CHAPTER 6

# Finger Millet: *Eleusine coracana*

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Contents



Abstract

Finger millet (*Eleusine coracana*) is a grass crop grown in Africa, India Nepal, and many countries of Asia. The plant and grain is resistant to drought, pests, and pathogens. It is rich in polyphenols and particularly in calcium. The double headed trypsin, α-amylase inhibitor from this grain has been isolated and characterized extensively. One major use for the grain is the making of fermented beverages after malting. α-Amylase and β-amylase are produced

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during germination. Food made from malted ragi is traditionally used for weaning and has been the source of low viscosity weaning foods that can deliver more energy per feed than those based on gelatinized starch. There is some evidence that foods from finger millet have a low gylcaemic index and are good for diabetic patients. Decortication, puffing, extrusion, and expansion are some of the new uses that the grain has been put to.

Dr. Aykroyd, Director of Nutrition Research at Coonoor, recently delivered an instructive lecture at Bangalore. From the lecture, as published by the press, it appears that a well balanced diet need not cost more than Rs. 4 per month. Thus the lecturer said that: ''The dietary requirements of an adult man per day were 16 ounces of *ragi* 1, two ounces of soya bean2, an ounce of jaggery, four ounces each of spinach and amaranth, an ounce each of potatoes and colacasia, 1.5 ounces of coconut oil and six ounces of buttermilk—all costing about two annas.'' M. K. Gandhi in the *Harijan* (12-10-1935)

## I. INTRODUCTION

Ragi, also known as finger millet or the *Eleusine*, is a grain grown for food in Africa and in India. It is consumed as staple in parts of Africa and India and is also used as a fermented beverage. It is a small grain, even smaller than that of rice or sorghum. In 1981–1985, globally 3,730,000 tonnes of ragi were produced annually out of which 2,613,000 were from India and 122,000 from Nepal. India is the largest producer of this crop (de Wet, 2006). In India, the production of ragi had declined to 2,608,100 and 2,374,600 tonnes in 1998–1999 and 2001–2002, respectively (FAO). It is also cultivated in Burma, the southern parts of Tibet, Nepal, Malaysia, Sumatra, Sri Lanka, Philippines, Indochina, Japan, China, Java, Iran, and Afghanistan.

## II. TAXONOMY

The Chloridoideae are seen as anatomically distinct in the grass family and are delimited by spherical, inflated bicellular microhairs, the Kranz syndrome (the vascular bundle enclosed in a ring of bundle sheath cells which in turn is enclosed by another ring of mesophyll cells), distinctive leaf-blade anatomy and the ''Asterad'' embryo (Bhanawara, 1988) which is generated by the division of the terminal and basal cells of the two-cell proembryo (Raghavan, 1997). The subfamily Chloridoideae comprises approximately 1360 species, in about 150 genera worldwide. Ayyangar

*et al.* (1932) describe the different types of fingers on the finger millet head. Philips (1972) has published a description of eight African species of the genus *Eleusine*. The species *coracana* has been described as non shattering spikelets bearing plump, usually brown grains that are exposed between the lemma and palea. Grains of the *Eleusine* are ornamented and are enclosed in a thin pericarp. Variation in grain shape helps distinguish closely related species. Different head shapes have also been described by Hilu and de Wet (1976). de Wet *et al.* (1984) defined the *Eleusine coracana* subspecies *coracana* as that which ''includes all cultivated finger millets. Plants are annual, tufted, erect, or with geniculately ascending culms that are upto 165 cm high and sometimes root from the lower nodes. Culms are commonly branched from the upper nodes to produce secondary inflorescences. Leaf-blades are linear to linear-lanceloate, up to 70 cm long and 20 mm wide. Inflorescences are digitate, often with one or more racemes some distance below the main cluster of 4–19 branches (Fig. 6.1). Inflorescence branches are slender to robust, up to 24 cm long, reflexed when slender or in curved at the tip when robust, sometimes with secondary branches. Spikelets bear 6–9 flowers and are 6–10 mm long, overlapping and mostly arranged in two rows along one side of the rachis. The grain is white, red, brown, or black; up to 2 mm long, more or less globose, with the surface finely striated. Inflorescence shape is variable. The digitately arranged branches may spread out and became reflexed, or they may be erect and incurved, often forming a fist-like structure.'' The inflorescence structures allowed the grouping of different land races into groups.

Six races based on inflorescence morphology have been delineated: Eragrostideae, Cynodonteae, Sporoboleae, Pappophoreae, Aeluropodeae, and Zoysieae. Roodt-Wilding and Spies (2006) investigated differences in the sequence from the chloroplast trnL (UAA) 5_exon-trnF (GAA) region and the nuclear ribosomal internal transcribed spacer regions of 38 species of African chloridoid grasses. They concluded that a combined analysis using both sequences could resolve the subfamily into three clades with the *Eleusines* being consistently in the cynodonteae clade.

Liu *et al.* (2007) using both floral morphology and sequences of the trnL intron and that of the rps16 intron from nine chloridoid grasses found that they formed three clades. Clade I consists of plants with one fertile floret per spikelet, while clade II (Cynodon, Dactyloctenium, and *Eleusine*) consists of plants with two to many fertile florets per spikelet.

Peterson *et al.* (2010) have used the sequence from six plastid DNA sequences (ndhA intron, ndhF, rps16-trnK, rps16 intron, rps3, and rpl32-trnL) and a single nuclear ITS to distinguish between 246 species of the Chloridoideae. The sequences were grouped into four tribes: Triraphideae, Eragrostideae, Zoysieae, and Cynodonteae. Eleusininae is a subtribe in the Cynodonteae and contains the *Eleusine* genus.

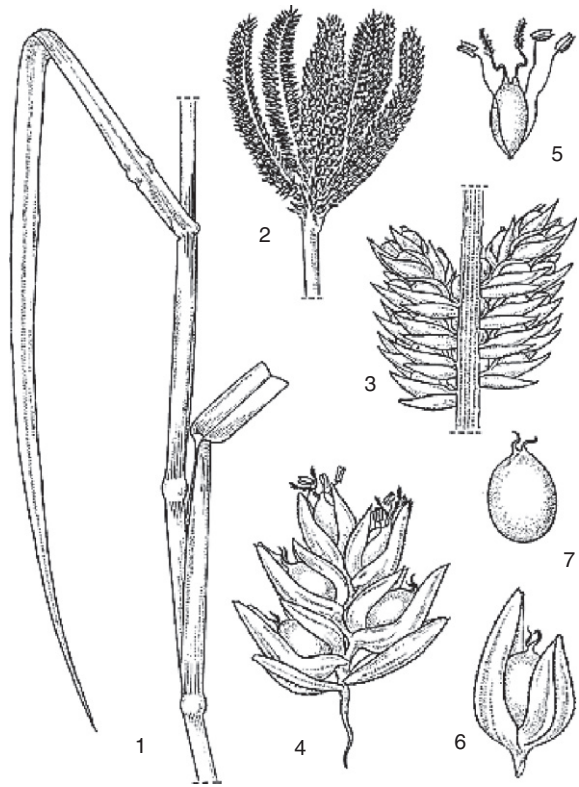


**FIGURE 6.1** Leaf, inflorescence, and grain of *Eleusine coracana* depicted. 1, Stem part with leaves; 2, Inflorescence; 3, Part of inflorescence branch; 4, Spikelet; 5, Floret without lemma and palea; 6, Grain within lemma and palea; 7, Grain (de Wet, 2006; Reproduced with permission from PROSEA).

The genus *Eleusine* belonging to the family Poaceae and subfamily Chloridoideae comprises nine species. *E. indica* is found now in many places of the world and is treated as a weed and has been found in various archeological sites in the United States. Only *E. tristachya* is found in South America, the rest being endogenous to Africa. The domestication of *E. coracana* is discussed in Hilu and De Wet (1976). They reported that the occurrence of *E. africana* and *E. indica* overlaps in Africa. The two ''species'' interbreed and the hybrids are weed-like.

Krishanswami and Rangaswami Ayyangar (1935) counted the haploid chromosome number to be 9 in *E. indica*, 18 in *E. coracana*, 18 in *E. brevifolia*, and 17 in *E. aegyptica*, while Bisht and Mukai (2000) counted 36 chromosomes in *E. coracana*, 18 in *E. indica* and *E. tristachya*, and 16 in *E. multiflora*. All these are annuals. For the perennials, *E. floccifolia* has 18, *E. intermedia* 18, and *E. jaegeri* 20 chromosomes. Laser flow cytometry was

used in the measurement of DNA content in the leaves and roots of various *Eleusine* species. The 2C DNA content of *E. indica*, *E. tristachya*, *E. jaegeri*, *E. multiflora*, and *E. floccifolia* ranged from 1.51 to 2.65 pg, while that of the polyploid species *E. coracana* subsp. *coracana*, *E. coracana* subsp. *africana* ranged from 3.34 to 3.87 pg. A large proportion of nuclei contained DNA four times that of the haploid DNA content in the diploid species, while the DNA content was eight times the haploid DNA content in some cells of *E. coracana* subsp. *coracana* (Mysore and Baird, 1997).

Bishit and Mukai (2000, 2001a,b), in a series of FISH ( fluorescent *in situ* hybridization) studies, reported that there were three diploid species. The two tetraploid species *E. africana* and *E. coracana* are related to one another. They also suggested that *E. multiflora* was a distinct species and that the two diploid species *E. indica* and *E. floccifolia* are probably the donors to the tetraploid species. This conclusion was based on the pattern of hybridization of a 5sRNA probe of the chromosomes of the species that they studied. However, phylogenetic analysis of sequence data does not appear to support this hypothesis; none of the ITS found in *E. coracana* closely related to the ITS of *E. floccifolia* (Neves *et al.* 2005).

