al. 2000; Kessler and Baldwin 2001), although such advantages have not been realized in all the cases. The maize volatile blend consists of indole, products of the lipoxygenase pathway, and a large number of mono- and sesquiterpenes (Kollner et al. 2004). Attempts to identify the compounds that are crucial for the attraction of parasitic wasps have been hampered by the complexity of blends and the difficulty of obtaining individual compounds with the correct chirality for bioassay (D'Alessandro and Turlings 2005, 2006). Although several physicochemical traits have been reported to be associated with insect resistance, their use in selection of insect-resistant cultivars has been underutilized.

8.4 Major Insect-Pests of Maize

In India, maize productivity is very low in comparison to its potential. The most important reason for its low productivity is improper management of the crop and insect-pest's infestation. The maize is damaged by an array of insect-pest groups, viz., soil dwellers, foliage feeders, stem borers, flower feeders, and cob feeders under field conditions, and rice weevil, Khapra beetle, lesser grain borer, and grain moth under storage conditions (Kumar et al. 2001). The insect-pest's status too is under continuous change in both inter- and intra-agroclimatic conditions due to unsuitable agroclimatic conditions, cultivation of high-yielding but insect-susceptible hybrids or varieties, indiscriminate use of pesticide, reduction in natural enemies' population, use of excess dose of fertilizers, change in cropping pattern, availability of alternate hosts, and increase in maize growing throughout year. The important insect-pests of maize are described below:

Maize stalk borer (*C. partellus*): Among all the insect-pests of maize, stalk borer is the most serious pest found throughout India. The yield losses due to this pest have been recorded up to 35–80 %. Sowing time influences the stalk borer attack as crop sown during the first fortnight of March shows higher infestation than that sown in the second fortnight of February or the second fortnight of June and October. The stem borer infestation ranges between 4.7 % and 24.0 % in summer (February to March) and 9.5 and 37.4 % in winter (November to December). Higher maximum temperature (>13 °C) and relative humidity (>70 %) favor for stem borer attack, and every 1 °C reduction in minimum temperature decreases 1.7 % stem borer damage at 30 days after sowing.

Pink stem borer (*S. inferens*): This is a major pest of wheat and also causes serious losses in maize. Infestation of this pest has increased gradually in North India and causes considerable yield losses in maize, while the losses due to this pest in South India are comparatively higher (25–80 %).

Shoot fly (*Atherigona* spp.): Shoot fly is a serious pest of maize in spring maize in northern India. It attacks the crop at the seedling stage. Three species of shoot fly are found in the country, of which most prominent are *Atherigona naqvi* Steyskal (in Ludhiana, Punjab), *Atherigona orientalis* Schiner (in Pantnagar), and *Atherigona soccata* Rondani and *A. naqvi* (in Delhi). Maximum temperature of 30–34 °C, minimum temperature of 24–25 °C, and relative humidity of 40–75 % are favorable for severe attack, while for minimum incidence, maximum temperature of 24–25 °C, minimum temperature of 21–22 °C, and relative humidity of 80–86 % in the second and third week after sowing are favorable.

Cutworms/armyworms: Severe infestation of cutworms can be seen in *Rabi* maize mainly in the Terai area of Uttar Pradesh and Uttarakhand where it causes loss throughout the year, which has now extended to Bihar, Delhi, Haryana, Rajasthan, Madhya Pradesh, West Bengal, Orissa Punjab, and Jammu and Kashmir in all seasons of the crop. The armyworm damage was earlier confined mainly to northern India on *Rabi* maize, which has also now been recorded to damage *Kharif* maize of Himachal Pradesh, Punjab, Karnataka, Tamil Nadu, Maharashtra, Manipur, Orissa, Gujarat, and Uttar Pradesh.

Sugarcane leafhopper (*P. perpusilla*): This is a serious pest of sugarcane, which has now been recorded infesting *Kharif* maize in the northern states of India mainly Punjab, Haryana, Maharashtra, Uttar Pradesh, and Uttarakhand. Indiscriminate use of insecticides and change in climatic conditions causing heavy reduction in parasitoids of hopper might have increased severity of the pest during recent years.

Shoot bug (*P. maidis*): Adults and nymphs suck sap, resulting in unhealthy, stunted, and yellow plants. The leaves whither from top downwards, and plants die if attack is severe. Honeydew excreted by shoot bug causes growth of sooty mold on leaves, midribs of leaves turn red due to egg laying and may dry subsequently. Shoot bug is occasionally a serious pest on maize. It has been found attacking maize in South India and Madhya Pradesh with peak activity during August–October.

Corn leaf aphid (*R. maidis*): The aphid colonies may completely cover emerging tassels and the surrounding leaves, preventing pollen release. This insect also acts as a vector of the sugarcane mosaic virus, maize dwarf mosaic virus, and maize leaf fleck virus. The aphid infestation in young plants seriously impairs ear production, while severe outbreak at tasseling stage affects seed set. The aphid is widely distributed and appears in serious form on the water-stressed crop and particularly during drought years.

Cob borer (*H. armigera*): Initially it started with infestation on *Kharif* maize, but currently it damages all seasonal maize crops in Uttar Pradesh, Bihar, Punjab, Haryana, Rajasthan, Himachal Pradesh, Karnataka, Andhra Pradesh, Maharashtra, and Delhi. Cob borer alone causes up to 50 % crop loss.

Flower-eating beetle: The insect was reported as occasional pest infesting tassel of maize in the Hyderabad region, while in recent years, it has also become a serious problem of maize during tasseling stage in northern India.

Termites (*Macrotermes* spp. and *Odontotermes* spp.): Termites have emerged as one of the major problems in maize production across India, including traditional maize growing states, viz., Andhra Pradesh, Karnataka, Bihar, and Uttar Pradesh. Termites occasionally cause partial or total defoliation of maize seedlings, but mature plants experience sever damage. The termite infestation at the seedling stage shows wilting symptoms in the young seedlings, and complete damage to the root system results in irreversible drying of the plants. There is no certain pattern of wilting; it could be on the field boundaries, in a certain direction across the field, in patches across the field, etc., depending on the movement of the termites in the field. In older plants, termites begin to attack the main root system, prop roots, stems, and cobs and eventually pack the stems with soil and cover them with galleries or tunnels made of thin sheets of soil. As plants mature, the amount of damage increases rapidly and so does the likelihood of lodging, brought about directly by termite injury or by wind. Severely damaged plants may lodge and be completely destroyed by termites.

8.5 Management of Insect-Pests in Maize

The maize crop is damaged by an array of insect-pest groups with different feeding habits right from the seedling stage to maturity of the crop,

and no single strategy is sufficient to manage such complex group of insect-pests. Thus, hereunder are some tactics for the management of insect-pests of maize:

- Collection and burying of stubble and stalks or plowing and destroying crop residue and removal of infested plant parts or infested plants through hoeing are effective in reducing the carryover populations of stem borers and termite menace in the forthcoming crop season.
- Selection of insect-resistant maize varieties followed by seed treatment with carbofuran (5 %), thiamethoxam (9 ml/kg seed), or imidacloprid (4 g/kg seed) improves plant stand and seedling vigor and reduces the damage by stem borer and corn leaf aphid.
- Application of phorate 10G at 15 kg/ha in furrows before sowing or application of 5–7 granules of carbofuran 3G in the central whorl of the plants one week after germination is effective for the management of stem borers and shoot fly in maize.
- Growing maize in association with legumes or intercropping with soybean significantly reduces damage by stem borers.
- Conserve egg parasitoid, *Trichogramma chilonis* (Ishii), and larval parasitoid, *Apanteles flavipes* (Cameron), for the management of maize stem borers.
- In case of heavy infestation of stem borers, spray the crop with cypermethrin 25 EC at 0.05 % or deltamethrin 2.8 EC at 0.02 %.
- The aphid and shoot bug populations can be effectively controlled by the coccinellids [C*occinella septempunctata* L. and *Menochilus sexmaculata* (Fab.)] if available; otherwise, spray the crop with monocrotophos or dimethoate at 0.1 %.
- Apply fipronil granules at 20 kg/ha followed by light irrigation. Application of imidacloprid or thiamethoxam drop by drop in the water channel at the source while irrigating the field also provides satisfactory reduction in damage by termites.

8.6 Conclusion

The hybrid initiative turned out to be exceedingly important for QPM efforts, and many countries in the developing world, including India, are becoming increasingly interested in QPM to reduce malnourishment and sustainable nutritional security. However, the yield potential of the maize genotypes deployed in India has not reached even half of that in the USA as a result of different stress factors mainly the insect-pests which cause huge crop losses. It is also likely that the increase in concentration and quality of nutritional compounds, particularly the protein in QPM, might favor the proliferation of insect-pests as they too prefer quality food for their growth and development and could be major constraints to increasing production and productivity of QPM. The grain yield, nutritional quality, and insect resistance are being addressed individually; however, collective action to handle all the three problems has been lacking. Therefore, development and deployment of high-yielding and insect-resistant QPM genotypes under the umbrella of integrated pest management system might help in narrowing down the yield gap by reducing the crop losses caused by insect-pests and could be the best fit R&D model for food and nutritional security, environmental safety, and sustainable maize production.

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Physiological Response of Maize Under Rising Atmospheric CO_2 and Temperature

9

Anjali Anand, Sangeeta Khetarpal, and Madan Pal Singh

Abstract

The projections for future climate change may have a strong influence on agricultural productivity. Maize, being a C_4 plant, has evolved to adapt to the atmospheric CO_2 concentration with higher photosynthetic efficiency than C_3 plants. It is believed that C_3 plants would gain a competitive advantage under increasing CO_2, but studies indicate that C_4 plants sometimes perform better due to improved water use efficiency at the ecosystem level. C_4 plant species have higher temperature optima for growth than C_3 plants. Temperatures above this range can affect the photosynthetic machinery, thereby decreasing growth. Despite the indication about the improvement in growth of C_4 plants under increasing CO_2 levels, the contribution of other factors still remains unclear in maize. This compilation is an attempt to highlight the factors and processes affected by climate change in maize and the areas of research that need to be strengthened to understand the underlying mechanisms.

9.1 Introduction

Rise in atmospheric CO_2 with parallel increase in temperature is posing to be a serious threat to the farming community. During the last 12 years, the increasing rate of CO_2 has been 1.9 μl l^{-1} $year^{-1}$ and is predicted to reach the level of 570 μl l^{-1} by the middle of this century (IPCC 2007). A predicted consequence of this rise in CO_2 and other greenhouse gases is warming of the earth's surface, which can cause significant changes in crop productivity through direct or indirect effects on crops, soils, pests, etc. Environmental factors during crop growth period determine the yield and quality of the produce, which in turn result from the physiological processes occurring during plant growth and development. All the physiological processes are affected by the change in the environment, which finally limits productivity of the crop. Elevated CO_2 causes partial stomatal closure, thereby decreasing leaf transpiration, while at the same time the carbon assimilation is increased (Morison 1998). The

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extent to which a species can acclimate to the changing environment by maintaining an optimum balance in enzymatic reactions of carbon fixation thus gains prime importance. In this chapter, various components affecting the growth and yield of maize and other cereal crops under elevated CO_2 and temperature are discussed.

C_4 plants account for a small percentage of the angiosperms, albeit they make an important contribution to productivity as many agriculturally important crops and grasslands are C_4 in nature. In C_3 plants, the current atmospheric CO_2 levels limit photosynthetic capacity and growth. An increase in CO_2 levels increases photosynthesis by reducing oxygenase activity of the photosynthetic enzyme, ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco) and photorespiration, if photosynthesis is not feedback-inhibited by limited utilisation of the products of photosynthesis (Bowes 1996; Drake et al. 1997). C_4 plants possess two types of cells, mesophyll and bundle sheath cells, and possess a mechanism that concentrates CO_2 in the bundle sheath cells to levels that have been estimated to be 3–8 times more than the atmospheric CO_2 concentration (Kanai and Edwards 1999). Ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), a photosynthetic enzyme, is located in the bundle sheath cells where the increase in the ratio of CO_2/O_2 helps in reduction of photorespiration. This led to an interpretation that an increase in CO_2 level may have insignificant effect on growth of C_4 plants (Reynolds 1996). However, there are many reports of substantial increase in growth of some C_4 species under CO_2 enrichment. A 28 % increase in growth of *Panicum antidotale* (Ghannoum et al. 1997) and 24–29 % in *Bouteloua gracilis* (Chen et al. 1996) were reported under double the ambient levels of CO_2. Daily average temperature of 23–26 °C resulted in 3–25 % increase in growth accompanied with 4–30 % enhancement of photosynthesis under levels of CO_2 that were double to that of ambient conditions (Ziska and Bunce 1997). Further, the magnitude of growth stimulation of C_4 plants to elevated CO_2 increases with reduced water availability, temperature and light intensity (Seneweera et al. 1998). The plants exhibit reduced stomatal conductance and transpiration rate, thereby increasing water use efficiency (Ghannoum et al. 2000). A differential response to increased CO_2 levels was observed in maize with studies showing no enhancement in growth (Hunt et al. 1991) to 50 % stimulation (Rogers and Dahlman 1993). The differences were dependent on species, cultivars and environmental factors like temperature, light intensity, water and nutritional status of the plants (Drake et al. 1997). However, these studies gave one clear indication—that maize has the potential to respond to elevated CO_2.

9.2 Elevated CO_2 and Photosynthesis in Maize

The basis of increased biomass production was studied in maize plants grown in temperature-controlled conditions under high light and CO_2 (1,100 μl l^{-1}). The plants exhibited 20 % increase in biomass and 23 % in leaf area under elevated CO_2. Light-saturated rates of photosynthesis on leaf area basis indicated higher rates of carbon fixation and dark respiration under high CO_2 levels. The plants grown at high CO_2 levels had lower carboxylation efficiencies (23 %) with lower stomatal densities in both young and mature leaves. Maize plants benefitted from increased CO_2 by decrease in activity of C_4 enzymes, NADP-malate dehydrogenase exhibiting the greatest decrease, without loss in the rate of carbon assimilation. This decrease could be explained by either difference in activation state of transcriptional or translational control of synthesis of specific proteins. The activities of enzymes required for sucrose and starch formation, fructose 1,6-bisphosphatase and ADP-glucose pyrophosphorylase, increased significantly under elevated CO_2 condition by 8 and 36 %, respectively (Fig. 9.1). The increased capacity to synthesise sucrose and starch and to utilise these end products of photosynthesis to

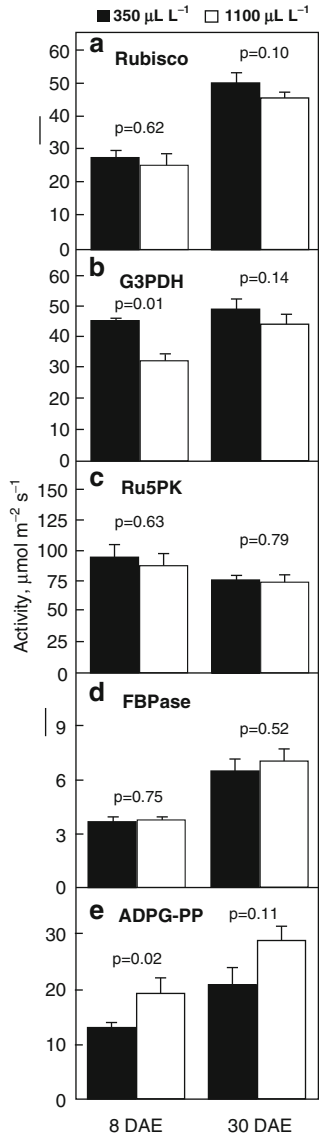

Fig. 9.1 Activities of key enzymes of the C_3 cycle, sucrose and starch biosynthesis in maize leaves at 8 DAE (second leaves) and 30 DAE (sixth to seventh leaves) from plants grown under ambient (350 µL L^{-1}, *closed bars*) and three times ambient (1,100 µL L^{-1}, *open bars*) CO_2. (**a**) Rubisco, ribulose 1,5-bisphosphate carboxylase/oxygenase. (**b**) G3PDH, glyceraldehyde-3-phosphate dehydrogenase. (**c**) Ru5PK, ribulose-5-phosphate kinase. (**d**) FBPase, fructose 1,6-bisphosphatase. (**e**) ADPG-PP, ADP-glucose pyrophosphorylase (Source: Maroco et al. 1999)

produce extra energy could explain the increased growth of maize under CO_2 enrichment (Maroco et al. 1999).

9.3 Elevated Temperature and Photosynthesis in Maize

9.3.1 Chlorophyll Fluorescence

Various components of photosynthesis have been studied to elucidate the most sensitive component to high temperature. The chlorophyll fluorescence traits of maize leaves were similar to that reported for C_3 plants under high temperature (Law and Crafts-Brandner 1999). The effect was evident in non-photochemical fluorescence quenching (qN) well before it could be realised in the maximum quantum yield of photosystem II (*Fv*/*Fm*) (Figs. 9.2 and 9.3). Thylakoid energisation was observed due to increased qN at moderate leaf temperatures of 32.5–37.5 °C, which was associated with increased ratio of ATP to ADP. The higher increase in temperature (42.5 °C) caused significant inhibition of photosystem II (PSII), which is otherwise considered to be the most heat-sensitive component of photosynthesis (Heckathorn et al. 1998) (Fig. 9.3).