Hilu (1988), based on the restriction pattern analysis of chloroplast DNA, concluded that *E. indica* was a progenitor of *E. coracana* and that *E. coracana* subsp. *coracana*, *E. coracana* subsp. *africana*, and *E. indica* share a common pattern, while that of the other diploid species *E. tristachya* was different. Hilu and Johnson (1992) digested DNA isolated from 73 individual plants representing 50 accessions of domesticated and wild finger millet and five other species of *Eleusine* with *Bam*HI, *Hin*dIII, and *Dra*I and probed after transfer to nitrocellulose membrane with probes spanning the interspacer and flanking regions of rice 17S and 25S rRNA. There was very little polymorphism among the *coracana* lines selected. The RFLP pattern of the *indica* lines was similar to that of *coracana*. Restriction patterns of DNA from *E. multiflora, E. jaegeri* and *E. floccifolia* were different from that of *E. coracana. E. tristachya* provided a unique pattern though with many bands in common with that of *E. coracana*. The data appeared to corroborate that *E. indica* was one of the parents of *E. coracana*. Salimanth *et al.* (1995) used RLFP (three restriction enzymes and eight different probes) as well PCR using random primers and those for inter simple sequence repeats in 22 accessions derived from five species. The 17 accessions of *E. coracana* were derived from India, Nepal as well as from east and south Africa. Polymorphism among bands obtained from different *E. coracana* lines as seen by RLFP was ~10% and ~25% using ISSR making it possible to differentiate between them. *E. indica* and *E. coracana* shared the most number of bands in any of the three methods. *E. indica* and *E. tristachya* shared markers, while the patterns obtained with *E. floccifolia* and *E. compressa* diverged from those

obtained with the other three species. The data presented, did not support the isolation of *E. compressa* into an independent genus. These workers suggested the use of ISSR markers to distinguish within and between species.

Neves *et al.* (2005) sequenced amplicons representing ITS1 spacer, 5.8S, ITS2 spacer, ITS region *trnT–trnL* spacer *trnL* intron, *trnL–trnF* spacer, and the *trnT–trnF* region from 33 accessions and nine species of *Eleusine* (*E. coracana* subsp. *coracana*, *E. coracana* subsp. *africana*, *E. floccifolia*, *E. indica*, *E. kigeziensis*, *E. tristachya*, *E. intermedia*, *E. jaegeri*, *E. multiflora*). Sequences of the ITS were most informative. Two sets of sequences were obtained only from *E. coracana* which differed from each other because of 18 substitutions and 1 bp indel. One set of sequences was related to that of *E. indica* and was considered to be that of ''A'' sequences (one half of the genome). The sequences containing the mutations were considered to belong to the ''B'' group (the other half of the genome). Pseudogenes belonging to the ''B'' group were detected in some *E. coracana* accessions. The sequences of locus A strongly supported ''CAIKT'' clade, encompassing *E. coracana* subsp. *coracana* and *africana*, *E. indica*, *E. kigeziensis*, and *E. tristachya*. Thus, the CAIKT clade is also morphologically similar in the number and type of glumes, nerves on the lemma and the presence of a winged keel. *E. coracana* is distinct in producing larger grains and has an upright habit. The ITS sequences from *E. tristachya* was related to that of *E. indica*. These workers subsumed *africana* as a subspecies of *E. coracana*, not considering them as a distinct species. The ITS sequences of *E. kigeziensis* was unlike that of the other tetrapolid, *E. coracana* and indicated that it is an autotetraploid.

Dida *et al.* (2007) used RFLP, SSR, EST, and SSRS to identify linkage groups in a mapping population of *E. coracana* and *E. africana*. They first established that there was greater variability among the *E. africana* accessions they studied than among the *E. corcana* accessions. Twenty-two linkage groups, arranged in nine homology groups were found. *E. indica*, a presumed A genome donor, was used to distinguish between A and B genomes. Many RLFP markers were able to distinguish between A and B donors. However, the marker position in both genomes was similar except for some possible rearrangement on one of the homology groups.

Dida *et al.* (2008) analyzed the divergence of 79 cultivated finger millet accessions (*E. coracana* subsp. *coracana*) from 11 African and five Asian countries and 14 wild *E. coracana* subsp. *africana* lines from Uganda and Kenya with 45 SSR markers developed by Dida *et al.* (2007). The cultivars grouped into three sets referred by them as African coracana, Asian coracana and africana subpopulations corresponding to the African lines, the ragi grown on the Indian plains, and the ragi found in Northern India, Nepal, and Pakistan. They hypothesized that finger millet was first domesticated in the African highlands, then in the south, and finally was

brought to India. They also noticed that the Asian coracana cultivars were shorter in plant height, lower in yield, and the grain paler than material derived from African coracana plants (Fig. 6.2). Das *et al.* (2007) used RAPD markers to distinguish between 30 ragi genotypes. The lines originating from Orissa were distinguishable from those derived from the south of India. Hammer *et al.* (2009) suggest that Oman could have been on the crossroads during the movement of *E. coracana* and other crops from Africa to Asia.

Tsehaye *et al.* (2006) published the results of a survey they had conducted in the north of Ethiopia in the Tigray region. A total of 37 farmers were contacted and a number of landraces collected from them. These were divided into three groups: Tsa'ada (meaning white seeded), Tselim (black seeded), and Keyih (red seeded). The white grains were preferred for making injera though red varieties are often used. The black ragi grains, which are supposed to be particularly resistant to birds and pests, were used in the making of areki (strong distilled local alcohol) and swa (unfermented local drink) (de Wet, 2006). The maytayta, an unfermented drink, was made by the Muslim farmers in the area. The white grains could be threshed easily. The white and red ragi were late in maturing. Bezaweletaw *et al.* (2007) examined over 64 accessions of landraces collected from different parts of Ethiopia and from Eritrea. They analyzed the accessions for diversity of plant type, seed color, seed shape, smoothness of the outside, persistence of pericarp. Plants with decumbent and prostrate types were found in Ethiopia, while erect types were found

**FIGURE 6.2** Maps of Africa and India showing modern distribution of races on *Eleusine coracana* (taken from Fuller 2003). There are differences between the *Eleusines* grown in the plains and in the hills in both Africa and India. The Indian plain *Eleusines* may have been derived from those of the African plains and the Indian Hill *Eleusines* from the hills of Africa (Dida *et al.*, 2008 © 2008. Reproduced with permission from Springer).

in the Eritrea collection. Ear shapes were variable varying from droopy to straight. There was variation in the persistence of the pericarp. Dark grains were found more often than red-grained *Eleusine* landraces, while those producing white seed grains were found less often. Overall, they concluded that there was great variability for different characters in the germplasm accession.

Srinivasachary *et al.* (2007) using homology between the sequences of markers (a total of 332 loci detected by 266 probes or primer pairs organized into 26 linkage groups) on a linkage map and the arrangement of orthologous sequences on the rice chromosome as may be seen in Table 6.1 compared nine linkage areas with the sequences on the rice chromosome. The sequences on some chromosomes are highly syntenic while genes on other sequences are not. There is indication of duplication,

**TABLE 6.1** Synteny of genes on chromosomes of *Eleusine* and *Orzya* (Srinivasachary *et al.* 2007)

| Linkage | Rice | Extent of synteny (%) | Nonsyntenic areas |
|---|---|---|---|
| 1A, 1B | Rice 1 | 85 | LG 1Aa and rice chromosome 5 are orthologous[a], a second nonsyntenic locus, *Xrgc58.1* is duplicated on finger millet linkage groups 1B and 6A |
| 2A, 2B | 2 long arm, 10 long arm | | Finger millet LG 10, which spans 16.5 cM four markers with homology to sequences on rice chromosome arm 2S |
| 3A | 3 | 91 | Location of *Xlfo112.1* is due to a single gene translocation |
| 4 | 4 | 48 | |
| 5 A, 5B | 5, 12 | | Possible insertion of rice 5 to rice 12*Xpse139* and *Xpse162.2*, detect closely linked copies in rice that are duplicated on rice chromosomes 4 and 5 |
| 6 | 6, 9 | 85–100 | Carries the waxy locus |
| 7 | 7 | 85 | |
| 8 | 8 | 90 | |
| 9 | 11 | 81 | |

[a] Orthologous genes in different species have a common ancestor.

deletion, and translocation. It is interesting to note that there is a difference between the *Eleusine* linkage groups 1A and 1B in this regard.

## III. ANTIQUITY OF CULTIVATION OF THE *ELEUSINE*

Fuller (2002) in a very exhaustive review of the work carried out in India and elsewhere on the origin of *Eleusine* in India points to its African origin and to the root *de'gi in a number of languages from southern Tanzania to northern Malawi which may be the source of the word ragi and its variants in India. He points out that ragi pericarp is ornamented (Fuller, 2006a) and many of the Indian finds do not appear to show this character. Fuller is skeptical of most of the reported finds of ragi in the ancient strata (personal communication) and considers only the single grain isolated from Halur 900 BC, the few grains obtained from Mahar (800 BC–1600 BC) and Hulashakara and those found in the south at Mangudi, Kodumanal, and Perur to be authentic and to be grown by neolithic settlers. These statements are reinforced in Fuller (2006b). At Perur, the presence of *Eleusine* has been attributed to the Iron Age (Cooke *et al.*, 2005). Pokharia (2008) indicated the absence of ragi in a site close to Mahar and refers to their work indicating the presence of ragi at Raja-Nal-ka-tila but dating to the Pre-Iron phase (prior to about 500 BC) and Early-Iron phase. Fuller (personal communication) has identified finger millet from the early historic period at Paithan in Maharashtra and from Rwanda [Africa] from around the 10th c. AD (Fig. 6.3).

Walshaw (2006) in a posting reported the presence of three grains of *Eleusine* in excavations in an Island off the coast of Africa (Tanzania) and this was dated to a rather recent period.

## IV. SEED DEVELOPMENT

Khosla (1946) compared gametophyte and seed development among *Setaria italica*, *Panicum miliaceum* L., *Penniseturn typhoideum*, and *E. coracana*. *Eleusine* has a camplyotropus embryo (right angles to stalk). During endosperm formation numerous nuclei are formed and fill the embryo sac. Cell wall formation occurs near the embryo. The outer integument disappears and deposits tannin. Narayanaswami (1952, 1955) has worked on microsprogenesis and caryopsis development in *Eleusine*. The ovary wall is five to eight cells thick in the beginning. The cells of the middle layer disintegrate. The two cells of the outer wall remain as a thin papery membrane around the seed coat. The seed coat is derived from two layers of the inner integument, which enlarges and becomes thicker and some brownish material is deposited in them. Soon after triple fusion, the product undergoes free nuclear divisions. Walls are laid down

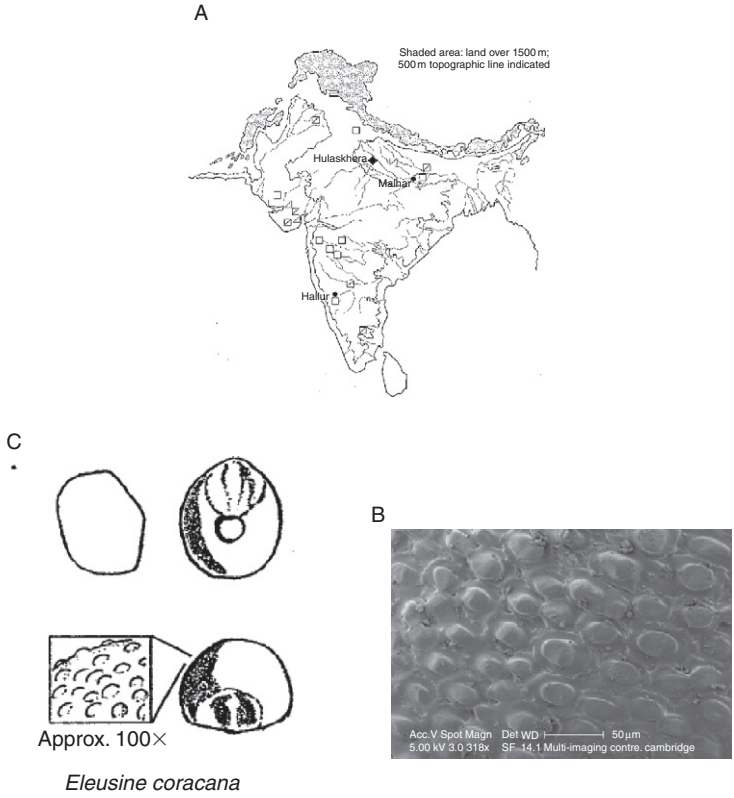


**FIGURE 6.3** (A) Figurative indication of archeological finds of *Eleusine* in India. (B) Scanning electron micrograph of the surface of the grain obtained from Hallur identified as ragi and estimated to be from the second millennium BC. (C) The pusticulate surface of *Eleusine coracana* and that the hilum is visible on one side and not on the other side perhaps resulting in misidentification (Fuller, 2002, 2003, 2006a. Reproduced with the permission of Dr. Dorian Fuller).

around the proembryo first and then toward the chalazal portion. Cell division becomes confined to the periphery layer. The outermost cells become the aleurone, and the inner cells become filled with starch. Cells of the pericarp abutting the endosperm are often empty (Chandra, 1963).

McDonough *et al.* (1986) have published a detailed report on the microscopic structure of the grain. Finger millet is unique in its grain characteristics as it is an utricle instead of a true caryopsis like other cereals. The utricle characteristic means that the pericarp is not completely fused with the testa (Philips 1972; McDonough *et al.*, 1986). This allows the pericarp to be removed by simply rubbing the dry grain or rubbing it after soaking in water. Finger millet has a five-layered testa which can be red to purple.

### A. Expressed sequence tags

Sivakumar *et al.* (2007) worked out a method for the isolation of RNA from little (*Panicum sumatrense* Roth.) and finger millet (*E. coracana* Gaertneri) grains. There are 1966 sequences from developing finger millet grain in the EST database. A large number of sequences belonging to α-amylase inhibitors are present. These have been deposited by Sorensen and Rasmussen (2005). These have not been analyzed critically. Figure 6.8 plots the relatedness of different Bowman Birk inhibitors in ragi.

## V. PROXIMATE COMPOSITION

Total lipids in seven cultivars of ragi varied from 1.85 to 2.10 g/100 g with chloroform-soluble lipids from 1.70 to 1.90 g/100 g. Neutral lipids constituted the bulk of the lipid varying from 1.35 to 1.46 g/100 g, glycolipids from 0.23 to 0.27 g/100 g, and phospholipids from 0.10 to 0.12 g/100 g. Palmitic acid varied from 25% to 30%, oleic acid from 49% to 50% (61% to 64% in the glycolipid fraction), and linoleic acid from 8% to 12% of the glycolipid fraction and 23% to 27% of the other fractions (Mahadevappa and Raina, 1978). Fernandez *et al.* (2003) reported that Nigerian *E. coracana* contained 1–2 g/100 g total fatty acid, ''42% of which was oleic acid (C18:1n-9), 21% palmitic acid (C16:0), 25% linoleic acid (C18:2n-6), and 4% α-linolenic acid (C18:3n-3).'' Sridhar and Lakshminarayana (1994) compared the lipid content and composition of Foxtail millet (*S. italica*), Proso millet (*P. miliaceum*), and finger millet (*E. coracana*) grains. Ragi contained 5.2%, while the hexane extractives were 2.2%. Triacyl glycerol accounted for 80% of the total lipid while phospholipid and glycolipid accounted for 14% and 6% of the total lipid, respectively. Phosphatidylglycerol, phosphatidylethanolamine, phosphatidyl choline, and digalactosylmonoglycerides predominated in the phospholipid and glycolipid fractions. Palmitic, oleic, and linoleic acid were the major fatty acids in the different lipids. The concentration of digalactosylmonoglyceride was highest in finger millet among the three millets studied. Ragi lipid contained more palmitic acid than did lipid from the other millets.

Babu *et al.* (1987) reported that, among six hybrid varieties of finger millet, nitrogen varied from 1.3 to 1.5 g/100 g and calcium from 293 to 390 mg/100 g. Barbeau and Hilu (1993) observed that protein ranged from 7.5% to 11.7%, calcium from 376 to 515 mg/100 g, and iron from 3.7 to 6.8 mg/100 g. All values were higher for *E. africana* than for *E. coracana*.

Ravindran (1991) estimated the protein content of ragi to be 9.8%, that of calcium, oxalate, and phytic acid to be 0.24%, 0.44 mg%, and 0.48%, respectively. Hadimani and Malleshi (1993) compared the protein, lipid, ash, calcium, phosphorus, and dietary fiber contents of seven native and milled millets. The protein content of ragi milled flour decreased by 61%

on removal of the bran from 9.4% to 5.85%; this change was not so much in the other millets studied. Decreases in the contents of ash, calcium, and phosphorus occurred simultaneously on milling. The total dietary fiber (nonstarchy polysaccharides) content was 11.8%, while that of the water-soluble component was 6.5 g/100 g. Only fox tail contained less total nonstarchy polysaccharide at 9.6%. Ragi ''bran'' contained 40% dietary fiber, only 9% of which was soluble in water.

Vadivoo *et al.* (1998) measured the protein and calcium content in 36 genotypes including brown seeded, white, and copper red. The calcium content of the genotypes ranged from 162 to 487 mg/100 g, while the protein content of the grain ranged from 6.7 g/100 g of grain to 12.3 g/100 g of grain. There was a negative correlation between the calcium and the protein content and between the protein content and yield.

Antony and Chandra (1999) estimated the mineral content of a white (CO 9) and a brown (C0 13) variety of *E. coracana* grain. The total content in mg/100 g of Ca was 365 and 383, P was 210 and 240, Zn was 2.2 and 2.23, Mn was 7.44 and 5.84, respectively. Ragi flour was either fermented or treated with a combination of hemicellulase and cellulase prior to fermentation. The amount of acid extractable minerals especially calcium increased in the brown variety after a combination of autofermentation and enzymatic treatment.

Admassu *et al.* (2009) measured the proximate composition of six varieties of finger millet. The values ranged from 6.26 to 10.5 g/100 g for protein content and from 50 to more than 300 mg/100 g for calcium content. Iron varied from about 4.5 mg/100 g to more than 50 mg/100 g and phosphorus varied from less than 4 to about 147 mg/100 g.

Hemalatha *et al.* (2007) using a simulated method showed that the availability of iron increased during germination of finger millet from 24.8% to 29.5% while that of zinc actually decreased from 3.9% to 2.4 % after 48 h of germination. They obtained similar results with green gram and chickpea.