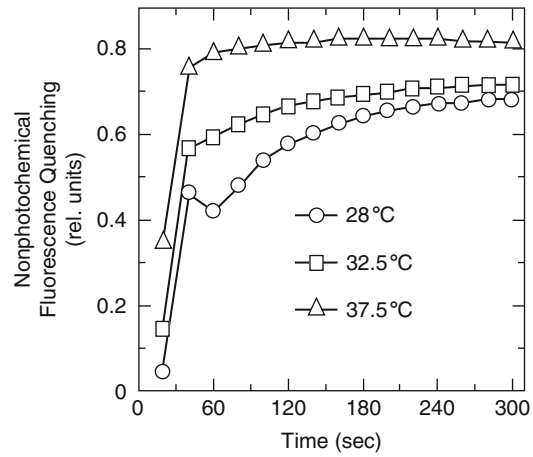

Fig. 9.2 Effect of leaf temperature on qN of maize leaves. An attached leaf was dark-adapted for 1 h at 28 °C prior to conducting non-photochemical quenching analysis. Subsequent measurements were made on the same leaf tissue after increasing the leaf temperature at 1 °C min^{-1} in the dark to 32.5 °C for 1 h and then to 37.5 °C for 1 h. Each *curve* represents the mean ± SE of three independent measurements. At 28 °C, qN was the same for plants that were dark-adapted for 1 and 3 h (data not shown) (Source: Maroco et al. 1999)

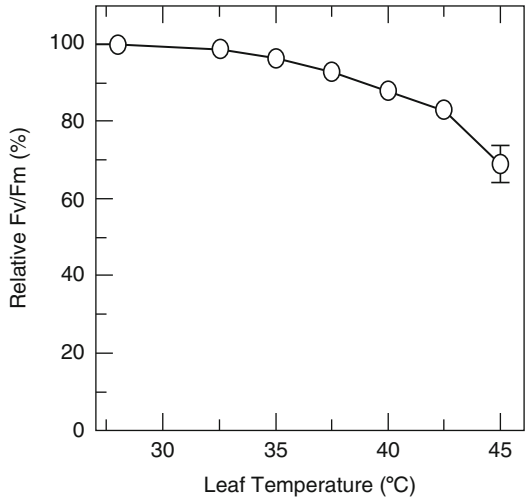

Fig. 9.3 Effect of leaf temperature on the *Fv*/*Fm* of maize leaves. Intact leaves were dark adapted for 1 h at 28 °C, *Fv*/*Fm* was measured, leaf temperature was increased at 1 °C min^{-1} to the indicated temperature, and *Fv*/*Fv* was measured again. Different plants were used for each temperature treatment, and the data points represent the mean ± SE of three independent measurements (Source: Maroco et al. 1999)

This inhibition was not associated with decline in photosynthesis up to 37.5 °C in maize leaves (Crafts-Brandner and Salvucci 2002). The decrease in photosynthesis at higher temperature could be through a limitation in the activation state of Rubisco. In C_3 plants, the increase in qN due to heat stress is correlated with decreased photosynthesis and gives an indication on inhibition of Calvin cycle activity (Feller et al. 1998).

9.3.2 Enzymes of C_4 Pathway

Phosphoenolpyruvate carboxylase (PEP carboxylase) and pyruvate phosphate dikinase (PPDK) are the key enzymes of C_4 pathway, and inhibition of either by heat stress, especially PPDK due to its low activity (Furbank et al. 1997), may decrease the supply of C_4 acids available for decarboxylation. This would further limit the supply of CO_2 to Rubisco. In maize, the activity of PEP carboxylase and PPDK was not affected by increase in leaf temperatures up to 45 °C, but activation state of PEP carboxylase was marginally sensitive to temperature of 40 °C or higher. In general, C_4 plant species have higher temperature optima for photosynthesis than C_3 plants due to the presence of a CO_2-concentrating mechanism (Edwards and Walker 1983). In C_3 plants, an increase in the ratio of oxygenase to carboxylase activities of Rubisco under high temperature leads to inhibition of net photosynthesis. Considering all these limitations, the catalytic turnover rate of Rubisco increases minimally with temperature. Thus, in C_3 plants photosynthesis declines at leaf temperature above 32 °C because the slight advantage in catalytic turnover is negated by a decrease in Rubisco activation state (Crafts-Brandner and Salvucci 2000). As temperature increases, the ratio of dissolved O_2/CO_2 and the specificity of Rubisco for O_2 increase (Jordan and Ogren 1984). Rubisco has low affinity for CO_2 and needs to be activated by a chloroplastic protein, Rubisco activase, at physiological concentration of CO_2 and Mg^{2+} (Portis et al. 1986). Rubisco activase, however, has been shown to be quite sensitive to high temperature (Salvucci and Crafts-Brandner 2004). In maize, the activation state of Rubisco decreased at temperature exceeding 32.5 °C, with complete inactivation at 45 °C. The inactivation of Rubisco is the primary constraint on the rate of photosynthesis of maize leaves as leaf temperature increased above 30 °C. High temperature denatures Rubisco activase rendering it unable to fit correctly onto Rubisco. Consequently, inactive Rubisco is not converted to active form (Crafts-Brandner and Salvucci 2000). Rokka et al. (2001) reported that high temperature induces the formation of high molecular mass aggregates of activase. The high level of CO_2 in the mesophyll chloroplasts allowed for a substantial temperature-dependent increase in the catalytic turnover rate of activated Rubisco, thus counteracting the effects of decreased activation state until temperature approached 40 °C. Levels of 3-phosphoglyceric acid and ribulose-1,5-bisphosphate decreased and increased, respectively, as leaf temperature increased, consistent with the decrease in Rubisco activation. When leaf temperature increased gradually, Rubisco

activation acclimated in a similar manner as photosynthesis, and acclimation was associated with the expression of a new activase polypeptide.

9.4 Elevated Temperature and Grain Filling

During grain-filling stage, availability of photosynthetic assimilates and the ability of the grains to utilise them for the synthesis of reserves are two major activities. The optimum temperature for dry matter accumulation in starch-storing crops ranges between 20 and 30 °C, and it is very unusual because this temperature is lower than the level of temperature on which proteins start denaturing. The main reason for the low temperature optimum for maximum grain yield of cereal crops has been due to the effect of temperature on both grain-filling rate and its duration (Muchow 1990; Brooking 1993). High temperature reduces grain size due to the steady decrease in the duration of grain filling combined with a failure of compensation by increased rate of dry matter accumulation above a threshold temperature.

Amylose to amylopectin ratio and reduced grain density are two important components of the grain yield, and changes in them due to any environmental stress can also affect the grain yield (Lu et al. 1993). Early stages of grain development are sensitive to high temperature than later ones (Tashiro and Wardlaw 1991), but this can also have direct effects on starch deposition (Bhullar and Jenner 1986), which is related to a limitation in the supply of assimilates to the grain (Wardlaw et al. 1980). Rijven (1986) on the biochemical basis of the effect of temperature identified the enzyme, soluble starch synthase (SSS). Later studies in potato and barley suggested that the site of damage in starch synthesis is extra amyloplastic (Mohabir and John 1988) or involves sucrose synthase (MacLeod and Duffus 1988). Keeling et al. (1993) have also demonstrated that part of the effect of high temperature on starch synthesis is linked with SSS. Starch deposition in amyloplast involves ADP-glucose pyrophosphorylase, a starch synthase and branching enzyme (Emes et al. 2001; Zeeman et al. 2010).

Glucose-1-phosphate is converted to ADP-glucose by the enzyme AGPase, which is then utilised by starch synthase. Various genetic and biochemical studies have reported that AGPase is a rate-limiting and regulatory enzyme in the pathway for starch synthesis. Maize endosperm mutants, shrunken-2 (sh-2) and brittle-2 (bt-2), which were deficient in AGPase, showed deficiency in starch content (Dickinson and Preiss 1969). These mutations involved the genes for small and large subunits, respectively, of AGPase (Tsai and Nelson 1966). Antisense inhibition of AGPase showed reduction in starch content in potato tubers (Muller-Rober et al. 1992). AGPase is allosterically activated by 3-phosphoglycerate (3PGA) and inhibited by Pi. The ratio of 3PGA/Pi regulates the catalytic activity of AGPase in the leaves (Preiss and Sivak 1996). Studies using maize endosperm mutants affecting branching enzyme (BE) and sucrose synthase showed that there was only minor control over flux by these enzymes (Singletary et al. 1997). Further, studies with the bt-2 mutant, which affects ADP-Glc pyrophosphorylase activity, showed that there is minimal control of flux by this enzyme. The effects of reduced ADP-Glc pyrophosphorylase on starch synthesis are related with a reduction in grain-filling duration rather than in grain-filling rate. SSS has been shown to be a major site of control of flux through the pathway of starch synthesis in both developing wheat and maize grains (Keeling et al. 1994).

Multiple isoforms of starch synthase that transfers glucosyl moiety of ADP-glucose to the non-reducing end of the pre-existing α-1,4-glucan primer through an α-1,4 glycosidic linkage have been reported in all the plant tissues (Ball and Morell 2003; Dian et al. 2005). In cereal endosperm there are about five SS (starch synthase) isoforms that have been categorised according to conserved sequence relationships. Four isoforms, named as SS I, SS II, SS III and SS IV, are believed to have unique functions during the amylopectin synthesis, but their exact roles have not been identified.

It has been reported that granule-bound starch synthase (GBSS), which is encoded by the waxy (Wx) locus in cereals, is involved to elongate amylose (Shure et al. 1983). The waxy mutants of maize containing wild-type amount of starch with no amylose are defective for GBSS (Nelson and Rines 1962). The enzyme GBSS binds tightly with the starch granule and is linked with amylose synthesis (Zeeman et al. 2010).

The level of temperature above 25 °C has been reported to affect the grain yield adversely because the enzyme SSS is very heat sensitive. Two different properties of SSS have been identified which are affected by heat treatment and limit the grain yield. Firstly, the rate of enzyme activity is adversely affected by elevated temperature, and this effect is reversible on returning to a lower temperature. The effect of high temperature on enzyme rate has been quantified using enzyme Q_{10} and found to be suboptimal above 20 °C temperature. Secondly, due to prolonged period of exposure to high temperature, loss in enzyme activity occurs due to thermal inactivation and is not freely reversible. Thermal inactivation of enzyme occurs when temperature exceeds 20 °C in wheat, but for similar response temperature more than 30 °C is needed for maize SSS (Fig. 9.4).

Normally, the inherent stability or Q_{10} characteristics of other enzymes involved in starch synthesis pathway are not affected by high temperature except for branching enzyme, which has minimal flux-control strength. Similarly, SSS thermal inactivation may not be a major problem in field-grown maize for developing grain, because temperature rarely is high enough to cause enzyme inactivation. However, the effect on enzyme Q_{10} is more physiologically relevant, since maize SSS is operating suboptimally as temperature exceeds 20 °C and selection of a high-temperature-tolerant form of maize SSS will be a better approach, using enzyme Q_{10} as a selection trait (Keeling et al. 1994). The transfer of this trait from the tropical donor to other lines through backcross breeding can be useful to produce a heat-tolerant line.

Other enzymatic proteins are also sensitive to heat stress as it denatures the enzyme and causes a loss of enzyme activity. For thermostability in the

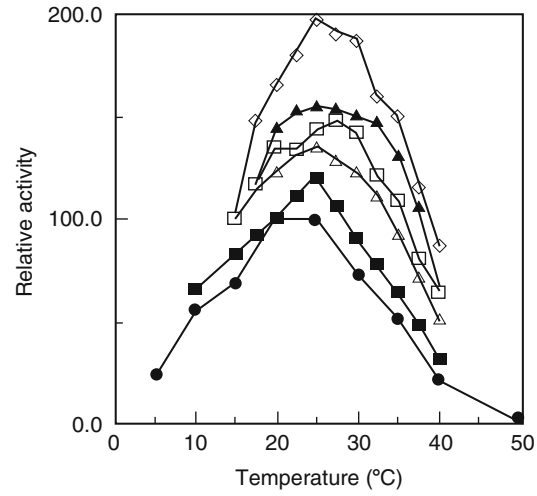

Fig. 9.4 Effects of temperature on SSS activity obtained from the endosperm of various crops. Data are presented as a percentage of the activity obtained at 25 °C. Results are given as the mean of duplicate assays. Maize (Δ), rice (▲), sorghum (□), potato (■), millet (◇), wheat (●) (Source: Keeling et al. 1994)

protein, chaperones, a specific class of proteins, have been known for assisting other proteins in post-translational folding and maintaining them in a proper functional state (Ellis 1990). Heat-shock proteins (HSPs) are well-known to work as molecular chaperones; they aid in refolding proteins denatured by heat and prevent them from aggregating and play a very significant role in improving tolerance to high temperature and other stresses. Among the various classes of HSPs, LMW-HSPs represent a set of homologous proteins in the range of 15–30 kDa (DeRocher and Vierling 1994). During stress conditions, LMW-HSPs may accumulate up to 1 % of the cellular protein (Vierling 1991). They are involved to protect the proteins associated with respiratory electron transport in mitochondria and PS II electron transport in thylakoid membrane of the chloroplast. A 22 kDa HSP in the chloroplasts of *Chenopodium album* has been shown to interact with the thermolabile oxygen-evolving complex of PS II and protect it from heat stress damage (Sun and Montagu 2002).

These reports suggest that transfer of thermally stable forms of these enzymes to cereals can enhance their starch-synthesising ability and

also stabilise the grain yield. Apart from above, there are some transgenic approaches being currently used to stabilise the starch biosynthesis in the following ways: (a) insertion of a gene or additional copy or copies of the gene of the wild-type enzyme with enhanced activity, (b) modification of the enzyme by protein engineering to achieve alterations in the kinetic and/or allosteric behaviour of the enzyme reaction in order to achieve thermal stability and (c) modification of the promoter sequences to achieve enzyme over-expression.

9.5 Elevated Temperature and Grain Quality

Temperature plays a crucial role in determining the quality of cereal crops. High temperature during the grain-filling stage results in smaller grains. While the quality of cereal crops like wheat and rice has been extensively studied under normal environment, there are no reports available on the effect of changing climate on quality of maize. Processing industries require grains possessing specific quality attributes. The important quality parameters in wheat are grain hardiness, grain size, milling, dough strength, and protein and starch quality. These attributes are genotype-specific and also depend upon its interaction with climate, growing temperature and soil fertility. Grain protein and oil content are used as grading factors in cereal trading.

The kernel size, composition and protein concentration are governed by post-anthesis environmental conditions. Over 80 % of nitrogen in the crop is taken up before flowering and stored in stems and leaves. It is deposited in the grain in the form of protein at a rate that reaches its maximum during the early part of grain filling. Carbohydrate in the form of starch is accumulated in the grain, but its rate reaches a maximum later during the grain-filling process. In general, grain nitrogen concentration increases with increase in temperature and reduced rainfall. In Australian variety trials extending over 27 years, grain nitrogen concentration was positively associated with the number of hours above 35 °C during grain filling (Blumenthal et al. 1991). In China, grain nitrogen concentration in durum wheat was found to be positively associated with average daily temperature during grain filling and negatively correlated with growth period (He et al. 1990). Under controlled environmental conditions, Ahmad et al. (1989) have reported similar relationship in experiments conducted at Lahore, Pakistan. Increasing temperature can reduce carbohydrate accumulation more than nitrogen accumulation as the rate of senescence is increased, thereby reducing photosynthesis and grain growth. The rate of protein synthesis in the grain is also promoted by warmth more than the rate of starch synthesis such that protein concentration increases (Spiertz 1977).

9.6 Elevated CO_2 and Grain Quality

There is scarcity of information on the effects of rising atmospheric CO_2 on nutritional value and grain quality. In wheat, elevated CO_2 reduces the protein content of grain and flour by 9–13 % (Rogers 1996). Plants grown under elevated CO_2 in field, open top chambers and FACE resulted in decreased protein concentration under elevated conditions (Fig. 9.5). Changes in protein concentration affect dough strength and bread making, thus being a major determinant of grain prices (Lawlor and Mitchell 2001). The total gluten concentration as well as concentrations of dry and wet gluten also decreased under high CO_2 (Bencze et al. 2004).

Elevated CO_2 has been reported in various studies to cause alteration in microelements. Concentration of all microelements decreases up to 18.3 %, whereas macroelements are not affected under elevated CO_2 (Hogy and Fangmeier 2008). Quality of wheat flour is determined by a proper balance of protein and non-protein components of grain. Poor dough of lower extendibility and decreased loaf volume was found in grains grown under high CO_2 levels (Blumenthal et al. 1996) without affecting the physiochemical properties of wheat starch during grain filling (Tester et al. 1995). Grain quality is a function of the sum of the contributions of grain constituents such as proteins, starch and lipids to dough strength,

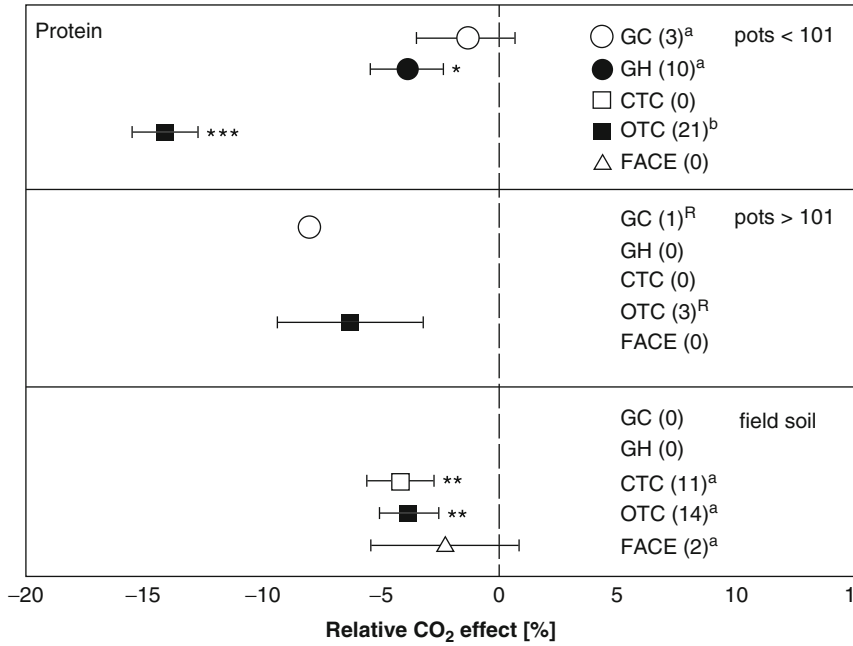

Fig. 9.5 Relative average changes (± SE) in protein concentration of wheat grains due to CO_2 enrichment (550 vs. 380 µmol mol^{-1}) in regard to exposure system and rooting volume. *GC* growth chamber, *GH* glasshouse/greenhouse, *CTC* closed field chamber, *OTC* open top chamber, *FACE* free-air CO_2 field enrichment. Significant CO_2 effects are denoted by ***($p<0.01$), **($p<0.01$) and *($p \leq 0.05$). Number in *parenthesis* indicates number of studies included (*k*); *letter* indicates significant differences between exposure systems (Source: Hogy and Fangmeier 2008)

loaf volume and also interaction between these components.

Oil deposition starts late during seed development and decreases with high temperature exposure resulting in decreased oil content. Fatty acid composition also gets influenced by production of saturated fatty acids under warmer conditions and polyunsaturated fatty acids under cooler moist conditions (Seiler 1983). High temperature increases the percentage of monounsaturated fatty acid (oleic acid) and decreases the polyunsaturated fatty acid (linolenic acid) in mustard (Triboi-Blondel and Renard 1999) and soybean (Rennie and Tanner 1989). Moreover, it affects protein content more than oil content, but oil concentration shows an inverse relationship with protein content. During reproductive period of the crop under Mediterranean-type environment, adverse effects of high temperature on oil content have been reported in canola by Gunasekera et al. (2006). In maize, such studies are lacking, and the quantification of increasing environmental temperature on quality parameters will be useful in planning agronomic interventions and in developing ideal plant type for future climatic scenarios. We need to urgently take steps to increase adaptive capacity by shifting our priority of research towards adaptive strategies accompanied by change in policies by the government.

9.7 Conclusion

The information on the response of maize and other C_3 crops to elevated CO_2 and temperature compiled above confirms that rising atmospheric CO_2 is beneficial for the growth and yield of C_3 crops compared to C_4; however, increasing temperature may negate these responses particularly during reproductive growth. On the contrary, elevated CO_2 may affect the nutritional quality of tissues as well as grains due to its dilution effect. Looking at agricultural perspective, rising

CO_2 may lead to reassessing the fertiliser management, as critical nitrogen concentrations in the tissues decrease. Since grain quality is negatively affected by CO_2 enrichment, we need to develop genotypes with efficient nitrogen uptake for high-CO_2 environment. Concurrent increase in air temperature may affect the quantity as well as quality of grains. C_4 crops are well-adapted to high temperature environment, but extreme weather phenomenon, as projected under changing climate, may affect their grain quality and yield. Hence, under changing climate, identifying the genotypes that can tolerate extreme weather events and introgression of these traits in breeding programmes can contribute to protect the seed yield and quality of maize and other cereal crops in future climate change scenarios.

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Part V

Value Addition in Maize

Maize Utilisation in Food Bioprocessing: An Overview

10

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Abstract

Fermented foods are considered as palatable and wholesome foods prepared from raw or heated raw materials and appreciated for their attributes such as pleasant flavour, aroma, texture, improved processing properties and better digestibility. Fermentation is carried out by the action of a single or a group of micro-organisms including homo- and heterofermentative lactic acid bacteria; moulds such as *Mucor, Rhizopus, Trichoderma, Aspergillus and Penicillium*; and yeasts such as *Saccharomyces cerevisiae, Schizosaccharomyces pombe, Hansenula anomala* and *Debaryomyces hansenii*. Globally, cereals rank number one as food crops as well as fermentation substrates. Among cereal crops, maize with annual worldwide production of 876.8 million metric tons during 2011–2012 has been utilised in the fermented form for hundreds of years as a stimulant, in traditional medicine, as well as in religious ceremonies. Maize is a good source of dietary fibre and protein, while being very low in fat and sodium. The endosperm consists of 72–73 % starch embedded in a protein matrix that makes the maize an excellent substrate for fermentation. Maize is processed, fermented and consumed in various ways. It is usually ground and pounded followed by boiling, baking or frying. Alternatively, the whole grain may be boiled or roasted prior to fermentation. Maize meal can be cooked with water either to provide a thick mush/dough or to provide gruel, porridge or soup. In this chapter, we elaborate the processing of maize for fermentation and illustrate the diversity, importance and microbiological features of some fermented maize products.

10.1 Introduction

Food products owe their production and characteristics to the fermentative activities of micro-organisms in order to bring a desirable change. Various raw materials have been

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subjected to the action of micro-organisms or enzymes in food fermentation, thereby converting carbohydrates into alcohol, carbon dioxide and organic acids (William and Dennis 2011). Fermentation is the "slow decomposition process of organic substances induced by micro-organisms, or by complex nitrogenous substances (enzymes) of plant or animal origin" (Walker 1988). It can be described as a biochemical change, which is brought about by the anaerobic or partially anaerobic oxidation of carbohydrates by either microorganisms or enzymes. This is distinct from putrefaction, which is the degradation of protein materials (Battcock and Ali 1998). Fermented foods originated many thousands of years ago when presumably micro-organisms contaminated local foods, but nowadays, these foods comprise about one-third of the worldwide consumption of food and 20–40 % (by weight) of individual diets.

Globally, cereals rank number one as food crops as well as fermentation substrates. Traditional fermented foods prepared from most common type of cereals, such as, rice, wheat, maize, millet and sorghum, are well known in many parts of the world, such as in Africa. Some are utilised as colourants, spices, beverages and breakfasts or light meal foods, while a few of them are used as main foods in the diet. Among cereal crops, maize, which has its origin in Mexico, has been consumed in the fermented form for hundreds of years. A number of traditional products based on maize were developed by the indigenous populations of Mexico and Peru in the pre-Columbian era and utilised as stimulants, in traditional medicine, as well as in religious ceremonies.

Maize is third behind wheat and rice, in terms of total area under cultivation in the world. About two-thirds of this area is in developing countries, where maize is widely grown for direct human consumption. As reported by Foreign Agricultural Service/USDA, total annual worldwide production of maize was 876.8 million metric tons in the year 2011–2012 showing 5.54 % increase over the previous year's production. The United States is the leading producer of maize in the world yielding about 35.8 % of the total or 313.9 million metric tons; China and Brazil follow on the second and third positions, respectively. India positioned at seventh place, after Mexico, having 2.4 % of world's total production or 21 million metric tons. Maize spread to the rest of the world due to its ability to grow in diverse climates. Sugar-rich varieties called sweet corn are usually grown for human consumption, while field corn varieties are used for animal feed and as chemical feedstock.

10.2 Common Uses of Maize

Maize can be used for many purposes as every part of the plant has commercial value. It is wet-milled to separate the grain into components like starch, oil, protein and fibre, which are then processed into multitude of food and industrial products including starch, sweeteners, corn oils, beverages and industrial alcohols as well as fuel alcohols. New bioproducts such as amino acids, antibiotics and degradable plastics are increasingly being synthesised using maize as a raw material. Maize is utilised in making cornbread, by mixing the meal with wheat flour. Immature cobs, preferably sweet corn, are boiled and eaten, while more mature cobs are roasted. Cornstarch (maize flour) is a major ingredient in home cooking and in many industrialised food products. It can be hydrolysed and enzymatically treated to produce syrups, particularly high-fructose corn syrup, a sweetener. Alternatively, hydrolysed cornstarch may be fermented and distilled to produce grain alcohol. Grain alcohol from maize is traditionally the source of Bourbon whiskey. Starch from maize can also be made into plastics, fabrics, adhesives and many other chemical products.

Further, maize has carotene, a type of carotenoids, otherwise occurring mainly in plants and algae. Carotene can be converted into vitamin A (retinol) by normal metabolic processes in human beings. Vitamin A is very important to human health, but most especially for vision, and as an antioxidant.

In the United States and Canada, maize grains are used as feed for livestock; however, the digestibility and palatability of maize silage, made by fermentation of chopped green corn

Table 10.1 Major benefits of fermentation

Effects of fermentation	Reference
Conditioning by steeping for wet milling of grains	Eckhoff (2004), Perez et al. (2003)
Affecting sensory properties such as aroma, taste, colour and texture	Sefa-Dedeh et al. (2003a, b), Muyanja and Namugumya (2009)
Generation of reducing sugars by saccharification prior to alcoholic fermentation	Tang et al. (2011), Godliving and Mtui (2012)
Preservation which relies mainly on acidification and or alcohol production	Auerbach et al. (2000), Driehuis et al. (2001), Owen (2002), Danner et al. (2003)
Lactic acid bacteria (LAB) isolated from fermented foods displayed probiotic properties such as hypolipidemic, hepatoprotective and antibacterial	Oyetayo and Osho (2004), Aderiye et al. (2007)
Enhancing food safety by inhibition of pathogens, such as *Burkholderia gladioli* which is responsible for bongkrek poisoning in products made from presoaked corn	Motarjemi and Asante (2002), Ejigui et al. (2005)
Removing anti-nutritional compounds such as phytate, enzyme inhibitors, polyphenols and cyanogenic compounds	Awada et al. (2005), Ejigui et al. (2005), Mbata et al. (2009)
Increase bioavailability of components by affecting physico-chemical properties of starch and associations of fibre constituents with vitamins, minerals or proteins	Awada et al. (2005), Chavan and Kadam (1989)
Removing undesirable compounds such as mycotoxins, endogenous toxins, cyanogenic compounds and flatulence-producing carbohydrates	Adegoke et al. (1994), D'Souza and Brackett (1998), Westby et al. (1997), Oluwafemi and Ikeowa (2005)

stalks, is greater than dried. Maize meal is also a significant ingredient of some commercial animal food products, such as dog food.

Stigmas from female maize flowers, popularly called corn silk, are sold as herbal supplements. The corn steep liquor, a plentiful watery by-product of maize wet-milling process, is widely used in the biochemical industry and research as a culture medium to grow many kinds of micro-organisms. Some forms of the plant are occasionally grown for ornamental use in the garden.

Maize cobs are also increasingly used as a substrate for biofuel production. Ethanol produced from maize can be mixed with gasoline to decrease the amount of pollutants emitted when used to fuel motor vehicles. Maize is widely used in Germany as a feedstock for biogas plants.

10.3 Benefits of Fermentation

Fermented foods can, in general, be described as palatable and wholesome foods prepared from raw or heated raw materials. They are generally appreciated for attributes such as pleasant flavour, aroma, texture and improved cooking and processing properties (Holzapfel 2002). In general, during the production process, the fermentation steps aim to achieve the properties detailed in Table 10.1.

10.4 Microbiology of Fermented Foods

Traditionally, the fermenting organisms came from the natural microflora or a portion of the previous fermentation and it is termed as starter culture. In many cases, the natural microflora is inefficient, uncontrollable and unpredictable or is destroyed during preprocessing stages such as pasteurisation. It is always beneficial to use previously characterised, well-identified starter culture (Table 10.2) as it can provide particular characteristics to the food in a more controlled and predictable manner.

The most important fungi involved in industrial fermentation are from two of the main classification groups: the aseptate zygomycota, which includes *Mucor* and *Rhizopus* and the septate deuteromycotina (fungi imperfecti), which includes the genera *Trichoderma, Aspergillus,*

Table 10.2 Commonly used starter cultures in fermentation

Microorganisms	Examples	Product
Yeast	*Saccharomyces cerevisiae, S. rouxii, S. ellipsoideus*	2 Ethanol + 2 CO_2 per hexose molecule
Homofermentative LAB	*Pediococcus, Lactococcus lactis, Streptococcus thermophilus, Vagococcus,* some *Lactobacilli*	2ATP + 2Lactate per glucose molecule
Heterofermentative LAB	*Carnobacterium, Oenococcus, Enterococcus, Lactosphaera, Weissells, Leuconostoc mesenteroides* subspecies *cremoris, Leuconostoc mesenteroides* subspecies *lactis,* some *Lactobacilli*	1ATP + 1Lactate + 1 Ethanol + 1 CO_2 per glucose molecule
Moulds	*Aspergillus oryzae, Erwinia dissolvens, Rhizopus oligosporus*	Variable

Penicillium, Aureobasidium and Fusarium. Yeasts are unicellular fungi that generally reproduce by budding; however, some exceptional species reproduce by binary fission such as *Schizosaccharomyces pombe*. *Saccharomyces* is the most widely used yeast in industrial fermentations and has applications in alcohol production and baking. All the strains ferment glucose and many ferment other plant-associated carbohydrates such as sucrose, maltose and raffinose but none can ferment lactose. *Kluyveromyces lactis*, which contains the necessary lactose-transporting and degrading enzymes, is particularly useful in production of alcohol and biomass from whey. *Zygosaccharomyces rouxii* is specially associated with fermentation of plant products at high salt concentration and low water activity. Many strains like *Hansenula anomala* and *Debaryomyces hansenii* can also grow in fairly concentrated salt solutions and the latter is frequently isolated from brined meat products and fermented sausages.

Lactic starters constitute the major group of fermentative organisms that includes bacteria having ability to convert sugars to lactic acid, for example, *Lactococcus lactis*. This group comprises 11 genera of gram-positive bacteria, that is, *Carnobacterium, Oenococcus, Enterococcus, Pediococcus, Lactococcus, Streptococcus, Lactobacillus, Vagococcus, Lactosphaera, Weissells and Leuconostoc*. The lactic acid bacteria are largely mesophilic and generally grow over a temperature range of about 10–40 °C, having a temperature optima between 25 °C and 35 °C. Alternatively, *Lactobacillus delbrueckii* subspecies *bulgaricus* is thermoduric and can grow above 40 °C. Similarly, the optimum pH range varies from 4 to 8, though, some can grow at a pH as low as 3.2 and as high as 9.6. Heterofermentative organisms like *Leuconostoc citrovorum* and *Leuconostoc dextranicum* are particularly desirable for development of flavour and aroma compounds such as acetyl aldehyde and diacetyl. A good starter culture will convert most of the sugars to lactic acid, increase the lactic acid concentration to 0.8–1.2 % (titratable acidity) and drop the pH to 4.3–4.5.

10.5 Biochemistry of Fermented Foods

Structurally, maize consists of an embryo (germ) and an endosperm enclosed by an epidermis and a seed coat (husk). While the germ is basically a package of nutrients such as amino acids, sugars, lipids, minerals, vitamins and enzymes, the husk is comprised mainly of cellulose, pentosans, pectins and minerals. Depending on the variety, maize may contain a number of important B vitamins, folic acid, vitamin C and precursor to vitamin A. Maize is also rich in phosphorus, magnesium, manganese, zinc, copper, iron and selenium and has small amounts of potassium and calcium. Maize is a good source of dietary fibre and protein, while being very low in fat and sodium. However, maize is naturally deficient in lysine and tryptophan, which are two of the eight amino acids regarded as essential for humans, but Quality Protein Maize (QPM) has been bred to be high in lysine and tryptophan. Maize lacks the

protein gluten of wheat and, therefore, makes baked goods with poor rising capability. The endosperm consists of starch granules of different sizes embedded in a protein matrix, which makes the maize an excellent substrate for fermentation. Starch constitutes, approximately, 72–73 % of the kernel weight, whereas other carbohydrates are simple sugars that present as glucose, sucrose and fructose in amounts that vary from 1 % to 3 % only. Chemically, this starch is a complex heterogeneous biopolymer composed of amylose and amylopectin, two high molecular weight components that may be present in different ratios. The former is a linear polymer containing 70–2,100 glucose units linked via α-1,4 glucosidic linkages, whereas the latter is a branched polymer with 4–6 % α-1,6 glucosidic linkages at branched points; the average length of branch chain is 20–25 glucose units (Stewart and Russell 1987).

Commonly used starter cultures of yeast and lactic acid bacteria lack the ability to transform this substrate to simple sugars. Clearly, it would require a hydrolysis to glucose by amylases, prior to fermentation. Western techniques use endogenous starch-degrading enzymes produced in the grains through the process of malting. In malting, the grain is moistened by steeping in water and is allowed to germinate. During germination, hydrolytic enzymes present in the aleurone layer surrounding the grain endosperm attack the endosperm, mobilising the nutrients and energy reserve, starch. Some native cultures in South America use salivary amylase for starch hydrolysis by simply chewing the substrate, while some others utilise enzymes produced by co-cultured, resident, or externally added moulds. These techniques, however, are not amenable at large-scale industrial production. Therefore, amylolytic enzymes, a group of starch-splitting enzymes, are of considerable importance to fermentation of grains like maize at industrial level. Starch cannot be converted to sugars easily, as it requires prior gelatinisation by heat treatment, liquefaction by α-amylase and saccharification by amyloglucosidase. The enzyme-mediated hydrolysis of starch is a rapid process with little contamination by reversion products and formation of fewer by-products. In addition, it is more specific and higher yields of sugars as well as alcohol and lactic acid are obtained.

Carbon, nitrogen and other nutrients from surroundings of micro-organisms enter the cell and transform into either new cell material or the product, through a process called metabolism. These transformations require energy, and since most of the micro-organisms involved in industrial fermentations are heterotrophs, therefore, this energy is obtained from breakdown of organic compounds. While in aerobic or respiratory processes, organisms are able to completely oxidise substrate into $CO_2 + H_2O$, resulting in maximum energy production, in anaerobic or fermentative metabolism, cells are less efficient in conversion of organic substrate into cellular material and usually excrete partially degraded intermediates, yielding lesser energy.

Inside the cell, the sugars are broken down by one of the three pathways: the Embden Meyerhof Parnas (EMP) pathway, hexose monophosphate (HMP) pathway and Entner Doudoroff (ED) pathway. The EMP pathway most widely occurrs in animals, plants and fungal, yeast and bacterial cells for glucose utilisation. A few bacteria including *pseudomonas* species, which do not metabolise glucose via EMP pathway, utilise ED pathway. HMP pathway is especially useful in generating precursors of aromatic amino acids and vitamins and the supply of NADPH and H^+ needed for many biosynthetic pathways. A significant product for all the three pathways mentioned earlier is pyruvic acid, which is channelled into tricarboxylic acid (TCA) cycle in aerobic metabolism and in the precursor for various acids, alcohols and other end products in anaerobic metabolism.

Lactic acid bacteria are gram-positive, non-spore-forming rods or cocci, mostly aero-tolerant anaerobes that lack cytochromes and porphyrins and are, therefore, catalase- and oxidase-negative. Some do take oxygen through the mediation of flavoprotein oxidase, which is used to produce hydrogen peroxide and/or to reoxidise NADH produced during dehydrogenation of sugars. Cellular energy is derived from fermentation of carbohydrates to produce peripherally lactic acid using two different pathways: homofermentative and heterofermentative (Fig. 10.1).