## VI. COLOR AND POLYPHENOLS

The grain color in ragi is determined by three factors (S, B1, and B2). Purple plants with brown grains contain the S and B factors. Factor D deepens the effects of the brown factors (Ayyangar *et al.*, 1931). Ayyangar *et al.* (1932) and Khosla (1946) noted that the brown pigment is confined to the seed coat. They identified some white-seeded plants also. Krishnaswami and Ayyangar (1942) noted that they were unable to obtain mutants varying in color by exposure of ragi seeds to X-rays. Gupta *et al.* (2010) reported that the use of RAPD and ISSR revealed great relatedness between white and brown-seeded parents and a golden-seeded hybrid progeny. The total polyphenol and tanin content as estimated by different workers in different lines of colored and white ragi is collated in Table 6.2.

**TABLE 6.2** Total polyphenol and tannin estimated in finger millet. (ND, not detectable)

| *N* | Total polyphenols | Tannin | References |
|---|---|---|---|
| Brown | | | |
| 26 | 0.08–0.96% | 0.85–3.47 | Ramachandra *et al.* (1977) |
| 12 | | 0.35–2.39 | Rao and Deosthale (1988) |
| 32 | | | |
| 18 | 0.34–1.84 | 0.02–2.08 | Siwela *et al.* (2007) |
| 3 | 0.55–0.59% | 0.17–0.32% | McDonough *et al.* (1986) |
| 1 | 0.1 | | Sripriya *et al.* (1997) |
| | | 0.74 ± 0.03 | Antony and Chandra (1999) |
| White | | | |
| 6 | 0.06–0.09 | 0.01–0.06 | Ramachandra *et al.* (1977) |
| 1 | 0.003 | | Sripriya *et al.* (1997) |
| | | 0.0 | Antony and Chandra (1999) |
| 4 | ND–0.09 | ND | Siwela *et al.* (2007) |

Antony and Chandra (1999) reported the absence of tannin (vanillin–HCl) in a white variety of ragi, while it was 0.74 g/100 g in a brown variety. The level of tannin decreased on autofermentation for 48 h to 0.44 g/100 g. The amount of tannin remained more or less constant when fermentation was combined with treatment of flour with hemicellulase and cellulase. The amount of nitrogen digested as a percentage of the total nitrogen (IVPD) increased during fermentation and more so when fermentation was combined with enzymatic treatment. Surprisingly, the increase in IVPD was more in the brown variety after such treatment surpassing that of the white variety similarly treated.

Rao (1994) reported a 46% fall in tannin during malting of brown seeded ragi. Rao and Muralikrishna (2002) reported that ragi contained about 53 and 21 mg/100 g of free and bound phenolic acids, respectively. Caffeic acid (1.64 mg/100 g), coumaric acid (1.23 mg/100 g), and ferulic acid (18.6 mg/100 g) were predominant. There was a steady decrease in concentrations of free and bound phenolic acids reaching 40 and 20 mg/100 g, respectively, after 96 h of malting (Rao and Muralikrishna, 2001). The antioxidant activity coefficient (AAC) was calculated from spectrophotometric measurements of β-carotene-linoleic acid emulsions at 470 nm in the presence and absence of ragi extracts and that of known phenolic acids. The AAC value was reported to be 770 per gram of flour before malting and 1686 per gram of flour after 96 h of malting. There was some discrepancy between these values and that calculated from the amount and types of antioxidants reported by them. This they concluded indicated the presence of other phenolic acids that were not detected by their HPLC method (Rao and Muralikrishna, 2004a).

Chethan and Malleshi (2007a) reported that water, acetone, methanol, ethanol, and propanol could extract 7.45%, 13.1%, 19.6%, 13.1%, and 10.0% of the total polyphenol fraction that could be extracted with acidified methanol and refluxing for 3 h. This solvent extracted 2.3% of the weight of the flour, estimated to be primarily polyphenols, using a version of the Folin–Ciocalteau method. It is interesting to note that about 7% of the amount extracted by acidic methanol and refluxing was extractable in acidified water. Raising the pH of the extract resulted in the formation of a precipitate which redissolved when an acidic environment was restored. The phenolics identified in pH 3.0 extracts were gallic acid, procatechuic acid, *p*-hydroxy beonzoic acid, *p*-coumaric acid, syringic acid, ferulic acid, and *trans*-cinnamic acid. At pH 7.0, gallic, syringic, ferulic, and *trans*-cinnamic acids were extracted, while, at pH 10.0, only gallic and syringic acids were detected. The precipitate contained gallic acid and procatechuic acid. Interestingly, the precipitate obtained from neutral extracts also contained 590 mg% of calcium, while the calcium content of the supernatant was 290 mg%. This is in comparison to the calcium content reported by these authors of 320 mg/100 g in the whole grain flour and of 770 mg/100 g in the ''seed coat fraction.'' Vadivoo *et al.* (1998) and Antony and Chandra (1999) reported that white-grained ragi was low in polyphenol content and also contained less calcium.

Siwela *et al.* (2007) measured the total phenolics and condensed tannin in four white and 18 brown-grained finger millet samples. The white-seeded grains had nondetectable levels to very low levels of total phenol and no detectable condensed tannin. The level of condensed tannin varied among the colored grains. The antioxidant activity was generally higher in grains with higher levels of condensed tannins. Two pigmented types included in the study also had very low levels of total phenols and condensed tannins. The four white and two pigmented grains did not become black on exposure to alkali and sodium hypochlorite while all other grains turned black. Hunter *a* values (redness) and total phenolic content were correlated. Grains with higher condensed tannin content are more likely to become black using this bleach test. The thickness of the testa was 14.6 and 13.4 μm for two varieties that contained tannins and was 9.2 and 9.7 μm, for varieties with no detectable condensed tannins. The tannins are located in the testa as is evident from the scanning electron micrographs and the stained transmitted light micrograph (Fig. 6.4).

Chethan and Malleshi (2007b) have reviewed the literature on polyphenols of ragi and report the presence of benzoic acid and its derivatives (gallic, procatecuic, *p*-hydroxybenzoic, vanillic, and ferulic acids) as comprising 85% of the total phenolics. Cinammic acid derivatives, syringic acid, *trans*-cinnamic acid, and *p*-coumaric acid were also reported to be present. The presence of the flavanoid, quercetin, was also indicated by them. Chethan *et al.* (2008a) reported that the acid methanolic extracts of ragi

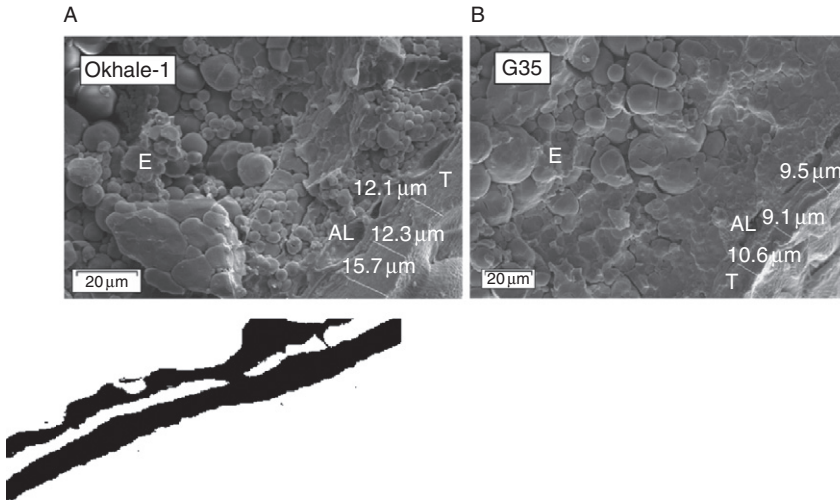


**FIGURE 6.4** Scanning electron micrograph and transmitted light micrograph of the ragi testa. (A) SEM showing differences in thickness of testa of a high tannin and a low tanin variety of finger millet (Siwela *et al.*, 2007 E, Endosperm; AL, ALeurone Layer; T, Testa. Figure reproduced with permission from Cereal Chemistry). (B) Sections of brown ragi stained with ferric chloride show that polyphenolics is concentrated in a region below a one cell layer pericarp/integument. In the black and white version of this picture the polyphenolic layer may be seen as a white layer between two black layers (Chethan and Malleshi, 2007b. Reproduced with permission from the American Journal of Food Technology).

seed coat contained ferulic (32.8%), *p*-hydroxy benzoic (17.9%), procatechuic (15.3%), gallic (12.6%), *p*-coumaric (4.4%), syringic (4.0%), vanillic (3.8%), *trans*-cinnamic (3.6%) acids, and quercetin (5.6%) as separated by C18 reverse phase HPLC. Having identified the composition of these phenolic acids, workers tested the ability of these compounds to inhibit the aldol reductase extracted from human cataracted eye lens. Aldol reductase converts glucose to sorbitol. Increased levels of sorbitol have been implicated in the development of high osmoticum in the eye lens during aging or on the onset of diabetes. Quercetin was found to be the most effective inhibitor of aldol reductase ($IC_{50}$ of 25.2 mg/100 g), followed by protocatechuic and *trans*-cinnamic acids. *p*-Hydroxybenzoic, vanillic, and ferulic acids were ineffective. Quercetin was found to be a noncompetitive inhibitor of the enzyme. It was suggested that dietary supplements of aldol reductase inhibitors might help in preserving the health of the eye lens.

Free radical quenching by extracts of brown ragi was 94% while that by germinated, fermented and white ragi was 22%, 25%, and 5%, respectively. Extracts from foxtail was equally effective while extracts from rice had a free energy quenching activity of 1.8 (Sripriya *et al.*, 1996). Mehta (2006) extracted ragi flour with methanol and added the dried powder to

ghee. The development of peroxides in the ghee was monitored in samples kept at 60 °C for more than 500 h. BHA (butylated hydroxy anisole) was added to other samples and used for comparison. The peroxide values increased most in untreated samples. The addition of ragi extract or BHA reduced the formation of peroxides drastically. This effect of ragi was attributed to both polyphenols and phospholipids.