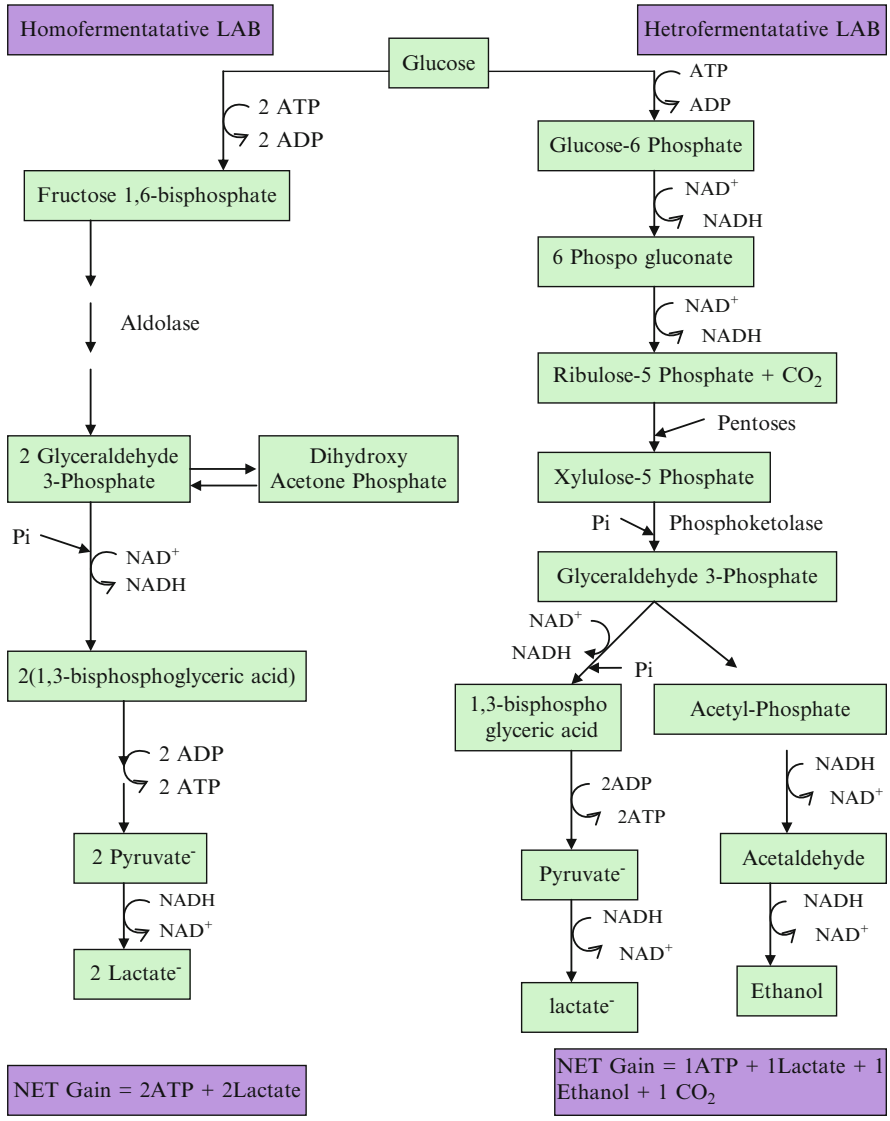

Fig. 10.1 Lactic acid fermentation using homofermentative and heterofermentative bacteria

Homofermentative lactic acid bacteria contain aldolase, a key enzyme of glycolysis, and produce virtually a single product, that is, two molecules of lactate per glucose molecules. They follow the Embden Mayerhoffs Parnas (EMP) pathway where the six-carbon molecule glucose is phosphorylated and isomerised before cleavage by enzyme aldolase into glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. These intermediates can be further converted into two pyruvate and finally two lactate molecules, generating 2 ATP by substrate-level phosphorylation. NADH produced during the oxidation of glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate is reoxidized to NAD^+ in the formation of lactate from pyruvate by the action of lactate dehydrogenases (LDHs).

Heterofermentative LAB lacks aldolase and thus cannot break down fructose bisphosphate to triose phosphate. Instead, they oxidise glucose-6-phosphate to 6-phosphogluconate and then decarboxylate this to pentose phosphate, which is then converted into triose phosphate and acetyl phosphate by key enzyme phosphoketolase. In

heterofermentative LAB, triose phosphate is converted ultimately to lactic acid with the production of one ATP molecule, whereas, to achieve redox balance, acetyl phosphate is reduced by NADH to ethanol, without the generation of any ATP. These heterofermentative bacteria can receive additional ATP molecules through conversion of acetyl phosphate to acetate.

10.6 Fermented Products of Maize

Maize is prepared and consumed in various ways. It is usually ground and pounded. The meal may be boiled, baked or fried. The whole grain may be boiled or roasted and it may be fermented. Maize meal can be cooked with water to provide a thick mush or dough. It may be cooked with water to provide gruel, porridge or soup. In this section, we illustrate the diversity, importance and microbiological features of some fermented maize products (Table 10.3).

10.6.1 Chicha

Chicha is a clear, yellowish, effervescent, alcoholic beverage prepared from maize, having alcohol content that varies between 2 % and 12 % (v/v), most popular in South and Central America. It has a flavour similar to that of cider and colour varies from red to purple depending upon the pigmented maize varieties used for its preparation.

The traditional production of chicha is a unique fermentation process where saliva serves as the source of amylase for converting starch to fermentable sugars. Alternatively, malting (germination) of maize kernels to produce the amylase required for starch conversion is widely used in modern-day processing. Frequently, a combination of both the processes can be used for malting to yield chicha (Steinkraus 1996).

For starch hydrolysis, dry-grounded maize is slightly moistened with water. The maize dough thus obtained is rolled into a ball, popped into the mouth and thoroughly mixed with saliva using the tongue to yield gob, which is then flattened against the roof of the mouth with the tongue and placed under the sun to dry. The dried product, termed muko, is then subjected to grinding in a stone mill. Muko production is generally carried out as a social event by groups of older women, sometimes with the help of young girls. In an alternative method the overnight soaked, maize kernels kept in the dark for about 3–4 days, under moist conditions for germination. With emergence of plumules of 0.25–0.5 cm in length, the kernels are heaped and covered with burlap for 1–2 days and the temperature is allowed to rise until it is uncomfortable to place the hand in the mass of kernels. The kernels become white, parched and covered with a thin layer of ash. Germinated grains are sun-dried, following which they are finely ground in a stone mill.

Methods of extracting the maize flour vary rather widely. In one of the methods, working capacity of an earthenware pot is filled with 1:2 ratio of maize flour and cold water and heated to approximately 75 °C, followed by thorough mixing for 1 h before allowing the mixture to settle and cool down. When *muko* is used, additional non-salivated flour may be added along with crude sugar or squash pulp. Three layers are formed during settlement: a top liquid layer called *upi,* a middle jelly-like layer and a bottom layer that contains coarse particles (*hanchi*). The *upi* is scooped and transferred to another earthenware pot. The middle layer is placed in a shallow pan, heated and concentrated to a sugar-like product. The *hanchi* is pressed and filtered and the filtrate thus obtained is added to the *upi*. The *upi* is caramelised by simmering for several hours and referred to as *misqui kheta*, which is then allowed to cool and ferment for 4–6 days. Since the fermentation pots are never cleaned, no inoculum is required, usually for such fermentations. After completion of fermentation, froth is removed with a cupped hand and the chicha is transferred to narrow-mouth pots for consumption.

The chicha is ready for consumption when the sweetness disappears and the flavour becomes semi-sharp. Brown sugar or molasses may be added in order to increase the alcoholic content of the chicha (Steinkraus 1996).

Table 10.3 Fermented food products from maize

Name of the fermented food	Description/ pretreatment	Microorganism	Nutritive value/applications	Reference
Chicha	Salivation and/or malting	*Saccharomyces cerevisiae, S. pastorianus, Mycoderma vini, Oidium lactis, Monilia candida, Leuconostoc, Lactobacillus, Acetobacter, Aspergillus, Penicillium*	Vitamins B	Steinkraus (1996)
Tesgüino	Malting and extraction of juice of maize stalk followed by boiling of juice	*S. cerevisiae, Lactobacillus, Streptococcus, Leuconostoc, Pediococcus, Saccharomyces, Candida, Cryptococcus, Hansenula, Brettanomyces, Pichia, Geotrichum* and *Penicillium*	Rich in vitamins and enzymes & serves as a refreshing alcoholic drink	Ulloa et al. (1987), Wacher-Rodarte (1995)
Umqombothi	Malting	Lactic acid bacteria and yeasts	Vit B mild alcohol, sour aroma	Bleiberg (1979), Coetzee (1982)
Busaa (Nigeria, Ghana)	Opaque maize beer	*L. helveticus, L. salivarius, Pediococcus damnosus, P. partulus, Candida krusei, S. cerevisiae*	Crude protein, thiamine and riboflavin	Farnworth (2003), Willis (2002), Obot (2007), Papas et al. (2010)
Atole (Mexico)	Steeping and milling of maize grains	Lactic acid bacteria	Sour nonalcoholic porridge, diacetyl provides sensory properties	Escamilla-Hurtado et al. (1993)
Pozol	Boiling of kernels	*Leuconostoc mesenteroides, Lactobacillus plantarum, L. confusus, Lactococcus lactis, L. raffinolactis, Candida krusei, Trichosporon cutaneum, Hansenula fabiani, Kluveromyces fragilis, Candida guillermondii, C. parapsilosis, C. tropicalis, S. Cerevisiae, Geotrichum candidum, Trichosporon cutaneum, Mucor rouxianus*	Control of diarrhoea; antagonistic to many pathogenic bacteria, yeast and moulds; acidic flavour and aroma impart the refreshing properties	Ulloa et al. (1987), Canas-Urbina et al. (1993), Nuraida et al. (1995)

(continued)

Table 10.3 (continued)

Name of the fermented food	Description/ pretreatment	Microorganism	Nutritive value/applications	Reference
Ogi (Nigeria, Benin)	Gelatinisation and Hydrolysis	*Corynebacterium (*hydrolyses), *L. plantarum, Saccharomyces cerevisiae, Candida mycoderma, Rhodotorula, Cephalosporium, Fusarium, Aspergillus, Penicilliums*	Soft or stiff gel Control gastroenteritis in animals and man, Rich in proteins, vitamins and minerals	Nkama et al. (2000), Achi (2005), Osungbaro (2009), Anigo et al. (2009), Muhimbula and Issa-Zacharia (2010), Aderiye et al. (2007), Adebolu et al. (2007), 2012
Mahewu (East Africa)	Based on corn meal	*Streptococcus lactis*, L. *cellobiosis*, L. *brevis*, *L. delbrueckii*, *L. Bulgaricus, Candida krusei, S. Cerevisiae, Candida guilliermondii*	Nonalcoholic sour beverage	Gadaga et al. (1999), Odunfa et al. (2001), Steinkraus (1996)
Pito (Nigeria and Ghana)	Meshing of malted maize	*Geotrichum candidum, Lactobacillus* sp., *Candida* sp.	Dark brown alcoholic liquid, varies in taste from sweet to bitter	Akyeampong (1995), Sefa-Dedeh (1999)
Uji (Sudan)	Grounded and slurrified	*Lactobacillus plantarum*, *L. fermentum, L. cellolbiosus, L. buchneri*	Sour maize gruel	Mbugua (1984)
Abati (Paraguay & Argentina)	Based on maize dough or flour	Lactic acid bacteria and yeasts	Alcoholic beverage	Haard et al. (1999)
Acupe (Venezuela)	Malting	Lactic acid bacteria and yeasts	Fermented and sweetened nonalcoholic beverage	Haard et al. (1999)
Cachiri (Brazil)	Based on maize, manihot or fruit and produced in clay pots	Lactic acid bacteria and yeasts	Fermented nonalcoholic beverage	Haard et al. (1999)
Agua-agria (Mexico)	Grinding of maize with water	Lactic acid bacteria and yeasts	Nonalcoholic beverage	Haard et al. (1999)
Atole agrio (Mexico)	Fermented black maize dough	Lactic acid bacteria and yeasts	Nonalcoholic porridge	Haard et al. (1999)
Fuba (Brazil)	Malting	Lactic acid bacteria and yeasts	Nonalcoholic beverages	Haard et al. (1999)
Ostoche	Based on maize juice and brown sugar	Lactic acid bacteria and yeasts	Alcoholic beverage	Haard et al. (1999)

10.6.2 Tesguino

Tesguino is a slurry-like, alcoholic beverage prepared by fermentation of germinated maize or maize stalk juice. It is consumed by several ethnic groups of northern and northwestern Mexico. Methodologies used in the preparation of *tesguino* vary among ethnic groups. In a most common approach, about 10 kg of dry maize kernels are soaked in water for several days,

drained and placed either in baskets in the dark or in a hole in the ground in order to allow them to germinate. The germinating kernels are protected from light, in order to prevent the formation of green and bitter sprouts. The germinated kernels are ground and boiled for about 8 h in water until the mixture turns yellow. The liquid portion is then transferred to a clay pot, and catalysts are added. The most common catalysts are bark (*batari*) or *kakwara* (*Randia echinocarpa*, *R. watsoni* and *R. laevigata*) and *kaya* (*Coutarea pterosperma*), which are chopped, ground and boiled for many hours prior to being added to the tesguino.

At higher altitudes where pine trees grow, the catalysts used are leaves of *Stevia serrata*, *Chimaphila maculata* and *Datura meteloides;* stems of *Bromus arizonicus*; and roots of *Phaseolus metcalfei* and *Plumbago*. The mixture is allowed to ferment for several days prior to consumption (Taboada et al. 1977; Steinkraus 1996).

In order to prepare tesguino from maize stalks, the raw material either fresh or dry is macerated by pounding with a club, in the depression of a rock. The macerated material is then placed on a sieve, water is slowly poured over and juice is collected in a hollow pumpkin. The juice is mixed with water and boiled for several hours prior to the addition of catalysts. The mixture is allowed to ferment in a dark place for 2–3 days, until it develops a pleasant appearance and flavour before it is consumed.

S. cerevisiae is an important micro-organism in the alcoholic fermentation of tesguino and is maintained on surfaces of the utensils and clay pots that are used exclusively for the preparation of tesguino. The bacteria produce the lactic and acetic acids which give tesguino some of its distinctively refreshing, acidic flavour. Abundant yeast species consistently identified at various stages of the fermentation are alcohol producers and include *Candida guilliermondii*, *Hansenula anomala*, *S. cerevisiae*, and *S. kluyveri*. *S. cerevisiae* and *S. kluyveri*. Other yeasts produce oxidative esters that contribute to turbidity, aroma and flavour. Compositionally, tesguino contains 73.9 % moisture, 2 % protein, 0.21 % crude fibre, 2.5 mg/100 g iron, 0.03 mg/100 g thiamine, 0.03 mg/100 g riboflavin and 0.29 mg/100 g niacin. During the fermentation, protein content increased by 58 % and lactic acid, acetic acid and ethanol concentrations were 0.41 %, 0.11 % and 3.73 %, respectively. At the end of the process, total protein concentration approached 13.2 % (Wacher-Rodarte 1995).

10.6.3 Umqombothi

Umqombothi is popular among the South African population. Maize and sorghum, used in combination, are the most common cereals used in South Africa to make *umqombothi*. It is a pink, opaque, mild alcoholic drink having yoghurt-like flavour and thin consistency. It is consumed in the active state of fermentation and therefore has a short shelf life of 2–3 days (Coetzee 1982). Equal amount of maize meal, crushed maize malt and crushed sorghum malt are mixed with warm water in a cast-iron pot. The mixture is left overnight for fermentation that generates a sour odour and bubbling. A small portion of the wort is removed and put to one side and the remaining mash is cooked until crusty sediment forms which can be eaten as porridge. When making beer, the sediment is left to cool for a day. After the mixture has cooled, it is poured into a large plastic vat along with the wort that was set aside. Some additional sorghum and maize malt is added to the vat and the brew is stirred properly. The vat is put in a warm place overnight, to encourage fermentation. Lactic acid bacteria and yeasts are thought to be the predominant micro-organisms during this fermentation. When the brew is ready, the fermented mash is filtered through a large metal strainer, to remove the spent grains. Strained beer is poured into a large communal drum and it is ready for consumption.

10.6.4 Busaa

Busaa is a Kenyan opaque maize beer having 2–4 % ethanol and 0.5–1 % lactic acid. It is considered more nutritious and superior as compared to clear lager beer due to its richness in

crude protein, thiamine and riboflavin and is typically made from the most plentiful source of grain, whether maize, millet or sorghum. Although brewing and selling traditional drink is illegal in Kenya, drinking traditional brew is a common activity during many social and religious ceremonies. For example, drinking busaa is integral to the custom of group genital circumcisions, weddings and funerals (Willis 2002). Once controlled by elder men, drinking of traditional brew has been largely transformed into a commercial enterprise that includes female and young consumers (Willis 2002; Papas et al. 2012) and is believed to be more frequently brewed and sold by women today (Holtzman 2001; McCall 1996; Obot 2007).

At the maize-souring stage, the micro-organisms involved are lactic acid bacteria and a few yeasts, mainly representatives of *L. helveticus, L. salivarius, Pediococcus damnosus, P. partulus, Candida krusei* and *S. cerevisiae*. The main fermentation is also governed by a mixture of yeasts and lactic acid bacteria including *C. krusei* and *L. casei* var. *rhamnosus*.

10.6.5 Atole

Atole is a sour porridge-type product prepared from maize, in Southern Mexico. It is produced by steeping maize grains in water for 4 days, followed by milling during which lactic acid fermentation takes place for 1 day. Lactic acid bacteria present in the atole form diacetyl, which contributes to the characteristic sensory properties of the product.

10.6.6 Pozol

Pozol is fermented maize dough formed into balls of various shapes and sizes. It is advantageous as it can be preserved without refrigeration under the tropical conditions, in which it is routinely eaten, owing to its low pH, along with having improved nutritional quality such as high protein, niacin, riboflavin, lysine and tryptophan, due to the development of certain bacteria, yeasts and moulds.

In the production process of pozol, 1–1.5 kg of kernels are boiled for 1 h in a pot containing 1–2 l of an approximately 10 % (w/v) calcium hydroxide solution. During boiling, the majority of micro-organisms associated with maize kernels are destroyed and swelling of the kernels takes place, thus allowing the pericarp to be relatively easily peeled off the kernels. The kernels are cooled, rinsed with water and termed as *nixtamal*. The *nixtamal* is ground to obtain coarse dough, which is manually shaped into balls. The balls are fermented for 1–14 days after wrapping in banana leaves to prevent desiccation. Ground cacao beans or coconut may be added to the dough prior to fermentation, to yield a fermented product called *chorote* (Canas-Urbina et al. 1993). During the initial 24 h (pH 7.3) of pozol fermentation, lactic acid bacteria (10^4–10^6/g) outnumber yeasts (10^2–10^4/g) and moulds ($<10^3$/g) and are probably responsible for the majority of acid produced. After incubation for 30 h at 28 °C, the counts increase to 10^{10}/g for lactic acid bacteria, 10^6/g for yeast and 10^4/g for moulds, while the pH decreases to 4.6 (Wacher et al. 1993).

Pozol is consumed in the southeastern states of Mexico, such as Chiapas, Tabasco, Campeche and Yucatan, and on a smaller scale in Veracruz, Oaxaca and Guatemala (Ulloa et al. 1987). Pozol balls are diluted with water in 1:2–1:3 ratios at various stages of the fermentation process to produce a whitish porridge that is consumed in the uncooked state as a basic food in the daily diet of large communities. Salt, toasted ground chilli pods, sugar or honey may be added as per the taste of consumers.

10.6.7 Ogi

Ogi porridge or sour gruel is obtained as a result of the submerged fermentation of some cereals such as maize in West Africa and sorghum and millet in the North. *Ogi* is usually a cooked gel of variable degree of stiffness and often marketed as a wet cake wrapped in leaves or transparent

polythene bags. Gelatinised *ogi* is an important indigenous, traditional weaning food common in the whole of West Africa.

Ogi is an example of traditional fermented food, involving a simple technique such as malting and fermentation, which has now been upgraded to a semi-industrial scale (Achi 2005). Microbiological and nutritional studies by Akinrele (1970) showed that the lactic acid bacterium *Lactobacillus plantarum,* the aerobic bacteria *Corynebacterium* and *Aerobacter*, the yeasts *Candida mycoderma*, *Saccharomyces cerevisiae* and *Rhodotorula* and moulds *Cephalosporium, Fusarium, Aspergillus* and *Penicillium* are the major organisms responsible for the fermentation and nutritional improvement of ogi. *Corynebacterium* hydrolyses the corn starch but the predominant starter in the fermentation production of lactic acid is *L. plantarum. Saccharomyces cerevisiae* and *Candida mycoderma* contribute to the flavour development (Odunfa and Adeyele 1995).

The production of ogi is often undertaken by unskilled female attendants of small-scale enterprise (Aminigo and Akingbala 2004). Therefore, these slurries have been known to exhibit health-promoting properties such as control of gastroenteritis in animals and man. This has been demonstrated by locals who drink the raw slurry when there is a case of diarrhoea (Oyetayo and Osho 2004; Aderiye et al. 2007; Adebolu et al. 2012). Ogi liquors prepared using cold and hot water methods were effective in inhibiting the growth of most of the test organisms except *Enterobacter* sp and *Staphylococcus aureus;* the growth inhibition, however, was not as wide as that of some of the antibiotics such as ciprofloxacin, gentamicin and ofloxacin but in most cases superior to that of tetracycline, nitrofurantoin and cotrimoxazole (Adebolu et al. 2007).

DogiK, an improved ogi formulated to aid the control of diarrhoeal diseases, was produced by using *Lactobacillus* starter cultures with antimicrobial activity against diarrhoeagenic bacteria and also possessing amylolytic activity. The survival of diarrhoeagenic bacteria was investigated in locally fermented ogi and in DogiK. The foods were inoculated with cell suspensions of *Salmonella, Shigella, Campylobacter, Aeromonas, Pleisiomonas*, Enteropathogenic and Enterotoxigenic *Escherichia coli, Yersinia enterocolitica* and *Vibrio cholerae*. None of the diarrhoeagenic bacteria were detected in DogiK after 6 h, whereas in the local ogi *Salmonella, E. coli* and *Shigella* survived for 24 h or more but showed a sharp decrease in numbers, while *V. cholerae* survived for 12 h. DogiK is active whether cooked or uncooked and exhibited inhibition of pathogens at neutral pH. It gives consistent quality. Preliminary investigation indicates possession of a better shelf life. Thus, DogiK may have a potential use in the prevention and treatment of diarrhoea (Olukoya et al. 1994).

10.6.8 Mahewu

Mahewu (*amahewu*) is an example of a non-alcoholic sour beverage made from corn meal, consumed in Africa and some Arabian Gulf countries. It is known by various names, like *amahewu*, *amarehwu, emahewu*, *metogo*, *machleu* and *maphulo*; the most commonly used, however, is *mahewu*. It is prepared from maize porridge, which is mixed with water. Sorghum, millet malt or wheat flour is then added and left to ferment (Odunfa et al. 2001). *Lactococcus lactis* subsp. *Lactis,* the natural flora of the malt, is the predominant micro-organism in the spontaneous fermentation carried out at ambient temperature (Steinkraus 1996; Gadaga et al. 1999).

Mahewu is known to offer some advantages over ogi as the former consists of coarse maize particles while ogi contains very fine pasty maize particles. The initial wild fermentation by fungi, in case of mahewu, is eliminated by boiling. Furthermore, it is pre-cooked and requires only mixing prior to consumption.

10.6.9 Pito

Pito is the traditional beverage, very popularly consumed throughout Nigeria and Ghana owing to its refreshing nature and low price. The

preparation of pito involves soaking cereal grains, maize, sorghum or a combination of both in water for 2 days. This follows malting for 5 days in baskets lined with moistened banana leaves. The malted grains are ground, mixed with water and boiled. The resulting mash is allowed to cool and later filtered through a fine mesh basket. The filtrate thus obtained is allowed to stand overnight, or until it assumes a slightly sour flavour, following which it is boiled to a concentrate. A starter from the previous brew is added to the cooled concentrate that is again allowed to ferment overnight. Pito, the product thus obtained, is a dark brown liquid which varies in taste from sweet to bitter. It contains lactic acid, sugars and amino acids and has an alcohol content of 3 %. Organisms responsible for souring include *Geotrichum candidum* and *Lactobacillus* sp. while *Candida* sp. are responsible for the alcoholic fermentation.

10.6.10 Uji

It is primarily a sour maize gruel from East Africa; however, alternatively some sorghum or millet may also be added in 4:1 ratio (Mbugua 1984). The finely ground raw cereal is slurried with water and allowed to ferment for 2–5 days at 25 °C temperature. During fermentation, pH of the slurry is reduced to 3.5–4.0 in 40 h. The product is diluted to about 8–10 % solids and boiled followed by further dilution and sweetening with sugar before consumption. *Uji* fermentation is characterised by the sequential growth of the dominant micro-organisms, initiated by the growth of coliforms and later succeeded by the growth of lactic acid bacteria. Early acid production at high concentrations by the lactic acid bacteria rapidly restricts coliform activity, thereby eliminating the problems of off-flavours and flavour instability. *Lactobacillus plantarum* is mainly responsible for souring of uji, although early activity of heterofermentative strains of *L. fermentum, L. cellolbiosus* and *L. buchneri* during the fermentation is evident (Onyango et al. 2003, 2004).

10.7 Limitations Associated with the Use of Fermented Maize Products

In most parts of Nigeria and Africa, children are fed with mashed adult food or gelatinised cereal flour slurries to complement breast milk from 4 to 6 months of age. These slurries absorb a large quantity of water and swell up greatly when mixed either with cold or hot water. The foods are therefore bulky due to the high viscosity. Due to the fact that high bulk reduces food intake by a child often resulting in malnutrition, therefore, development of nutritionally balanced calorie-dense, low dietary bulk and easily digestible weaning foods are being sought for. Moreover, soaking of maize in alkali water (made with ashes and calcium oxide) liberates the B-vitamin niacin, the lack of which was the underlying cause of the condition known as pellagra. Once alkali processing and dietary variety were understood and applied, pellagra disappeared in the developed world. Besides the lack of niacin, pellagra was also characterised by protein deficiency, a result of the inherent lack of two key amino acids in premodern maize, lysine and tryptophan. A range of indigenous fermented foods is prepared from cereals in combination with legumes, thus improving the overall protein quality of the fermented product. Cereals are deficient in lysine but rich in cysteine and methionine. Legumes, on the other hand, are rich in lysine but deficient in sulphur containing amino acids. Thus by combining cereal with legumes, the overall protein quality is improved (Campbell-Platt 1994). Consequently, number of leguminous seeds including soya beans and okra seeds are used to fortify and improve their protein, iron, calcium and fibre content (Osungbaro 2009; Anigo et al. 2009) to eliminate incidences of anaemia and stunted growth, often associated with malnutrition (Muhimbula and Issa-Zacharia 2010). Further, maize contains lipid transfer protein, an indigestible protein that survives cooking and has been linked to a rare and understudied allergy to maize in humans.

Above all, poor handling, inappropriate safety measures and ill manufacturing practices will

ensure contaminated product that makes it source of infections such as cholera, typhoid fever, hepatitis and gastroenteritis, in infants and adults. For example, microbial assessment at critical parts of ogi fermentation revealed contamination of fungi like *Aspergillus flavour, Aspergillus niger, Penicillium oxalicum, Fusarium oxysporium* and *Rhizopus stolonifer*; yeast like *Candida albicans* and *Saccharomyces cerevisiae*; enteric bacteria like *Escherichia coli* and *Klebsiella aerogenes;* and non-enteric bacteria like *Lactobacillus plantarum, Pseudomonas aeruginosa* and *Staphylococcus aureus* (Oyelana and Coker 2012). The inclusion of fumonisin and aflatoxin in maize has been linked to certain fungal species such as *Aspergillus, Penicillium, Rhizopus* and *Fusarium* (Omemu et al. 2005). The carcinogenic effect and other health implications of these toxins were extensively discussed by Shephard et al. (2002), Barug et al. (2004) and Gadaga et al. (2004).

10.8 Conclusion

Fermented foods were discovered before mankind had any knowledge of micro-organisms, but these were simple observations that certain way of storing food effected desirable changes in its characteristics such as shelf life. These characteristics, in the industrial world, became less important with the advent of alternative preservation methods such as canning, chilling and freezing. Modern technology, however, in no way has diminished the sensory appeal, contributed by peculiar flavour and aroma, of fermented products. Fermented foods are considered beneficial over non-fermented products as microbial action not only improves shelf life by producing acids, alcohols and bacteriocins but also provides better digestibility, nutritional supplementation, retention of micro- and macronutrients, improved aroma and flavour characteristics to foods.

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Maize Malting: Retrospect and Prospect

11

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Abstract

Malting is the process of converting cereal into malt. Barley is the most preferred malted grain because of its high enzyme content needed for the conversion of grain starch to malt. Wheat, rye, oats and rice are the other cereal grains used for this purpose. Maize kernels are rich in starch (≈70 %). The abundance of starch in maize stimulates researchers to evolve improved technological interventions for the better conversion of maize to malt. Inadequate diastatic power and the immature breakage of plumule before the complete endosperm modification of maize kernel are the major hurdles in selecting maize for malting purpose. Maize is used as an adjunct in the production of beer. Breeding maize for malting and the evolution of improved technology for proper endosperm modification are needed for the efficient conversion of maize to malt.

11.1 Introduction

Maize is an important crop in the Indian agricultural system that provides food, feed and fodder and serves as a source of basic raw material for a number of industrial products including starch, oil, syrups, alcoholic beverages and biofuel. In the last 5-year plan, maize achieved the highest growth rate (6.7 %) amongst cereals as against the required growth rate of 4.7 % set by the planning commission of India. The production as well as productivity of maize is increasing continuously for the last few years. As a result, India moved from being importer to exporter of maize. Considering the changing climatic scenario and the impact of single-cross maize hybrids, it is estimated that production and productivity of maize will further increase in the near future. In the present utilization pattern, a major part of maize produced is used as animal feed followed by food. A significant amount of maize produced is used as an industrial raw material. At present, around 20 % of maize produced is utilised in the starch industry for the production of starch along with maize oil, bran and gluten meal as by-products. As a result of increasing

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maize production, its industrial utilisation is also increasing, resulting in further expansion of this segment. Since maize is one of the most important multipliers of starch in this universe, its use as a raw material in the malting industry could effectively be explored. To date, barley is primarily being utilized for malting purpose. The abundance of starch in maize, however, prods the researchers to explore improved technological interventions as well as instrumentation for the efficient conversion of maize into malt. In this chapter the nutritional quality of maize is being discussed followed by its strengths as well as weaknesses with respect to its conversion to malt for brewing purpose.

11.2 Structure of the Maize Kernel

Maize kernels vary in size and shape in different kinds and varieties. They may be only an eighth of an inch long and near round in popcorn to a half-inch long and a flattened-cylinder shape in some other kinds. Maize kernels develop on the female inflorescence called the ear. The ear is a long cylindrical structure that may hold from 200 to 1,000 single kernels depending on the number of rows, diameter and length of the cob. The number of rows may vary from 12 up to 22 depending upon the genotype, as well as quantum of nutrients applied. There are four major physical structures of the kernel: the outermost called pericarp, hull or bran; the germ or embryo; the endosperm; and the tip cap (dead tissue found where the kernel joins the cob). The endosperm is the largest component comprising about 80–85 % of the kernel weight, whereas germ is about 10 % and the pericarp around 5 % of the kernel weight.

11.3 Chemical Composition

The different components of maize kernel differ significantly in terms of chemical composition (Table 11.1). The pericarp is characterised by a higher concentration of crude fibre (87 %), consisting mainly of hemicellulose (67 %), cellulose (23 %) and very small quantities of lignin (0.1 %) (Burge and Duensing 1989). These components are considered indigestible for humans but help in the proper movement of bowl. However, ruminants can efficiently digest the bran and, therefore, it is used in animal feed. Endosperm, the major component of maize kernel, contains high levels of starch (≈85–88 %) and protein (≈8 %). The germ is rich in fat, averaging about 30 %. It also contains relatively high levels of protein (≈18 %), sugar (≈11 %) and some minerals.

Table 11.1 Chemical composition of the main parts of maize kernels (%)

Chemical component	Pericarp	Endosperm	Germ
Protein	3.7	8.0	18 4
Ether extract	1.0	0.8	33.2
Crude fibre	86.7	2.7	8.8
Ash	0.8	0.3	10.5
Starch	7.3	87.6	8.3
Sugar	0.34	0.62	10.8

Source: Watson (1987)

Table 11.2 Gross chemical composition of the different types of maize (%)

Maize type	Protein	Sugar	Oil	Starch
Normal (HM 4)	11.8	3.3	3.5	70.3
QPM (HQPM 4)	10.8	3.9	3.3	67.7
Sweet (WOSC)	13.6	13.4	12.1	50.0
Pop	13.7	2.5	5.7	66.0
High oil (HKI-162-2)	11.0	3.5	6.2	69.9

Source: 53rd Annual Progress Report, DMR, (2010); Cortez and Wild-Altamirano (1972)

The chemical composition, however, varies amongst different types of maize. A wide variability is observed in each major nutrient component. Table 11.2 summarises data on various types of maize. The variability observed is both genetic and environmental. It may influence the weight distribution and individual chemical composition of the endosperm, germ and hull of the kernels.

Starch is the major carbohydrate of the maize kernel, averaging around 67–74 %. Other carbohydrates are simple sugars, mainly sucrose and very small amounts of glucose and fructose. Starch is an odourless, white powder with a bland taste, which is extensively used as an industrial

material for the synthesis of a number of products. Maize is one of the most important multipliers of commercially produced starch. Chemically, starch is a polysaccharide consisting of a large number of glucose units joined by glycosidic bonds. It consists of two types of molecules: the amylose and the amylopectin. Amylose is a linear polymer in which the α-D glucose units are linked through α-1,4 glycosidic bonds. Amylopectin is a branched structure in which apart from α-1,4; α-1,6 glycosidic bonds are also present. The composition of maize starch is genetically controlled. In normal maize, amylose constitutes up to 25–30 % of the starch and amylopectin up to 70–75 %. Maize that contains 100 % amylopectin is called waxy maize. An endosperm mutant called amylose-extender (ae) has an increased amylose proportion of the starch induced up to 50 % and higher. Other genes, alone or in combination, may also modify the amylose-to-amylopectin ratio in maize starch (Boyer and Shannon 1987). The waxy as well as *ae* lines have different applications in the starch industry. Waxy maize is mainly utilised in the food industry primarily due to its viscous and gel-forming properties. High amylose maize, on the other hand, is hard due to its compact chemical structure and is used for making starch threads, etc. Starch is extensively used in the food, pharmaceutical, textile, paper, adhesive and a host of other industries. It can also be converted into a range of starch derivatives such as liquid glucose, dextrose monohydrate, dextrose anhydrous, maltodextrin and sorbitol.

Protein is the second largest and an important component of maize kernel. In normal maize, the protein content varies from 7 % to 13 % of the kernel weight. Most of the protein is located in the endosperm. It is made up of at least five different fractions (Landry and Moureaux 1970, 1982). Albumins, globulins and non-protein nitrogen amount to 18 %, whereas prolamine fraction contributes 52 % of the nitrogen in the kernel. Prolamine 1 or zein 1 is found in the largest concentration (42 %), whereas prolamine 2 or zein 2 is found to be around 10 %. Glutelin fraction 2 amounts to about 8 %, while glutelin 3 amounts to about 17 % for a total glutelin content of 25 % of the protein in the maize kernel (usually a small amount, about 5 %, is residual nitrogen). In the normal maize, zeins predominate in the endosperm, which are primarily responsible for the poor quality of protein of normal maize. To the contrary, the quality protein maize is characterised by higher content of non-zeins, which are better balanced in terms of protein quality.

Although a by-product, corn oil occupies a significant position in human nutrition. The oil comes mainly from the germ. Its concentrations are genetically controlled, with values ranging from 2 % to 20 %. Maize oil has low level of saturated fatty acids. On the other hand, it contains relatively high levels of polyunsaturated fatty acids, mainly linoleic acid (24 %). Very small amounts of linolenic and arachidonic acids have been reported. Furthermore, maize oil is rich in vitamin E, which makes it relatively stable. Maize oil is highly regarded for human consumption because of its fatty acid profile, mainly oleic and linoleic acids.