Asharani *et al.* (2010) compared the antioxidant activity (measured as α-tocopherol units per gram) of methanolic extracts from different varieties of finger millet (*E. coracana*), little millet (*P. sumatrense*), foxtail millet (*S. italica*), and proso millet (*P. miliaceum*). Extracts from ragi averaged 15.3 ± 0.6 while those of little millet, foxtail millet, and proso millet were 4.7 ± 1.1, 5.0 ± 0.4, and 5.1 ± 0.8, respectively. The total tocopherols in these millets were 4.1 ± 0.2, 1.3 ± 0.2, 1.2 ± 0.008, and 3.6 ± 0.1 mg/100g flour.

Singh *et al.* (2008) analyzed six varieties of ragi for different polyphenols and found gallic acid at about 12–33 μg/g, tannic acid at 0–4.7 μg/g, and cinammic acid at 0–0.93 μg/g. After cooking, gallic acid content decreased to 2–6 μg/g. Sridevi *et al.* (2008) reported the presence of about 0.6% of polyphenol in brown ragi and 0.14% in white ragi. The phytic acid content of white ragi was 1.6%, while this figure was reported to be 3.95% for brown ragi (Singh *et al.*, 2008).

Chethan *et al.* (2008b) reported that about 44% of polyphenols was lost during the first 24 h of germination and another 40% was lost during the next 48 h.

Viswanath *et al.* (2009) reported that there were more polyphenols in the husk-enriched fraction of ragi (6.1 g/100 g) than in the whole flour (2.6%). Gallic acid predominated in both husk and whole flour fractions while syringic acid was more in extracts from whole flour. Husk polyphenols were more effective antioxidants than those from the whole flour. Polyphenols from husk and from whole grain inhibited growth of *Bacillus cerus* and *Aspergillus flavus*. The MIC was 305 for the husk extracts and 505 for the whole flour extracts. Antony *et al.* (1998) established that extracts of fermented finger millet could suppress the growth of *Salmonella* and *Escherichia coli* in Macconkey agar. Chethan and Malleshi (2007b) observed antimicrobial activity of ragi extracts against a number of pathogenic bacteria including *Bacillus cereus, Staphylococcus aureus, Yersinia enterocolitica.* There is a report regarding the presence in some ragi samples of toxins produced by the fungus *Alternaria* (Ansari and Shrivastava,1990).

The *in vitro* protein digestibility of 13 finger millet varieties decreased with an increase in tannin content in the grain, varying from 85.1% to 55.4% in flours of grains with tannin content varying from 0.06% (Hamsa) to 3.47% (IE 927) (Ramachandra *et al.*, 1977). An examination of the inhibitory effect of different finger millet polyphenols on malt amylase revealed that gallic acid (67.7%), vanillic acid (71.9%), the flavonoid quercetin (73.5%), and *trans*-cinnamic acid (79.2%) were potent inhibitors. The purified inhibitors,

however, inhibited the enzyme in an uncompetitive manner (affecting $v_{max}$) while the millet polyphenol extract was a noncompetitive inhibitor affecting both $k_m$ and $v_{max}$. This was taken to mean that polyphenols inhibited the enzyme by binding both to the active site and to another site (Chethan *et al.*, 2008b). Shobana *et al.* (2009), using ESI-MS, identified a number of polyphenols present in acidic methanolic extracts of ragi. Gallic acid, caffeic acid, ferulic acid, 4-*O*-methyl gallic acid, naringenin, epicatechin, and catechin gallates were thus identified. These were shown to be noncompetitive inhibitors of rat intestinal and porcine pancreatic amylase. These authors attribute the lower gylcemic index of ragi to this ability of inhibiting enzymes involved in starch hydrolysis.

Hegde *et al.* (2005) incorporated ragi at a 55% level into rat diets supplemented with casein, oil, minerals, vitamins, and corn starch that were fed to adult male rats made diabetic with alloxan. During the feeding period of 28 days, body weight increased by 43 g in the control group, by 6 g in the diabetic rats fed corn starch and casein, and by 28 g in the group of diabetic rats fed finger millet. Blood glucose was 83 mg/dl in the control, 212 mg/dl in the diabetic rats, and 137 mg/dl in the diabetic rats fed finger millet. The total cholesterol in blood and the level of glycated collagen were lower in the diabetic rats fed finger millet. The activity of catalase, glutathione peroxidase, and glutathione reductase was higher in the ragi-fed rats rather than the diabetic rats fed corn starch and casein. They attributed these increased enzymatic activities to the antioxidant and protective properties of the ragi grain.

Finger millet seed is used to treat dysentery, possibly a refection of it's antimicrobial properties. In southern Africa, the juice of a mixture of finger millet leaves and those of *Plumbago zeylanica* L. is used in the treatment of leprosy (de Wet, 2006). An infusion of the aerial parts of *E. indica* is in the treatment of influenza and pneumonia in Brazil. De Melo *et al.* (2005) demonstrated the ability of two flavonoids isolated from the leaves, schaftoside (6-C-b-glucopyransoyl-8-C-a-arabinopyranosylapigenin) and vitexin (8-C-b-glucopyrnaosylapigenin) to prevent the recruitment of neutrophils in *Balb* c mice inhaling lipopolysaccharide (inflammatory) from Gram negative bacteria. Lans (2006) in a survey of the plants used by traditional healers in Trinidad and Tobago noticed the use of the leaves and roots of *E. indica* for the treatment of urinary infection.

## VII. CARBOHYDRATE

### A. Starch

The gelatinization temperature of ragi starch varied from 62 to 70 °C. Palmitic acid was the predominant fatty acid in starch granules while oleic acid was predominant in the bound fraction (Wankhede *et al.*, 1979). The size of

the ragi starch granule varied from 4 to 7 μ (39%), 8 to 12 μ (42%), and 14 to 22 μ (19%). The number of granules in the 4–7 μ size range increased on germination. The intrinsic viscosity decreased for the raw starch from 1.52 to 1.35 and 1.18 after 24 and 96 h of germination. The swelling power in water at 45 °C increased from 0.5 to 0.7 g/g, while at 85 °C, it was 9.0 g/g. The solubility of starch extracted from malted ragi in DMSO was higher than that extracted from native ragi. The peak viscosity for malted starches was lower than for starch from ungerminated ragi. The susceptibility to β-amylolysis decreased during germination, while susceptibility to the action of α-amylase remained unchanged (Malleshi *et al.*, 1986c).

Jideani *et al.* (1996) reported that ragi starch granules varied in size from 3.3 to 14.3 μ with an average value of 7.3 μ. The amylose content was estimated to be 19.3%. Isolated amylose was shown to consist of both branched and unbranched regions. The degree of polymerization fell from 4640 to 3470 in β-limited amyloses and the $DP_w$ distribution fell from 380–17,400 to 300–11,200 after treatment of amylose with β-amylase. The molar ratio of branched to unbranched molecules was 0.4–0.60. The amylopectin from ragi was separated into two fractions, and one of the fractions was labeled as micro gel-like amylopectin (MGA). The isolated amylopectin was debranched with isoamylase. The debranched molecules were separated on size exclusion HPLC. Several fractions were obtained. The average chain length was 1300 for the first fraction and 300, 98, 95, 41, and 16 for the subsequent fractions. The trend was similar to that of other starches. In the MGA, the high molecular weight fraction from HPLC accounted for 4%. On the rotaviscometer, ragi starch exhibited a peak viscosity comparable to that of other starches. The viscosity did not fall on cooking and hence could be said to experience very low breakdown during cooking (Fig. 6.5).

The ability of ragi starch to swell more than rice starch was shown by its ability to absorb greater amounts of water at 90 °C and by the shape of

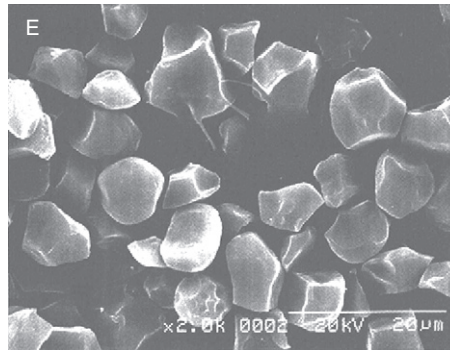


**FIGURE 6.5** Scanning electron micrograph of starch granules isolated from *Eleusine* (Jideani *et al.*, 1996. Reproduced with permission from Cereal Chemistry).

the pasting behavior in a visco-analyzer. Ragi starch had a higher peak viscosity and a larger breakdown than did rice starch. Ragi starch also had a greater setback viscosity. This would indicate that ragi starch granules swell more, break down more easily under shear and the released molecules reassociate better than do molecules released from rice starch granules. An examination of the data on the fractionation of starches using Sepharose 2b showed that ragi starch contained higher molecular weight void volume amylopectin as well as higher molecular weight amylose. This is also shown by a higher $\lambda_{max}$ of the ragi starch iodine complex ($603\lambda_{max}$) than from rice ($611\lambda_{max}$). The fragments obtained by the action of human salivary α-amylase were larger with gelatinized ragi than with gelatinized rice starch (Mohan *et al.*, 2005).

Muralikrishna and Gopal (2008a) comparing the properties of maize, ragi, wheat, and rice starches noted that the enthalpy of gelatinization, degree of crystallinity, and resistance to digestion by pancreatic α-amylase for ragi starch were higher than for wheat and rice starches but lower than that for maize starch.