Dietary fibre consists of indigestible portions of carbohydrates. It comes mainly from the pericarp and the tip cap, although small quantities are also contributed by endosperm cell walls and germ cell walls. Maize bran is composed of 75 % hemicellulose, 25 % cellulose and 0.1 % lignin on a dry-weight basis. Small amounts of sugars (2–6 %) are also found in mature maize kernel with sucrose, the major component, found mostly in the germ. Higher levels of monosaccharides, disaccharides and trisaccharides are present in maturing kernels.

Apart from this, minerals also contribute between 1 % and 2 % of the kernel weight. Germ provides about 78 % of the whole kernel minerals. The most abundant mineral is phosphorus, found as phytate of potassium and magnesium. All the phosphorus is found in embryo. Maize is low in calcium and trace minerals.

11.4 Malting

In the simplest term, malting is the controlled germination and kilning of grain. The grains are made to germinate by soaking in water and are then halted from germinating further by drying

with hot air. Malting grains develops the enzymes (diastatic enzymes) required to modify the grain starch into sugars, including monosaccharides, such as glucose or fructose, and disaccharides, such as sucrose or maltose. It also develops other enzymes, such as proteases, which break down the proteins in the grain into molecules that can be used by yeast. The malting of cereal grains involves controlled germination, and finally biological activities are arrested through gradual heating. Though, the most preferred grain for malting is barley, but other grains like wheat, maize and sorghum are also used for making malt.

11.5 Malting Process

Malting is a three-step process consisting of steeping, germination and finally kilning. A brief detail of all the three steps is outlined in the following:

11.5.1 Steeping

The basic aim of this process is to increase the moisture content of the grain while maintaining seed viability. This is required for the germination of the grain and for uniform diffusion of enzymes throughout the endosperm. On an average, the moisture content is raised to more than 40 %. This operation is done in conical vessels or flat-bottomed tanks. Conical vessels have limited capacity of around 50 t, while flat-bottomed tanks have capacity of around 300 t. The grains are initially dipped in water, and a temperature of 14–18 °C is maintained for about 6–8 h. During this step, the dirt, floating kernels and some undesirable compounds get removed. Since the metabolic activity in the grain gets started during this period, the dissolved oxygen in the steep water gets depleted. To supply the oxygen and to remove the excess carbon dioxide, air is bubbled through water, and after the immersion cycle, water is drained and fresh air is circulated, and this phase is known as the air rest stage. The air rest stage is followed by again steeping or immersion stage and then again air rest stage. The duration of each stage and total steeping depends upon the kind of grain and variety of grain being used, as at the end of the steeping period, the grain is usually in chitted state.

11.5.2 Germination

This process is done to achieve modification of the endosperm reserves, especially starch. During this process, hydrolytic enzymes, that is, amylolytic and proteolytic ones, become active and break down complex molecules into simple ones. The cell walls of endosperm cells also get disintegrated during this process. The commercial germination unit consists of a large compartment to accommodate 50–300 t of grains, with a provision to provide cool and humidified air and slow turning of grains. Turning of grains is essential to separate the growing roots so that the flow of air through the bed is uniform. Temperature is kept between 16 and 18 °C to minimise the respiratory losses, which will ultimately lead to decreased malt yield. Again germination period varies from 3 to 7 days depending upon the kind of grain and variety being used.

11.5.3 Kilning

The major objective of kilning is to dry the germinated grains to the moisture content of 4–5 % and to achieve development of malt colours and flavours. The kiln has provision of gradual temperature increase and air circulation, and energy is conserved to the maximum extent possible. The kilning is usually done for 16–40 h with gradual increase in the temperature. The temperature is raised from 45 °C to 70–80 °C gradually to preserve the enzyme activities. During the kilning process, the temperature of grains is usually lower than the heating air temperature because of moisture loss from the grains. After the kilning process, the grains are cooled and rootles removed. The malt is cleaned and packed in airtight bags for shipment.

11.6 Maize Malting: Problems and Future Prospective

The malting potential of different cereals has been studied over time (Malleshi and Desikachar 1989). Barley is the most preferred grain for brewing all over the world. It is the fourth largest cultivated cereal crop in the world. There are many advantages of barley over other cereal grains for malting process. It has a short germination period suited for malting process. It has sufficiently high starch content and low protein which reduces haze in beer. However, the most important characteristic that makes it suitable for malting is perhaps its high diastatic power (high level of diastatic enzymes) needed for conversion of starch (Kneen 1944; Engel 1947; Norris and Lewis 1965; Palmer 1989; Muoria 1998). Secondly, barley grain is covered by husk which has advantage in malting since during germination, the endosperm modification is determined by the length of the acrospire. The growth of the acrospire is used as a rough guide to the progress of malting. Usually, the lengths of the acrospires of a number of grains are noted as fractions of the grain lengths. Thus, in traditional practice the green malt is kilned and growth terminated when most acrospires are 0.75–1 of the grain's length. In barley, the acrospires develop beneath the husk, whereas in corn the plumule sprouts upward making it vulnerable to break immaturely before the completion of the endosperm modification process. The steeping and germination is usually carried out in big tanks where a grain bed is formed for this process. The grain bed is continuously agitated to avoid deoxygenation. During the process, the breakage of plumule is the most common problem with cereals other than barley.

The second important disadvantage with corn is its diastatic power. In a preliminary study carried out at the Directorate of Wheat Research, Karnal, it was observed that corn produces around 50–60 % of malt after steeping and germination period spanning 6–7 days (unpublished data). The maize kernel consists of hard endosperm, which is surrounded by a thin and uniform layer of pericarp. This increases the steeping period. Usually, it takes around 3–4 days to gain maximum moisture content, and the total period for germination is around a week. To date, corn is being used as adjunct along with barley in the malting process (Iwouno 2012). In order for enzymatic conversion to occur, grain starches must be *gelatinised*, which means their structure must be broken down by heat and water. Each grain has a different temperature at which gelatinisation occurs. Corn has a higher gelatinisation temperature than barley, which needs special processes to make the starch available for conversion. Maize generally has little or no diastatic power and so must be used with base malts for starch conversion (Takaku 1988). Malting of maize for use as a major source of hydrolytic enzymes required for brewing purposes has received less attention (Eneje et al. 2004). Earlier works have shown that cereal grains could differ significantly in their modes of amylases development (Kneen 1944; Lauriere 1992; Ziegler 1999). The low activity of amylases in most cereals other than barley malt has been defined as one of the most serious obstacles to their use as replacement for barley malt (Takaku 1988; Okungbowa et al. 2002). However, the gluten-free characteristics of maize make it a good substitute for barley malt especially for people suffering from celiac disease (Sweeney 2004).

However, considering the magnitude of starch available from maize, efforts are continuously being made to explore the experimental variables for better maize malting. In an earlier study, 27 yellow maize varieties were malted using varying steeping time (30 h, 36 h, 42 h), germination time (3 days, 4 days, 5 days) and kilning temperature (50 °C, 55 °C, 60 °C). Results revealed that 42 h of steeping, 5 days of germination and 50 °C kilning temperature give the best maize malt (Wouno and Ojukwu 2012). However, maize malt showed low diastatic power when compared with barley malt as reported by Okafor and Aniche (1980).

The use of maize as malting grain attracts little attention in India. However, with the increasing production and productivity of maize, a lot of opportunities are available whereby we can

explore the possibility of a good quality beer produced from corn. Since maize possesses a sufficiently high quantity of starch, efforts could be diverted towards exploring new ideas and scientific know-how to use maize as an efficient grain towards better beer production.

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Value Addition in Maize

12

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Abstract

Globally, maize is the most important coarse grain cereal and well known as "poor man's nutricereal" due to presence of high content of carbohydrates, fats, proteins, and some of the important vitamins and minerals. On the basis of its unique characteristics and nutritional composition specialty, corn is classified into quality protein maize, baby corn, sweet corn, pop corn, green eared corn, high oil corn, etc. Specialty corn has diverse end uses and a number of value-added products can be prepared from it. Quality protein maize differs from normal maize as it contains added amount of essential amino acids such as tryptophan and lysine. Baby corn is the ear, harvested young when the silks have just emerged and no fertilisation has taken place. In sweet corn, the taste of kernels is much sweeter than normal corn. In popcorn when kernels are heated, they explode and produce large puffed flakes (popping). The value-added products prepared from specialty corns are traditional foods, infant foods, health foods, snacks and savory, baked products, etc. Each specialty corn has different recipes. Apart from these products, maize is used to prepare industrial products such as starch, specialty chemicals, ethanol, refined corn oil, sorbitol, cake mixes, candies, carbonated beverages, and cosmetics.

12.1 Introduction

Maize [*Zea mays* L.] is a major cereal crop for human nutrition. With its high content of carbohydrates, fats, proteins, and some of the important vitamins and minerals, maize has acquired a well-deserved reputation as "poor man's nutricereal." Several million people, particularly in the developing countries, derive their protein and calorie requirements from maize. The incomparability of corn is due to its wide range of uses and highly useful products into which it can easily be transformed. Maize crop has a special place in Indian agriculture and is a staple food of people of low socio-economic groups.

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Table 12.1 Nutritional composition of quality protein maize and normal maize (dry matter basis)

Particulars	HQPM-1	Shaktiman-1	HM-4	HM-5
Moisture (g/100 g)	6.90	7.34	7.37	7.23
Crude protein (g/100 g)	11.48	11.65	10.04	10.15
Crude fat (g/100 g)	4.40	4.52	4.43	4.29
Crude fibre (g/100 g)	2.41	2.34	2.40	2.65
Ash (g/100 g)	1.46	1.55	1.34	1.58
Total carbohydrates units	80.28	79.94	81.97	81.33
Energy (Kcal/100 g)	465.2	407.04	375.67	–
Total soluble sugars (g/100 g)	2.34	2.81	0.14	2.58
Calcium (mg/100 g)	15.39	17.78	17.76	17.53
Phosphorous (mg/100 g)	200.63	200.78	197.89	197.63
Iron (mg/100 g)	2.74	2.78	2.73	2.50

Source: Kawatra and Sehgal (2007)

Corn is largely classified into six types based upon the quality, quantity, and composition of endosperm. These types are known as dent, flint, flour, sweet, pop and pod. Each has different properties with various end uses. In India, much work has been done in the area of specialty corns such as sweet corn, popcorn, baby corn, quality protein maize, green-eared corn, high-oil corn, waxy corn and fodder maize.

12.2 Quality Protein Maize and Its Value-Added Products

In spite of diverse uses, maize has many drawbacks, especially imbalance in amino acids composition. To overcome these problems, scientists have discovered the maize mutant *opaque-2*, which is superior in its nutrient composition than common maize and is named as quality protein maize (QPM).

QPM itself is value-added maize as it contains increased amount of essential amino acids such as tryptophan and lysine. The discovery of QPM was initiated in the early 1960s, when scientists at Purdue University discovered a peculiar gene that significantly increased the level of two essential amino acids, lysine and tryptophan, in the maize grain. QPM looks and tastes like normal maize and yields as much or more, but it contains nearly twice the quantity of the essential amino acids, lysine and tryptophan, which are essential building blocks of proteins in humans and monogastric animals like poultry and pigs (CIMMYT 2000).

The QPM has better protein quality than common maize because it contains double the amount of lysine and tryptophan and no change in other amino acids except low level of leucine. Decrease in leucine is considered particularly desirable as it makes the leucine–isoleucine ratio more balanced, which in turn helps to liberate more tryptophan for niacin biosynthesis and thus helps to combat pellagra (Prasanna et al. 2001). Other nutritional benefits of QPM include higher niacin availability due to higher tryptophan and lower leucine content, higher calcium and carbohydrate and carotene utilisation.

12.2.1 Nutrient Composition of Maize/QPM

A number of QPM varieties are being released with wide variability in nutrient composition. Two varieties of QPM (HQPM-1 and Shaktiman-1) and two varieties of normal maize (HM-4 and HM-5) have been analysed at CCSHAU Hisar for their nutrient profile, which is presented in Table 12.1. Protein, fat and ash content ranged from 10 to 11 %, 4.25 to 4.53 %, and 1.34 to 1.58 %, respectively.

Fig. 12.1 Value-added products from blends of maize and legume

12.2.2 Value-Added Products of Quality Protein Maize

In India, maize is generally consumed in the form of chapati, popcorn, roasted fresh cob, etc. A wide variety of products (Fig. 12.1) have been developed in the Department of Food and Nutrition, CCSHAU, Hisar, RAU, Pusa (Bihar) and UAS, Mandya (Karnataka) which can meet the nutritional need of the vulnerable section. To further improve the nutritive value of the QPM, legumes can be supplemented to prepare blends to improve the protein quality of the products. Many products have been developed by using flour blends prepared with addition of soya bean and green gram:

Traditional products	Ladoo, halwa, kheer, chapati, sev, mathi, pakora and cheela
Baked products	Bread, nan khatai and cake
Extruded products	Vermicelli and pasta
Convenience foods	Instant idli mix, instant dhokla mix, and porridge mix; sprouted products — sprouted chat, QPM vada, QPM sevian and QPM flour
Infant food	Infant food-I, infant food-II, infant food (flavoured), infant food (enriched with vitamin A) and infant food (flavoured and enriched with vitamin A)
Health food	QPM mix-I, QPM mix-II, QPM ladoo, honey maize chocolate, maize coconut chocolate, maize coconut toffee, maize groundnut toffee, choco maize bar and honey maize water
Snacks and savoury item	QPM biscuit salted, QPM biscuit sweet, choco maize biscuit, honey maize chikki, maize matthi, namak para, sev, shakarpara, QPM burfi, QPM halwa, suji upma, suji kheer, sevian (sweet), sevian (upma), QPM chatni powder-I, QPM chatni powder-II and QPM chatni powder-III
Specialty foods	High-quality protein mix, low-quality protein mix, quality protein mix for elderly, QPM honey liquid, and honey maize water (Singh 2006)

Quality Protein Maize was blended with soya bean/green gram in the ratio 70:30 and the product developed includes the following:

Traditional products	Cake, biscuit
Baked products	Halwa, upma, dalia, cheela, namak para, sattu, khichri, burfi
Dehydrated products	Vadi and fryums

All the products have been found acceptable to people in the acceptability trials. Value-added products were nutritious, rich in protein, energy, fat, etc. Products made with addition of legumes have better protein in terms of both quantity and quality. The products can be stored well up to period depending on nature of the product (Kawatra and Sehgal 2007).

12.3 Baby Corn and Its Value-Added Products

Baby corn, as the name suggests, is the young corn cob at its earliest stage of development. Baby corn is the ear, harvested young, when the silks have either not emerged or just emerged and no fertilization has taken place. Baby corn is a very delicious and nutritious vegetable and has been considered to be a high-value agriculture produce in national and international markets. The story of its production technique is approximately three decades old when in 1972, the government of Thailand's agricultural research policy focused on baby corn research and development in order to improve the deteriorating economy of the country. As a result, in a record time, this crop became popular in the whole of Thailand and the farmers collected valuable foreign exchange through its production. In India, baby corn industry is still at the juvenile stage. At present, baby corn production is confined to *peri*-urban areas as its consumption is mostly confined to big hotels and restaurants. The nutritive value of baby corn is comparable to several high-priced vegetables like cauliflower, cabbage, French bean, spinach, lady finger, brinjal, tomato and radish. Besides being nutritious, it has several other advantages too. It is free from the harmful effects of pesticides as baby corn is covered tightly with husk and due to early harvesting; it is devoid of harmful pests and diseases. In addition, baby corn has a great potential for providing higher economic benefits to farmers as it is possible to have three or more crops from the same piece of land as this crop matures in less than 60 days during *kharif* and farmers also get good price for it. Baby corn is highly nutritive as it contains sufficient quantities of carbohydrates, proteins, minerals, and vitamins, particularly beta carotene (Table 12.2).

Table 12.2 Nutritional composition of baby corn (fresh matter basis)

Parameters	Content (g/100 g)
Proximate composition	
Moisture	90.03 ± 0.16
Crude protein	17.96 ± 0.40
Crude fat	2.13 ± 0.13
Total ash	5.30 ± 0.08
Crude fibre	5.89 ± 0.34
Available carbohydrates	
Total soluble sugars	23.43 ± 0.23
Reducing sugars	1.96 ± 0.03
Non-reducing sugars	21.47 ± 0.13
Starch	15.60 ± 0.02
β-carotene (mg/100 g)	670
Ascorbic acid (mg/100 g)	5.43
Phosphorous (mg/100 g)	898.62

Source: Kawatra and Sehgal (2007)

12.3.1 Processing of Baby Corn

Baby corn can be processed to improve its shelf life. Main processing methods which can be used to improve shelf life are as follows.

12.3.1.1 Canning

Canning is a commonly used method for processing baby corn. Baby corn can be canned in brine, stored for months together, and transported to far-off places. Farmers can be trained to establish the canning plants or made aware regarding various processing units available in the nearby areas, so that they can get good price for their crops. Canning is a simple process following a sequence of steps:

Peeled baby corn → cleaning → boiling
→ soaking → grading
→ canning → brining
→ exhausting → lid covering
→ cooling → quality inspection

12.3.1.2 Dehydration

Dehydration can be used to increase the shelf life of baby corn for a long period. Baby corn can be cut into 0.5 cm round pieces and dried (in air oven, vacuum oven, or can be solar dried). Dried baby corn packed in polythene pack/vacuum pack/tetrapack can be stored well for a longer period. Shelf life of dry baby corn stored for 3 months has been studied on trial basis. Non-significant changes in moisture, crude protein,

Fig. 12.2 Baby corn products

crude fat, total ash, crude fibre, minerals, protein content and starch digestibility have been observed after 3 months. Dehydration results in significant losses of ascorbic acid, which further reduces during storage of dehydrated baby corn. Similarly, β-carotene content is also lost during dehydration and storage of baby corn. Dehydrated baby corn can be rehydrated by soaking in water and can be used in preparation of food products. Products developed using dried baby corn have been found acceptable organoleptically, like those prepared from fresh baby corn.

12.3.1.3 Freezing

Baby corn can be frozen and stored for long period like other frozen vegetables, maintaining cold chain. Storage of frozen baby corn for 3 months produced non-significant changes in proximate principles. Storage of frozen baby corn in cold storage produced significant reduction of 6.95 %, 9.02 % and 11.06 % in ascorbic acid content on 30th, 60th and 90th day of storage, respectively. β-carotene content has been found to be reduced by 10–11 % on 3 months of frozen storage. Frozen baby corn can be used effectively for preparation of food products. Preparations like soups and vegetables prepared from frozen baby corn are as acceptable as from fresh baby corn. Frozen baby corn can be directly used in various preparations.

12.3.2 Value-Added Products of Baby Corn

Baby corn can be eaten raw. It can be included in diets in a number of ways as salads, Chinese and Italian preparations. A wide range of food products have been developed at CCS HAU, Hisar, which are not only acceptable but can be easily prepared at home (Fig. 12.2):

Traditional products	Pakoda, cutlet, chat, salad, dry vegetable, kofta, mixed vegetable and raita
Sweet products	Halwa, kheer and burfi
Preserved products	Jam, chutney, pickle, candy and murraba
Chinese products	Soup, Manchurian, baby corn chilli, chow mein, and sweet and sour vegetable

12.4 Sweet Corn and Its Value-Added Products

Sweet corn (*Zea mays L. saccharata*) is one of the most popular vegetables in countries like the USA and Canada. It is becoming increasingly popular in India and other Asian countries. It is consumed in immature stage of the cob. Sweet corn varies from normal corn essentially due to gene(s) that affects starch synthesis in the seed endosperm wherein one or more simple recessive

alleles alter the carbohydrate content of the endosperm and elevate the level of water soluble polysaccharides (sugars) and decrease starch. Thus, the kernels of sweet corn taste much sweeter than normal corn, especially at 18–21 days after pollination. The total sugar content in sweet corn ranges from 25 % to 30 %. Sweet corn matures early and can be harvested in 75–80 days after planting. Thus, it can fit easily in multiple or intercropping systems. Due to the absence of starch in the kernels (seeds), the dried seed is shriveled in appearance.

Modern sweet corn cultivars arose in the nineteenth century when a single gene (*su)* mutated in field corn. Plants descending from this mutant had kernels with a sugary coating rather than a pericarp (seed coat) than normal corn, making it tender. In earlier history of sweet corn, lines with only the sugary *(su1)* allele on chromosome 4 used to be referred to as sweet corn. Currently, at least eight genes that affect carbohydrate synthesis in the endosperm are being used either singly or in combination to breed for sweet corn varieties. Depending on the gene combination used, endosperm types differ in eating quality, shelf life of both fresh and processed sweet corns, and their field emergence. Thus, they often require specialised seed production, planting techniques, and either distance or time isolation to prevent cross-pollination. These types are referred to as super sweets, although technically this term only describes cultivars with the *shrunken 2 (sh2)* characteristic.

12.4.1 Value-Added Products of Sweet Corn

A number of sweet corn products have been developed for commercial use. Some of these include:

- Thai basil and sweet corn
- Salted green beans with shallots and sweet corn
- Organic speed chef—pizza with garlicky greens and sweet corn
- Sweet corn cake
- High summer scallops with sweet corn and couscous
- Sweet corn and tomato salad

12.4.2 Future Strategies for Sweet Corn in India

In spite of availability of promising varieties in India and potential of the technology, sweet corn has yet to assume popularity in the country. A major portion of the sweet corn is being utilised in hotels and big restaurants. The main reason behind this is non-existence of appropriate storage and marketing facilities in India. Thus, there is an urgent need to develop entrepreneurship to harness the advantages associated with sweet corn. Since sweet corn needs to be utilised immediately after harvest, its cultivation should be encouraged in *peri*-urban regions of the country. Market network must be developed to ensure household availability of sweet corn. This can only be attained with proper market leadership and policy support, which is presently lacking in India (Rakshit et al. 2003).

12.5 Popcorn and Its Value-Added Products

Popcorn (*Zea mays L. indurata*) is a popular snack food throughout the world. Kernels of popcorn range in colour from off-white gold to red, black and many colours in between. When kernels of popcorn are heated, they explode and produce large puffed flakes (popping) (Fig. 12.3). Once popped, popcorn has two basic shapes: snow flake or butterfly, which pops big and shapes like a cumulus cloud, and mushroom,

Fig. 12.3 Popcorn

which pops into a round ball. An added feature of popcorn is its light and crunchy texture.

Popcorn is used primarily for human consumption as fresh or as the basis of its confections. Besides their use as a popular snack, ground popcorn as flour or grits can be used in the preparation of many traditional dishes. Isolated planting is not necessary in case of popcorn, since there are no major xenia effects on popping expansion and much popcorn are cross-sterile with field corn. Although the conditions for growing popcorn are the same as for the dent corn, special harvesting, dry, and storage practices are necessary to maintain popping quality.

Popcorn plant type has some distinctive characters compared to normal corn. The plant type is lanky. The tassel is highly branched and the branches are droopy. The ear placement is higher up compared to normal corn. Often the prolificacy is more in fertile soils with good management. It is very common to find two ears per plant. In many popcorn types, there is tendency of tillering, and the brace roots are also fewer compared to normal corn. In popcorn, several grain colours are grown, viz., white, yellow, and red. However, yellow types are more common. There are two main types of popcorn: rice type and pearl type. The rice-type popcorn kernels are common in white-grain types. They are typically beaked, that is, long and pointed at the tips. Pearl-type popcorn is more common than the rice type. It has smooth and round kernels and is common in yellow-grain type. Based on the type of flakes produced on popping, popcorn is further classified as butterfly type (flat flakes) and mushroom types (compact globose flakes).

12.5.1 How Does Popcorn Pop?

The nutritional composition of popcorn kernel consists of carbohydrate (principally starch), protein, fat, and water. Water is stored in a small circle of soft starch in each kernel. As kernel is heated, the water heats, builds up pressure, and takes up any available room. The harder surface surrounding the starch resists the vapour pressure for as long as it can. When the outer surface gives way, the water vapour further expands, causing popcorn to explode. The soft starch pops out, the kernel turns inside out, steam inside the kernel is released, and the corn pops.

12.5.2 Nutritional Value of Popcorn

Popcorn is a good source of carbohydrate, energy and fibre. For individuals of normal weight, carbohydrate is the best source of body fuel. Popcorn is a rich source of fibre, which is an important component of human diet. There are two types of fibre: (i) soluble fibre which plays a role in regulating hunger, cholesterol and blood sugar; and (ii) insoluble fibre, which is important in gastrointestinal functioning. Popcorn has no artificial colour or flavour additives and is surprisingly low in calories. One cup of popcorn has 31 cal if eaten plain or seasoned with herbs; 133 cal if drizzled with a tablespoon of butter, margarine, or oil; 34 cal if sprayed with butter-flavoured oil; and 35 cal if sprinkled that butter substitute. It is a wholesome, fun food that aids digestion by providing necessary roughage. Health and medical associations regard popcorn as an excellent mealtime complement: sugar-free, fat-free and low in calories. The National Cancer Institute (NCI), USA, suggests that increased fibre intake is associated with reduced incidence of cancer. The American Dental Association includes popcorn in its list of recommended sugar-free snacks. The American Diabetes Association and the American Dietetic Association permit popcorn as a bread exchange on weight-control diets; the Feingold Diet for hyperactive children permits popcorn because it contains no artificial additives. Nutrition experts agree that popcorn is a tasty, economical and healthy food.

Popcorn is a whole grain and its nutritive value is retained inside the hull until it is popped. The US Department of Agriculture has estimated the nutritive values in popped popcorn (Table 12.3). The figures may vary because of the actual weight or size of the kernels. Popcorn with high-quality carbohydrate and high fibre content coupled with low calories makes it

Table 12.3 Nutritive value of popcorn

Weight	0.5 to 1 ounce
Calories	50 to 110 cal—refers to unbuttered popcorn
Carbohydrates	10 to 22 g
Protein	2 to 4 g
Fat	1 to 2 g
Calcium	2 mg
Iron	0.4 to 0.6 mg
Niacin	0.2 to 0.6 mg

Source: Rakshit et al. (2003)

longer to chew. Compared ounce for ounce with beef, popcorn provides 67 % as much protein, 110 % as much iron and an equal amount of calcium. A pint of popcorn contains three times as much phosphorus as a pint of milk. A cup of unbuttered popcorn contains fewer calories than half a medium grapefruit.

Popcorn is a very good dieting aid. Eaten just before a meal, it will take the edge off an appetite. The cellulose is excellent dietary roughage and compares to bran flakes. Digestible carbohydrates in the popcorn provide energy. When popcorn is being used as part of a diet programme, the butter may be skipped and just light salt with the popped kernels may provide a good taste.

12.5.3 Value-Added Products of Popcorn

A number of value-added products of popcorn have been developed. The following are some of the products developed for commercial use:

- Apple popcorn brittle
- Ballpark popcorn crunch
- Beach party popcorn
- Boston tea party popcorn
- Caramel corn crunch
- Caramel nut popcorn crunch
- Cherry almond popcorn clusters
- Chilli corn
- Red cinnamon popcorn
- Swiss onion popcorn
- Piña colada popcorn
- Patchwork popcorn party mix
- Kettle corn

12.5.4 Future Strategies for Popcorn in India

To date, no hybrid of popcorn is available in India. Thus, the produce has less uniformity, which in turn affects its market acceptance, particularly in international market. Therefore, there is a need to develop popcorn hybrids in India. A second problem associated with Indian popcorn cultivars is less popping ratio. Best popcorn variety has popping ratio of 20:1 as against 40:1 in the USA. Thus, there is scope for breeding popcorn cultivars with enhanced popping ratio and with soft texture and bigger volume. There is need to develop kernels with thick pericarp, hard coating with soft starch cavity inside. For this purpose, efforts may be made towards screening of germplasm and their utilisation in breeding.

Popping ratio of popcorn is very much dependent on storage as well as popping temperature and moisture. Therefore, appropriate drying and storage of popcorn grains are essential. In the USA, and other developed countries, food industries pack popcorn with appropriate oil, butter and salts in popping bags. These bags are ready to pop in the microwave. Such packings are very handy for end users and fetch very good market price. Such type of entrepreneurship is much needed in India. This will be possible only with support from the food industry and policy makers (Rakshit et al. 2003).

12.6 Industrial Products of Corn

Corn is used in more than 3,000 products which include adhesives, antibiotics, automobiles, baby food, breakfast cereals, canned vegetables, cheese spreads, chocolate products, printings, cosmetics, crayon and chalk, dessert powders, dyes, edible oil, finished leather, insecticides, ketchup, livestock feed, malted products, paper manufacturing, pharmaceuticals, drugs and carpets, shoe polish, soft drinks, textiles, wheat bread, yogurts, etc. (Fig. 12.4). Corn is subjected to wet and dry milling to separate the grain into its components, which are used as food ingredients.

Fig. 12.4 Various industrial products developed from corn

12.6.1 Dry Milling

Two different approaches have been used for dry milling of corn: degerming and non-degerming. Older systems did not remove the germ before grinding the corn into grits, meal and flour, and the resulting products had short shelf lives, being prone to developing rancid flavours and odours. Today, no more than 20 % of corn is milled by this traditional or unbolted dry milling process. Early systems used stone mills for particle reduction and sieves to separate products on the basis of particle size.

12.6.2 Wet Milling

The dry milling industry could not provide starch of sufficient purity to process into good syrups. The wet milling industry has grown rapidly in recent years due to demand for high-fructose corn syrup (to replace largely imported cane sugar used in soft drinks) and fuel ethanol (to increase octane of unleaded fuels, extend petroleum reserves and improve air quality).

12.7 Use of Corn in Feed and Food

- Corn as livestock feed: Corn is the most widely used feed stuff for rearing poultry, piggery and livestock because of its abundant, predictable and widespread supply; low cost; high digestibility; and feed conversion with minimal processing. Corn rarely contains any toxins, such as the microbial toxins aflatoxin and fumonisin.
- Corn as food: Corn may be converted with little processing into common foods in the American diet. Alternatively, dry and wet milling have evolved to take corn apart into its various components and convert them into a wide range of food ingredients used in today's highly engineered and processed foods. These ingredients are becoming more important as consumers demand processed and convenient foods with long shelf life. In Latin America, Africa, and Asia, it is a staple food comprising a high proportion of diets.

12.8 Use of Corn in Developing Intermediary Raw Products

(A) Starch: Two-thirds of the starch produced by wet mills is used for industrial products in the USA, such as adhesives in paper and building materials, paper coatings and sizing, textile sizing, charcoal binders, and recently in degradable plastics. Both native and modified starch are used depending upon the functional properties required. The starch include the following:
   (a) Maize starch: Maize starch exhibits all the properties of native starch. It improves efficiency in weaving, there is no danger of thinning down, and no foaming during cooking.
   (b) Dextrin: It is of two types—white and yellow. White dextrin is an intermediate product between starch and sugars derived from starch. Yellow dextrin is soluble gummy carbohydrate formed by partial hydrolysis of starch. Yellow

dextrin or canary dextrins, as they are frequently called, are made by roasting starch with less acid and more heat.

(c) Synthosize: Synthosize is a versatile product for sizing of blended synthetic yarn in modern textile industry. It not only helps in reducing droppings and breakages but also increases loom efficiency to a great extent, which is the demand of every modern textile unit. The use of synthosize completely eliminates the addition of PVA in size mix for sizing of blended yarn.

(d) Papyrox: Papyrox is extremely white, oxidized starch with high brightness. It is a highly versatile product that finds wide applications in paper, textile, adhesive, building material and other such industries.

(e) Thintrite-90: Thintrite-90 is a starch material in the form of superficially unchanged granules obtained by action of chemicals on starch.

(B) Specialty chemicals by chemical processes: In addition to modifying the starch, syrups can be converted by chemical processes into many specialty chemicals including organic acids, polyols, glucosides and glucose esters. For many years, corn cobs have been converted into furfural derivatives.

(C) Ethanol: Corn starch is an economical fermentation feedstock to produce organic chemicals. The most notable example is fuel ethanol. Although beverage alcohol has been produced by corn fermentation for many years, expansion has been motivated to extend petroleum reserves and motor fuels, especially during the early 1980s when the Organization of Petroleum Exporting Countries (OPEC) drastically reduced petroleum supplies and increased prices.

(D) Specialty chemicals by fermentation: Corn-derived syrups are easily fermented into a wide array of specialty chemicals including organic acids (acetic, citric, gluconic, malic, succinic, fumaric, propionic, and butyric), alcohols (ethanol, isopropanol, and butanol), ketones (acetone and glucosone), amino acids (methionine, lysine, and tryptophan), nucleotides (guanyl, inosinic, and xanthyl acids), biopolymers (xanthan, pullulan, alginate, and polyhydroxybutyrate), single-cell proteins and oils, enzymes, vitamins, antibiotics, and hormones.

(E) Refined corn oil: Corn oil is one of the most popular international cooking mediums, particularly popular in the USA and Europe due to its unique properties. Corn oil is a co-product of wet milling of corn. The crude corn oil is refined to remove free fatty acids and phospholipids. The product is decolourised and deodourised under sterile conditions to yield sparkling clear oil.

(F) Sorbitol: Sorbitol is a low-calorie refined sugar which is synthesized from glucose. Chemically, it is a hexahydric alcohol and is also known as D-glucitol. It is widely found in various types of fruits and berries such as strawberries, cherries, apples and pears.

(G) Glucopep (liquid glucose): Liquid glucose sold under the brand name of Glucopep is a thick syrup manufactured by hydrolysing starch with acids/enzymes followed by multiple stages of refining.

12.8.1 Use of Intermediary Raw Products of Maize in Developing Finished Product of Other Industries

- Beer: Beer manufacturing is a process of treating barley to convert and extract the barley starch to fermentable sugars using the amylolytic enzymes present in malt followed by yeast fermentation. However, demand for lighter, less filling beer, especially in the USA, has permitted use of more refined carbohydrate sources of two types:
  (a) Dry adjuncts, primarily dry milled corn grits, broken rice, refined corn starch, and more recently dextrose

(b) Liquid adjuncts, namely, corn syrups

- Cake mixes: Cake mixes use a pregelatinised corn starch that forms a paste in cold or warm water. In baked goods that use yeast for rising, dextrose is used as a yeast nutrient.
- Candies: Corn syrup is used in hard candies to provide a body, giving them chewiness and desirable mouthfeel without excessive sweetness. Coated candies use a pyrodextrin corn starch for the coating.
- Carbonated beverages such as Coke: High-fructose corn syrup (HFCS) blended with sucrose in a 50/50 blend is sweeter than the same concentration of sucrose. The use of HFCS in carbonated beverages is common throughout Canada and the USA.
- Cookies: Corn starch, corn flour, or dextrose are also used in cookies.
- Corn flakes: The flaking grits are cooked to a rubbery consistency with syrup, malt, salt and added flavours. After tempering, the cooked grits are flattened between large steel rolls followed by toasting in travelling ovens to a golden brown colour.
- Corn meal: Corn meal is a popular dry corn product because of its long shelf life. It is used to produce an assortment of chemically leavened bread and fried products like corn bread and muffins.
- Cosmetics: Corn cob when finely ground is relatively dust-free and very absorbent. This absorbency makes corn cobs useful carriers for pesticides, fertilizers, vitamins, hand soaps, cosmetics, and animal litters.
- Granola dips/granola bars: Some types of granola dips use dextrose as a sweetener.
- Gypsum wallboard: Starch containing corn flour is gelatinised during the manufacturing process. It functions by controlling the rate of water loss during drying of the board. Soluble carbohydrates migrate to the surface and control the rate of crystallisation of the gypsum, providing a strong bond between the gypsum and the liner.
- Instant coffee and tea: Maltodextrins are derived from the wet milling process. They are a dextrose-equivalent product having complete solubility but little or no sweetness. Maltodextrins are sprayed on instant tea and coffee to keep the granules free flowing. This solution is also used in instant soup mixes or other packages where the contents must be kept free flowing.
- Mars bar and Twix bar: Many candy bars contain corn syrup.
- Paint and varnish: Tetrahydrofurfuryl alcohol is a resin developed from processing corn cobs. These resins are useful in the paint and varnish industry as solvents for dyes, resins, and lacquers.
- Paper products: Paper products use raw starch in the manufacturing process. The properties of high paste viscosity and strong gels are useful in specially coated papers. Pyrodextrins are also used for paper manufacturing for the adhesive property on remoistenable gums for postage stamps and packaging tape.
- Pharmaceutical such as aspirin: An oxidised starch paste, which dries to a clear, adherent, continuous film, is spread in a thin layer over the aspirin. Some intravenous (IVs) fluids consist of dextrose and water solutions.
- Antibiotics: Preferred carbohydrate sources are corn syrup, dextrose, corn starch, lactose and sucrose. Corn steep liquor was early found to provide a ready source of soluble nitrogenous nutrients along with unknown growth factors that stimulate antibiotic production. Over 85 different types of antibiotics are produced using corn.
- Snack foods—corn chips and Doritos: These snack foods are generally made from whole corn (corn meal). The high starch content of corn meal and flour is important in giving a high puff in preparation of extruded (pressed) snack products in which a delicate corn flavour is desired.
- Spark plugs: Starch is used in the production of the porcelain part of spark plugs.
- Rubber: In the production of tyres, corn starch is sprinkled on the moulds before toothpaste as a low-calorie, water-soluble bulking agent.