## B. Sugars and nonstarchy polysaccharide

Ramachandra and Moneiro (1979) estimated glucose, arabinose, and xylose contents in water- and alkali-soluble extracts of α-amylase-treated ragi using paper chromatography for separation, followed by elution of the sugars, and estimation using phenol sulfuric acid. The yield of pentosan was 0.74% and 0.76% from white and brown varieties, respectively. There was more arabinose in the water-soluble fraction (arabinose/xylose: 2.21–2.27) than in the alkali-soluble pentosans (arabinose/xylose: 1.23–1.60) and less xylose in the white ragi. The pentose/hexose ratio was 0.69–0.78 in the water-soluble fraction from the white and brown-seeded ragi and 12.2 and 29.1 for the alkali-soluble fraction. The total pentosan content in ragi as estimated by these workers varied from 2.7 to 4.0%.

Rao and Muralikrishna (2004b) isolated a fraction of the arabinoxylan from ragi and determined its structure using a combination of methylation, enzyme digestion, NMR, and MALDI-TOF-MS. They determined that the arabinoxylan was a (1–4)-linked xylose backbone carrying arabinose at the C-3 position and an occasional glucuronic acid at the (1–3)-position. Xylose was also found in the side chain (Fig. 6.6).

Water-soluble polysaccharides extracted from native and malted (96 h) rice and ragi were purified using DEAE and Sephacryl columns. Malting resulted in a decrease in the molecular weight of the polysaccharides from being over 140 kDa to about 40 kDa, while the ferulic acid content increased from 161 to 950 μg/g. The arabinose/xylose ratio was 0.21 for the fraction purified from native ragi and 0.04 for that prepared from malted ragi. It would appear that malt xylanases cut through regions

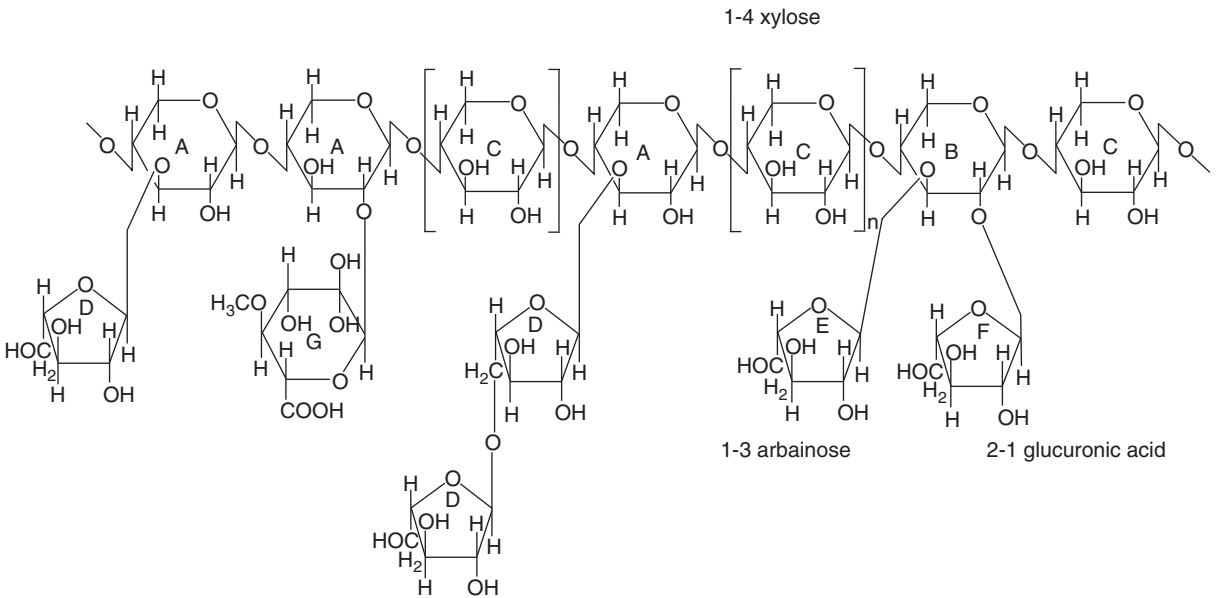


**FIGURE 6.6** Structure of the hemicellulose of ragi (Rao and Muralikrishna, 2004b. Reproduced with permission from Elsevier).

of the arabinoxylans in such a manner as to release fragments rich in ferulic acid. Since the ferulic acid residues are linked through a galactose moiety, the content of galactose was higher in water-soluble polysaccharides purified from malted ragi and rice. The fractions also contained glucuronic acid. The antioxidant activity of the polysaccharides was much higher than that of ferulic acid alone. The uronic acids were implicated in this function (Rao and Muralikrishna, 2006a, 2007).

Malleshi *et al.* (1986a) compared the composition of sugars extractable by aqueous ethanol from flours and malts derived from ragi, pearl millet, and foxtail millet. The amount of glucose, fructose, maltose, and sucrose increased during malting in all three grains. The extent of change was largest in ragi. The yield of water-soluble, nonstarchy polysaccharide increased during germination, while the concentration of pentose decreased during malting and the hexose concentration increased. This resulted in a lowering of both arabinose and xylose such that the arabinose/xylose ratio remained unaltered. The concentration of pentosan (hemicellulose A) increased during germination (Malleshi *et al.*, 1986b). Nirmala *et al.* (2000) reported a fall in the xylose and glucose contents of the nonstarchy polysaccharides during the malting process and an increase in the glucose/fructose ratio, sucrose, and maltose in the aqueous alcohol-soluble fraction.

Rao and Muralikrishna (2001) fractionated nonstarchy polysaccharides from raw ragi and ragi that had been germinated for 96 h. A decrease in the amount of arabinose was noted in all fractions with a concomitant increase in glucose and galactose content noted in the hemicellulose A and B fractions. The arabinose to xylose ratios increased during germination indicating debranching of this pentosan fraction. Degradation of cellulose was also indicated by a fall in glucose content of the alkali-insoluble residue. Rao and Muralikrishna (2004a) determined the composition of water-soluble and -insoluble nonstarchy polysaccharides from rice, maize, wheat, and ragi. The water-extractable, nonstarchy polysaccharide from ragi had a higher arabinose/xylose ratio (1:0.5) than rice (1:4.8), maize (1:1.3), and wheat (1:0.40). The water-insoluble residues had a similar ratios of arabinose/xylose (1:0.53–0.84). Rao and Muralikrishna (2006a) have reported the isolation of both hemicellulose A (precipitated from an alkaline extract by acid) and of hemicellulose B (precipitated from an alkaline extract by alcohol) from unmalted ragi and ragi malted for 96 h. Both fractions were further fractionated using different techniques. While there was a decrease in the yield of hemicellulose A on malting, there was a sixfold increase in the yield of hemicellulose B from germinated ragi. Generally, there was more arabinose in the fractions that were water-soluble than those that were not. The material fractionated on DEAE cellulose columns and requiring alkali for elution contained more arabinose than xylose than those that required water or ammonium carbonate for elution.

Hemicellulose B was fractioned using graded ammonium sulfate. The galactose content was highest in the fraction that did not precipitate at the highest concentration of ammonium sulfate. The amount of galactose in the sample increased on malting and that of arabinose also generally increased. One of the fractions (F-70) was loaded on a DEAE cellulose column and fractions eluted with different eluants. Generally, the amount of xylose appeared to decrease in this fraction. This is reflected in decreased intrinsic viscosity and a decrease in the molecular size of the fraction of hemicellulose B eluted from a DEAE-cellulose anion exchange column (Rao and Muralikrishna, 2006b).

## VIII. PROTEIN

The amino acid composition of ragi and some of the storage proteins contained in the grain is listed in Table 6.3. It has been noted by various workers that *E. coracana* is especially high in methionine and the isoleucine/leucine ratio is less than that of sorghum or maize proteins. The lysine content is also higher that of other grains. Nutrition studies with ragi have indicated that a diet of ragi alone supports better growth of rats than one of sorghum alone. On being complemented with legumes, as is typical in India, the ability of ragi to support growth is even better.

Finger millet has a relatively high content of high sulfur-containing amino acids. With fertilization, the contents of methionine and cysteine increased and then leveled off (Starbursvik and Heide, 1974). Mbithi-Mwikya *et al.* (2000) estimated the amino acid profiles of ragi and kidney beans after sprouting, autoclaving, and fermentation. Tryptophan and lysine increased after processing.

The albumin content was 0.7–1, globulins from 1.0 to 1.5, glutelins from 4.3 to 6.6 and prolamins (60% aqueous ethanol-soluble) from 1.9 to 3.4 g/100 g defated flour in six hybrids of finger millet (Babu *et al.* (1987). Hilu and Esen (1993) extracted prolamins from 23 chloridoid species from 24 genera belonging to six recognized tribes of the chloridoid (Eragristoideae) and raised antibodies to them. The prolamins were generally 15–30 kDa in size. There were differences in the banding pattern of *E. indica* and *E. coracana* prolamins. Antibodies to prolamins were used to distinguish between the different tribes. Virupaksha *et al.* (1975) fractionated the proteins from the seeds and endosperm of 12 varieties of finger millet. The protein content varied from 6.77% to 11.03%. The water- and salt-soluble proteins varied from 8% to 15% of the total proteins. The prolamins ranged from 35% to 50% of the total protein and comprised 29–41% of the weight of the endosperm. The final 12–28% of the total protein remained as insoluble residue. White-seeded varieties contained more prolamin than most brown-seeded ones. Ramachandra *et al.* (1978)

**TABLE 6.3** Amino acid composition of *Eleusine* flour and prolamin

| Amino acid composition (g/100g total amino acids) | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Asx | Thr | Ser | Glx | Pro | Gly | Arg | Ala | Cys | Val | Met | Ile | Leu | Tyr | Phe | His | Lys | Tryp | References |
| *E. indica* | 7.3 | 4.5 | 5.8 | 24.3 | 6.8 | 3.4 | | 6.5 | 1.0 | 5.2 | 3.5 | 3.7 | 9.9 | 4.4 | 5.9 | 2.3 | 3.7 | 0.0 | Yeoh and Watson (1981) |
| *E. coracana* | 6.6 | 4.5 | 6.1 | 22.8 | 7.2 | 3.5 | | 6.6 | 1.5 | 3.5 | 3.6 | 4.6 | 10.5 | 5.9 | 2.3 | 3.3 | 0.2 | 2.0 | Yeoh and Watson (1981) |
| *E. coracana*/ *E. africana* | 5.77–7.2 | 4.3–5.3 | 5.3–6.2 | 23.2–27.4 | 9.9–12.7 | 3.3–4.0 | 3.4–4.0 | 6.1–8.2 | ND | 6.3–8.9 | 2.9–3.8 | 4.3–5.0 | 10.8–13.6 | 3.6–4.1 | 6.0–7.7 | 2.3–3.0 | 2.2–2.8 | ND | Barbeau and Hilu (1993) |
| *E. coracana* Prolamin FM3 | 2.3 | 4.9 | 4.7 | 29.6 | 9.1 | 6.7 | 2.0 | 5.1 | 1.1 | 9.1 | 5.0 | 3.7 | 7.3 | 4.0 | 4.0 | 1.1 | 0.4 | ND | Tatham *et al.* (1996) |
| *E. coracana* Prolamin FM6 | 3.5 | 3.8 | 4.4 | 18.9 | 14.0 | 4.7 | 1.1 | 10.6 | 1.4 | 9.8 | 2.0 | 4.5 | 7.5 | 3.8 | 8.2 | 0.8 | 0.6 | ND | Tatham *et al.* (1996) |
| *E. coracana* Sprouted | 5.76 | 4.31 | 5.51 | 23.75 | 6.30 | 3.38 | 4.44 | 6.62 | 1.49 | 5.81 | 2.81 | 3.85 | 10.05 | 4.29 | 5.35 | 2.44 | 2.75 | 1.44 | Mbithi-Mwikya *et al.* (2000) |
| *E. coracana* | 7.69 | 4.38 | 5.30 | 17.13 | 6.26 | 4.77 | 3.68 | 7.42 | 1.43 | 5.36 | 4.02 | 3.66 | 9.28 | 4.53 | 6.07 | 3.15 | 2.01 | 1.35 | Malleshi and Klopfenstein (1998a,b) |

FM3 and FM6 are prolamin fractions. Prolamins were extracted from FM flour with 50% (v/v) aqueous propan-1-ol, 2% (v/v) acetic acid, and 2% (v/v) 2-mercaptoethanol, reduced and alkylated prior to separation on RP HPLC.

fractionated ragi flour proteins. The water/saline soluble proteins ranged in content from 9.6 to 12.2% of total nitrogen in flour. The prolamin content ranged from 22.1 to 29.7% of total nitrogen, with the lowest figure attributable to that from white ragi. These workers noted a major prolamin band in the range of 10–14 kDa. Glutamine, leucine, phenyl alanine, and iso leucine were the amino acids contained in the prolamins at higher concentrations than were other amino acids.

Tatham *et al.* (1996) extracted ragi and teff with 1 M sodium chloride, 70% ethanol, and then with 50% propanol, acetic acid, and 2-mercaptoethanol to obtain a prolamin fraction. Using SDS-PAGE, three major protein bands with $M_r$s of about 25.7, 24.5, and 21.9 kDa and minor bands 45.5 and 60.0 kDa were observed which resolved into a number of fractions on using reverse phase HPLC. The amino acid composition of some fractions and the N-terminal amino acids of the proteins therein were determined. The amino acid composition was similar to that of maize prolamins with a high isoleucine/leucine ratio, and high contents of glutamic acid and proline. The methionine content of the ragi prolamins was higher than that of α-zein, the main storage protein in the seeds of maize. Two major prolamin bands with $M_r$s of about 25.0 and 22.5 kDa were present in teff and three such bands were found in finger millet (Fig. 6.7, tracks e and i).

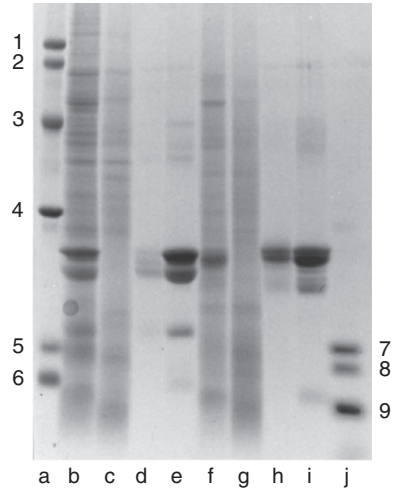


**FIGURE 6.7** SDS-PAGE of proteins extracted from teff and finger millet. Track a: $M_r$ markers, 1 = 76–78,000, 2 = 66,200d, 3 = 42,700d, 4 = 30,000d, 5 = 17,200d, and 6 = 12,300d. Teff: track b = total proteins; c = salt; d = 70% ethanol extracted; e = 50% propan-1-ol, 2% acetic acid and 2% 2-mercaptoethanol-extracted. Finger millet: track f = total proteins; g = salt extracted proteins; h = 70% ethanol-extracted proteins; i = 50% propan-1-ol, 2% acetic acid and 2% 2-mercaptoethanol-extracted proteins. Track j: $M_r$ markers, 7 = 16,900d, 8 = 14,400d, and 9 = 8100d (Tatham *et al.*, 1996. Reproduced with permission from Elsevier).

The propan-1-ol extracts also contained minor prolamin bands with $M_r$s of about 40.5, 42.5 kDa in teff and 48.5 and 60.0 kDa in finger millet (Fig. 6.7, tracks e and i).

The relationship between the prolamins of different millets are clear in the photograph taken from Shewry and Halford (2003) with the major bands from all of the grains being of the same size (Fig. 6.8).

Vidyavathi *et al.* (1983) measured protease activity in germinating ragi using the native trypsin inhibitor/α-amylase inhibitor as one substrate and hemoglobin as the other. Both activities peaked at 3 days of germination. The optimal pH for proteolytic activity was pH 2.5 for the former and pH 5.0 for the latter. The protease activity measured using the inhibitor as substrate required the presence of intact sulphydryl groups where as the protease activity against haemaglobin did not. Protease activity against the inhibitor was also curtailed by DNME (diazoacetyl-DL-norleucine methyl ester), DTNB (5,5′-dithiobis-nitrobenzoic acid) and cystatin. Thus, it appears that both a cysteine proteinase and an aspartic (carboxyl) protease are present in germinating ragi. Shivaraj *et al.* (1982) purified a trypsin/chymotrypsin inhibitor from ragi. This inhibitor like the α-amylase/trypsin inhibitor inhibited mainly the caseinolytic activity of trypsin and not its esterase activity. It was reported to be a glycoprotein. The antitryptic activity was heat stable, while the chymotrypsin activity was much less so. The antitrypsin activity was also stable to pepsin treatment. Trypsin was inhibited noncompetitively (reducing $t$ maximum rate ($V_{max}$)

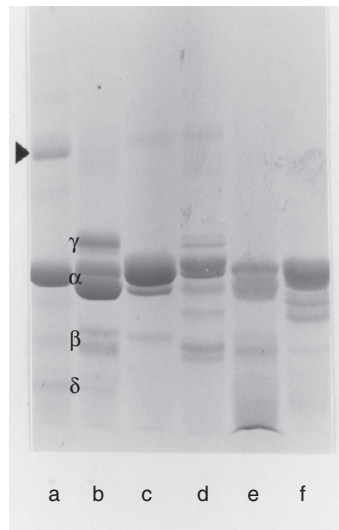


**FIGURE 6.8** SDS-PAGE of prolamin fractions from (A) pearl millet, (B) maize, (C) sorghum, (D) coix, (E) teff, (F) finger millet (Shewry and Halford, 2003). Reproduced with permission from Prof. Shewry.

without altering affinity of catalyst for substrate), while chymotrypsin was inhibited uncompetitively (increasing apparent affinity of the enzyme for substrate while decreasing maximum rate).

Beckles *et al.* (2001) showed the presence of a soluble cytosolic ADPG glucophosphorylase in barley. They then measured the ratio of the levels of ADPG to that of UDPG in the endosperm of many cereal endosperms including that of *E. coracana*, tomato seeds, bean cotyledon, and roots of yam and taro. The ratio varied from 0.30 to 0.64 (0.38 in the endosperm of *Eleusine*) and varied from 0.01 to 0.04 in the others. The higher ratio in the cereal endosperm was taken as an index of greater activity of the enzyme in these tissues.

Latha and Muralikrishan (2007) reported the purification of an acetic acid esterase from malted ragi. The $M_r$ was estimated to be 19.7 kDa. The enzyme was most active against wheat water-soluble polysaccharides and against synthetic substrates, such as *p*-nitrophenyl acetate and α-naphthyl acetate. The pH optimum for enzyme activity was between pH 6.0 and 9.0 and the temperature optimum was 45 °C. The enzyme was inhibited by $Ca^{2+}$ and activated by $Fe^{3+}$. A procedure for the isolation of acetic acid esterase from malted finger millet using buffers containing glutathione, calcium, Triton, and polyvinylpyrrolidine was developed (Latha and Muralikrishan, 2008a). These workers also showed that the enzyme activity doubled when gibberilic acid was added to the grain during germination.