- Whiskey: The major carbohydrate source in the production of whiskey is corn. A typical Canadian whiskey is made from a mixture of about 90 % corn, 5 % rye and 5 % barley malt.
- Yogurt: Some of the different brands of yogurt use corn syrup as a sweetener (Venkatesh et al. 2003)

12.9 Conclusion

The value-added products prepared from maize are not only nutritious but also easy to prepare. Keeping in view the nutritional profile of maize, the development of these products will not only diversify the uses of maize but also will be beneficial for human health especially in combating malnutrition. There is a need to develop and popularize value-added products based on quality protein maize and baby corn among housewives so that they include these preparations in their daily diet. The development of industrial products and dehydration, freezing, and canning of baby corn can be taken up as entrepreneurial activity by the entrepreneurs. The increased utilisation of maize will encourage farmers in improving the production of maize which may indirectly help in improving economic standards of farmers. Efforts towards commercialisation of maize-based value-added products through self-help groups, food industries, etc. are also required.

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Fodder Quality of Maize: Its Preservation

13

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Abstract

Green fodder is an important component of animal husbandry. The growth of dairy sector primarily depends upon the availability of nutritious fodder. Maize is one of the most nutritious non-legume green fodders. The high acceptability of maize as fodder can be judged from the fact that it is free from any anti-nutritional components. Maize is quick growing, yields high biomass, and is highly palatable. It contains sufficient quantities of protein and minerals and possesses high digestibility as compared to other non-legume fodders. It contains high concentrations of soluble sugars in the green stage, which makes it most fit for preservation as silage. The abundance of green fodder due to increasing cultivation of specialty corn could greatly help in boosting the prospects of dairy sector in the peri-urban regions of the country.

13.1 Introduction

The economics of milk production is largely dependent upon the quality of nutritious fodder fed to milch animals. Feeding of green forages compared to concentrates lowers the cost of milk production substantially. For optimum milk production, around 40 kg of green fodder is required to feed per animal per day. However, a huge deficit exits between the demand and supply of green fodder in India. At present, there exists around 63 % deficiency of green fodder and 23.5 % deficiency of dry fodder in India (Singh 2009). Therefore, to meet the needs of the ever-increasing livestock population, the production as well as productivity of fodder needs to be increased. The increasing cultivation of cereal and cash crops, however, contributed towards a decline in the area under fodder cultivation. There is a tremendous pressure of livestock on the available total feed and fodder, as the land available for fodder production has been decreasing. Moreover, green forages are rich and the cheapest source of carbohydrates,

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protein, vitamins and minerals for dairy animals. Therefore, by providing sufficient quantities of fodder, instead of costly concentrates, to the milch animals, the cost of milk production can be considerably reduced. Maize is one of the most important non-legume green fodders. It is a tall, leafy plant having biomass yields to the tune of 400–500 q/ha. It is a highly nutritious, palatable fodder, free from any unwanted anti-quality components. Green maize is rich in protein and possesses sufficient quantities of soluble sugars required for proper ensiling. In this chapter, the fodder quality of maize is discussed in comparison to other non-legume forages along with the methodology for its preservation as silage.

13.2 Green Fodders

Green fodder production is the most challenging activity in the dairy enterprise. It not only reduces the feeding cost but also keeps the animals healthy, reduces micronutrient deficiencies and increases milk production. Forages usually contain relatively large concentrations of cellulose, hemicelluloses, and lignin, as well as variable amounts of non-fibrous carbohydrates and proteins. Dairy animals obtain nutrients, viz., energy, protein, fibre, minerals, vitamins and water from the forages for the maintenance of their body and optimum performance. There are many legume and non-legume fodder varieties available for cultivation. Ideal characteristics of good fodder varieties are short duration, high biomass potential, nutritious and tasty fodder, suitability for preservation and negligible concentrations of anti-nutritional components. Green fodders can be broadly divided into two categories, viz., legumes and non-legumes. Some of the common green forages cultivated in India are listed in Table 13.1.

Table 13.1 Major legume and non-legume forages of India

S. No.	English name	Botanical name
Legume fodders		
1.	Egyptian clover	*Trifolium alexandrinum*
2.	Persian clover	*Trifolium resupinatum*
3.	Indian clover	*Melilotus* spp.
4.	Lucerne/alfalfa	*Medicago sativa*
5.	Cowpea	*Vigna unguiculata*
6.	Cluster bean	*Cyamopsis tetragonoloba*
Non-legume fodders		
1.	Maize	*Zea mays*
2.	Sorghum	*Sorghum bicolor*
3.	Pearl millet	*Pennisetum glaucum*
4.	Teosinte	*Zea mexicana*
5.	Guinea grass	*Panicum maximum*
6.	Oat	*Avena sativa*
7.	Ryegrass	*Lolium* spp.

13.3 Maize as Fodder

Maize (*Zea mays*) is one of the most important crops having wider adaptability to varied agroclimatic conditions. In India, it is the third most important food crop after rice and wheat. It is the crop with the highest per day productivity. The production as well as productivity of maize is growing in India. In the XI five-year plan, maize achieved the highest growth rate (6.7 %) among cereals as against the required growth rate of 4.7 % set by the planning commission of India. It is an excellent crop in terms of biomass production also. Since the production as well as productivity of maize is increasing, the availability of biomass from maize is also increasing by the same magnitude. Maize straw has been used as animal fodder since long. The fodder quality of green maize is considered best among non-legume forage crops. Maize is considered ideal forage because it grows quickly, produces high yields, is palatable, is rich in nutrients, and helps to increase body weight and milk quality in cattle (Sattar et al. 1994). As fodder for livestock, maize is excellent, highly nutritive and sustainable (Hukkeri et al. 1977, Iqbal et al. 2006). It is commonly grown as a *kharif* fodder in the north-western regions of India. Its quality is much better than sorghum and pearl millet, since both sorghum as well as pearl millet possess anti-quality components such as hydrocyanic acid and oxalate, respectively.

Table 13.2 Comparative nutritional quality of non-legume fodders

Fodder crop	Physiological stage	Harvesting stage Days after sowing (DAS)	Crude Protein (CP) (%)	In-vitro dry matter digestibility (IVDMD) (%)
Maize	Silk to milk stage	55–65	11–8	68–52
Bajra	Boot stage	45–55	10–7	62–55
Sorghum	Initiation of flowering	70–80	8–7	60–57
Teosinte	Pre-flowering	80–85	9–7	62–58
Sudax	Subsequent cutting after 30 days	65–70	11–7	60–55
Napier bajra hybrid	One meter height and Subsequent cutting after 30 days	55–60	11–7	60–55
Guinea grass	One meter height and subsequent cutting after 25–30 days	55–60	10–8	60–57

Source: Gupta et al. (2004)

13.4 Forage Quality

Forage quality is defined in various ways but is often poorly understood. Though important, forage quality often receives far less consideration than it deserves. Forage quality can be defined as the extent to which forage has the potential to produce a desired animal response. Adequate animal nutrition is essential for high rates of gain, ample milk production, efficient reproduction and adequate profits. However, forage quality varies greatly among and within forage crops. Analysing forages for nutrient content can be useful in determining whether quality is adequate and to guide proper ration supplementation. In recent years, advances in plant and animal breeding, introduction of new products and development of new management approaches have made it possible to increase animal performance. However, for this to be realised, there must be additional focus on forage quality. Many factors influence forage quality. Some of these include palatability (whether the animals eat the forage), intake (how much the animal will eat), digestibility (the extent to which forage is absorbed as it passes through an animal's digestive tract), nutrient content, anti-quality components (tannins, nitrates, alkaloids, cyanoglycosides, oxalates, estrogens and mycotoxins), and lastly animal performance, which is the ultimate test of forage quality.

13.4.1 Fodder Quality of Maize

Desirable forage characteristics include high dry matter yield, high protein concentration, high energy concentration (high digestibility), high intake potential (low fibre content), and optimum dry matter concentration at harvest for acceptable forage fermentation (Carter et al. 1991). Maize is an ideal forage crop as it is quick growing, high yielding, palatable and nutritious. Among the cultivated non-legume fodders, maize is the most important crop that can be grown round the year under irrigated conditions. It is free from any anti-nutritional components and is considered a valuable fodder crop. It contains high concentrations of protein and minerals and possesses high digestibility. Two maize varieties, namely, J-1006 and African tall, are developed and released for commercial cultivation of fodder in India. The nutritional quality of maize as compared with other non-legume fodders is expressed in Table 13.2.

13.5 Specialty Maize as Fodder

Apart from grain, maize is also grown for some special uses such as baby corn, sweet corn and popcorn. Maize cultivated for this purpose is called specialty maize. Specialty corn (baby corn and sweet corn) cultivation is gradually

Table 13.3 Green fodder yield, dry matter, crude protein, and crude fibre content of baby corn genotypes

Genotype	Use	GFY (t/ha)	DM (%) I harvest	DM (%) II harvest	CP (%) I harvest	CP (%) II harvest	CF (%) I harvest	CF (%) II harvest
JH-3459	Baby corn	38.12	21.19	21.13	8.72	7.43	24.77	28.67
Parkash		30.14	20.55	20.91	7.00	6.70	26.80	28.77
PMH-2		40.14	21.22	22.47	8.46	8.31	26.33	23.13
J-1006	Fodder	46.67	24.28	24.69	7.44	5.83	26.13	23.87
African tall		30.99	22.24	22.61	7.14	5.54	30.20	28.67

Source: Chaudhary et al. (2012)

Table 13.4 Neutral detergent fibre, acid detergent fibre, total ash, and in vitro dry matter digestibility of baby corn genotypes

Genotype	Use	NDF (%) I harvest	NDF (%) II harvest	ADF (%) I harvest	ADF (%) II harvest	Ash (%) I harvest	Ash (%) II harvest	IVDMD (%) I harvest	IVDMD (%) II harvest
JH-3459	Baby corn	64.13	66.63	40.80	43.13	8.00	6.23	58.23	55.90
Parkash		64.97	64.67	42.87	44.73	7.33	6.70	63.73	61.80
PMH-2		65.93	64.67	46.77	45.83	6.60	6.10	64.33	58.60
J-1006	Fodder	69.87	72.80	40.37	45.47	7.27	7.43	58.07	52.86
African tall		67.57	66.10	38.27	38.73	5.97	6.60	65.00	57.63

Source: Chaudhary et al. (2012)

increasing, particularly in the peri-urban regions of the country. Baby corn is a very delicious and nutritious vegetable and has been considered to be a high-value agriculture produce in national and international markets. The export potential of baby corn provides further boost to its cultivation. On the other hand, sweet corn is being utilised in hotels and big restaurants. Many value-added products made from baby corn and sweet corn are being used for human consumption. A significant quantum of green biomass is available from specialty corn cultivation, which can efficiently be used as animal fodder. Yield potential and nutritional quality of some promising genotypes such as JH-3459, Parkash, and PMH-2 grown for baby corn along with two most common fodder maize varieties, viz., African Tall and J-1006, were evaluated. Green samples were taken at I as well as II harvest of baby corn. After harvesting the baby corn, the stalk was used for forage quality analysis. The samples were analysed for various parameters of fodder quality such as green fodder yield (GFY); dry matter (DM); crude protein (CP); fibre components, viz., crude fibre (CF), neutral detergent fibre (NDF), and acid detergent fibre (ADF); in vitro dry matter digestibility (IVDMD); and total ash (TA) (Tables 13.3 and 13.4).

The data show that the nutritional quality of baby corn stalks is almost at par with the maize grown for fodder purpose. Although biomass from baby cornstalks was little less compared to fodder maize J-1006 and African tall, there is little difference in terms of crude protein and in vitro dry matter digestibility. The woodiness is also comparable. It means the baby corn is as good as fodder maize.

13.6 Ensiling

Forage conservation is a key element for productive and efficient ruminant livestock farms. It permits a better supply of quality feed when forage production is low or dormant. Forage conservation also provides farmers with a means of preserving forage when production is

faster than its adequate utilisation by grazing animals. This prevents lush growth from becoming too mature. Consequently, forage conservation provides a more uniform level of high-quality forage for ruminants. Ensilage has many advantages over the other methods for preservation of nutrients, particularly from forages. Silage is the material produced by controlled fermentation of nutrients under an anaerobic condition. The fermentation process is governed by micro-organisms present in fresh herbage to maintain anaerobic conditions and discourage clostridial growth with minimum loss of nutrients.

13.6.1 Ensiling of Maize

Maize is an excellent crop for ensiling. It possesses high energy value. For proper fermentation, the crop might possess sufficient quantities of moisture as well as soluble carbohydrates which are converted to lactic acid during the process of fermentation. Maize cultivated for green fodder and baby corn purpose possesses the required moisture and soluble sugars and, therefore, is most suitable for ensiling. Maize silage is becoming more important in dairy rations. Maize is valued because of its high yield and ability to make excellent silage, and it can be harvested in a single operation without significant leaf loss. Cows fed corn silage produced more milk and consumed more silage dry matter in both trials than those fed sorghum silage (Lance et al. 1964). Corn silage is used extensively for lactating dairy cows that require high-energy feed for maximum milk production (Marsalis et al. 2010; Irlbeck et al. 1993).

13.6.2 Methodology of Ensiling

The quality of silage depends on the stage at which the fodder is harvested. Maize is best suited to be ensiled when the grains are in the milking stage. In the tropics this is usually found in 60–70 days after sowing. The dry matter content of whole plant should be around 25–30 %. Maize is ready for ensiling if the dry matter in the grain has reached a value between 56 % and 60 %. Harvesting at this recommended time will ensure optimum compaction properties, reduced tendency to heating up, and mould formation. Silage making is a simple process which can be carried out manually in the farm area by employing a few labourers. The first and foremost thing in silage making is the digging of a pit. A rectangular pit is to be dug near the cattle shed whose size depends upon the number of animals along with the availability of fodder. If the fodder is sufficiently available, then the time duration of feeding could be considered in deciding the size of the pit. Usually one cubic meter pit can accommodate roughly 5–6 quintals of green fodder. Crop should be chaffed to 5–7 cm length before ensiling. For good silage, the chop length should be kept shorter. Chaffed silage is more palatable to livestock and has little chance of secondary fermentation. The next step is filling the pit with chaffed fodder. For this purpose fodder is spread up to a height of 1 ft in the pit followed by compression. This process is repeated till the pit is filled with fodder. The major precaution during this process is to exclude as much air as possible from the chaffed fodder by compressing it properly. This is executed by pressing the material through manual labour or mechanically by using a tractor. Care should be taken that material on the sides and edges are properly compressed. Raise the fodder heap above the ground level up to a height of around one meter. Finally, add some more fodder in the central portion of the heap and then trample it. Packing is important to create anaerobic conditions. It should be thoroughly pressed so that no air pocket is left in the silo; otherwise chances of mould formation will be there which will spoil the silage. After filling, silo should be covered with polythene sheet followed by a layer of soil, etc. Some cracks may develop in the covered soil over time. These are to be plugged immediately (Figs. 13.1, 13.2 and 13.3).

After 45 days of ensilage, the silage will be ready to use and, therefore, can be removed for

Fig. 13.1 Filling of pit

Fig 13.2 Huge stock of silage

feeding. Care should be taken in removing the silage from the pit. It should not be opened from one side only. Cover should be kept firmly in place as long as possible and the minimum face should be exposed at one time. The sugars, proteins, and lactic acid present in the silage are subject to attack by mould growth and oxidation as some air is allowed for fermentation and causes loss of feeding value and intake by the animals.

13.6.3 Silage Quality

Silage quality is determined mainly by the physical state, that is, colour and odour. Good quality silage should be light brown in colour and have the smell of vinegar. Poorly fermented silage will be dark in colour and foul smelling due to the production of butyric acid. Chemically, it should have the following characteristics: (i) pH below 4.2 (ii) ammoniacal nitrogen of

Fig. 13.3 Silage of baby corn

Table 13.5 Common end products of silage fermentation

Item	Positive or negative	Action(s)
pH	+	Low pH inhibits bacterial activity
Lactic acid	+	Inhibits bacterial activity by lowering pH
Acetic acid	–	Associated with undesirable fermentations
	+	Inhibits yeasts responsible for aerobic spoilage
Butyric acid	–	Associated with protein degradation, toxin formation and large losses of DM and energy
Ethanol	–	Indicator of undesirable yeast fermentation and high DM losses
Ammonia	–	High levels indicate excessive protein breakdown
Acid detergent insoluble nitrogen (ADIN)	–	High levels indicate heat-damaged protein and low energy content

total N, less than 10 % of total N; (iii) butyric acid, less than 0.2 %; and (iv) lactic acid 3–12 %. The end products of silage fermentation are often monitored to assess silage quality, and the composition of "normal silages" is presented in Table 13.5.

Thus, it could be concluded that maize is an excellent crop which could effectively be utilised as animal fodder. Specialty corn is going to play an important role in the socio-economic perspective of rural folk as the dual purposes of agriculture as well as livestock are fulfilled by its cultivation. Maize silage could greatly help towards reducing green fodder scarcity and provide a much desired boost to the dairy sector.

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