Ferulic acid esterase increased in activity during germination, peaking at 96 h. The purified enzyme was 16.5 kDa in size, inhibited by iodoacetamide and Para Chloro Mercuric Benzoate, and activated by the serine homolog, eserine. The optimal pH for enzyme activity was determined to be pH 6.0. $Ni^{2+}$, $Zn^{2+}$, $Co^{2+}$, $Cu^{+}$, $Cu^{2+}$, oxalic, and citric acid activated the enzyme, while 5 mM $Fe^{3+}$ inhibited the enzyme's activity of releasing ferulic acid from water-soluble polysaccharides from wheat, maize, and ragi (Latha *et al.*, 2007). The viscosity of wheat and ragi water-soluble nonstarchy polysaccharides and their ability to stabilize foams decreased after treatment with either enzyme (Latha and Muralikrishan, 2008b). Deacetylation of xanthan and locust bean gum solutions by the acetic acid esterase resulted in increased viscosity.

Upadhya *et al.* (1985) purified a carboxyesterase from germinating ragi using ion exchange and gel exclusion chromatography of ammonium sulfate precipitated extracts. The molecular weight of the enzyme as estimated by gel chromatography was estimated to be 70,000 and the PI determined to be 5.1. The enzyme was inhibited by dichlorvos and phosphamidon, two organophosphate insecticides.

Glycerolphosphatase and pyrophosphatase activities increased during germination. The optimal pH for these enzymes was pH 5.6 and 5.0, respectively, and the optimal temperature was 45 °C. Both enzymes were be inhibited by fluoride (Chandrasekhara and Swaminathan, 1954).

Chandrashekara and Swaminathan (1953b) examining crude extracts of ragi for the properties of the amylases contained therein. The pH optimum for enzyme activity was pH 4.6, the temperature optimum was at 60 °C, and no effect was observed for added salt. They reported that after heating to 70 °C for 15 min, a procedure claimed to destroy β-amylase, the enzyme activity was reduced by half.

Gimbi and Kitabatake (2002) used blocked *p*-nitrophenyl maltoheptaoside and *p*-nitrophenyl maltopentoside to measure α- and β-amylase activity during germination of finger millet. Highest α-amylase activity was obtained from grains germinated at 15 °C while the highest β-amylase activity was obtained in grains germinated at 30 °C. β-Amylase was inactivated at 70 °C for 10 min while α-amylase lost 90% of its activity at that temperature after 40 min. The pH optima was 5.4 and 6.0 for α-amylase and β-amylase, respectively. At least three α-amylase bands were visible on activity stained polyacrylamide gels.

Nirmala *et al.* (2000) noted a decrease in starch content in the grain from 65% to 43% after 96 h of malting. The activity of α-amylase increased between 24 and 72 h, declining thereafter. The activity of α-glucosidase peaked at 48 h, while that of invertase and xylanase increased after 48 h of germination. The levels of glucose, fructose, sucrose, maltose, and maltotriose increased as germination progressed. Nirmala and Muralikrisha (2003a) purified three α-amylases from ragi germinated for 72 h. These enzymes differed in their mobility on PAGE gels but all of these enzymes were 47 kDa in size. The enzymes were optimally active at 45–50 °C, and their pH optima ranged from 5.0–5.5. They were activated by $Ca^{2+}$ and inhibited by $Al^{3+}$, $Fe^{3+}$, and $Hg^{2+}$. The $K_m$ for the three enzymes using ragi as the substrate was 0.59, 1.1, and 0.53 while the $v_{max}$ (u/mg protein) was 2381, 1111, and 2778, respectively. The $K_m$ of these enzymes for ragi starch was the lowest. Nirmala and Muralikrishna (2002, 2003b) reported that the α-amylase, known as α-3, was the more active of the three amylases isolated by them from germinating ragi when starch granules from either ungerminated or germinated ragi was used as substrate. Maltose and maltose G7 was first released by the three amylases acting on starch isolated from ungerminated grain and the amount of G4 maltose subsequently increased. Starch isolated from germinated grain became more accessible to the enzymes and a greater amount of G3 and G4 maltoses were released from germinated than from ungerminated ragi starch particularly by the α-amylase, α-3. The $K_m$ of the three enzymes that were isolated by Nirmala and Muralikrishna (2003a) was lowest for ragi and rice starches and greatest for maize and wheat starches. The enzymes released G4, G5, and G7 and those greater than G7. The G3 content increased with time while that of G7 decreased (Nirmala and Muralikrishna, 2003a). Chithra and Muralikrishnan (2007) and Muralikrishnan and Chithra (2008) purified an endoxylanase from ragi germinated for 96 h.

The enzyme approximately 29 kDa in size, was activated by many divalent cations such as $Ca^{2+}$ and $Mg^{2+}$ and was inhibited by chloromercuricbenzoate, citric acid, and oxalic acid. It is interesting to note that the starch from germinating ragi unlike those of sorghum and bajra are not pitted during germination (Malleshi and Klopfenstein, 1998a). Chandrasekher *et al.*, 1981 compared the inhibitory activities of extracts from eight species of millet on amylase from human, bovine, porcine, and endogenous plant sources. Extracts from pearl millet and finger millet inhibited all four enzymes. The inhibitory activity of the finger millet extract was nondialyzasable, heat resistant, and inactivated by pepsin.

## A. Trypsin and α-amylase inhibitor

Shivraj and Pattabhiraman (1981) purified an α-amylase inhibitor 14.3 kDa and an 16.5 kDa α-amylase/trypsin inhibitor (RATI) in size, from ragi. The modification of the arginine residues with cyclohexane-1,2-dione resulted in a loss of 85% of the antitryptic activity. Modification of amino groups by 2,4,6-trinitrobenzenesulfonic acid resulted in an almost complete loss of amylase-inhibitory activity. Shivaraj *et al.* (1982) using enzyme affinity methods purified an α-amylase/trypsin inhibitor which inhibited the caseinolytic activity of trypsin but not its esterase activity. It was shown to be a glycoprotein. The amylase trypsin inhibitor inhibited trypsin uncompetitively. The α-amylase/trypsin inhibitor was most against human pancreatic and porcine pancreatic amylase and less against the human salivary enzyme. Saxena *et al.* (2007) standardized a three-phase system containing water, ammonium sulphate and *t* butanol to concentrate the α-amylase and trypsin inhibitor from flour extracts. Using repeated extractions, they were able to concentrate the α-amylase inhibitor and trypsin inhibitor activity 20- and 16- fold with an yield of 39.5% and 32%, respectively. The protein was found in a layer between water and *t* butanol. The purified protein showed as a 14 kDa band on SDS gel electrophoresis and contained both activities. Campos and Richardson (1983) sequenced the entire protein. Velanakar and Murthy (1984) noticed the similarities and differences in sequence between the ragi Bowman Birk Inhibitor and that of other cereals. José-Estanyol *et al.* (2004) reviewed the conserved nature of the eight cysteine motif in lipid transfer proteins and in protease inhibitors among various plants.

Strobl *et al.* (1998) determined the structure of the RATI when in association with the yellow meal worm α-amylase. They concluded that the N-terminal part of the RATI, especially the first serine residue and the pro52–Cys55, complexed with the active site of the enzyme: Asp185, Glu222, and Asp287. Arg 61, Val67–Ser70, Gly72, Thr107–Gly110, and Leu115–Leu117 of the RATI interacted with the amylase at a site above the N-terminal/active site interaction. The carboxy terminal of the

inhibitor may also be involved. Gourinath *et al.* (2000), after examination of crystalline RATI using X-ray at 2.2 Å resolution both in the presence and absence of α-amylase, observed that RATI contained four α helices and two short β strands. The trypsin-binding loop was determined by αI and αII helices. The N-terminal region appeared to form an extensive linkage with α-amylase.

Alam *et al.* (2001) studied the inhibitory action of CNBR fragments of the RATI on amylase 2 purified from porcine pancreas and reported that the fragment comprising the first 10 amino acids of the N-terminal segment inhibited amylase competitively. NH2-Ser-Val-Gly-Thr-Ser-Cys-Ile-Pro-Gly-OH was most effective. A serine to alanine transformation diminished the inhibitory effect. Blocking the N-terminus abolished the α-amylase inhibitory activity. It may be presumed that the abolition of the α-amylase inhibitory activity with 2,4,6-trinitrobenzenesulfonic acid as observed by Shivraj and Pattabhiraman (1981) may have been due to its interaction with cysteine. Figure 6.9 shows a representation of this ragi protein with its ability to bind both α-amylase and trypsin with its two arms.

Rocher *et al.* (1992) reported the presence of an 11-kDa protein from oat endosperm that displayed a great resemblance in sequence to that of the ragi bifunctional α-amylase inhibitor.

Among the EST database of ragi sequences, there are two groups of bifunctional proteinase inhibitor trypsin α-amylase from seeds of ragi sequences. The upper clade was further subdivided (Fig. 6.10). Wang *et al.* (2008) concluded that there was great diversity in the sequence of different Bowman–Birk inhibitors in emmer wheat both within and between populations.

## IX. PROCESSING AND UTILIZATION

White-colored grain is mostly preferred for porridge and the brown-colored varieties are used for traditional opaque beer brewing in southern Africa (Gomez, 1994). Ragi/finger millet is made into porridge (Ugali/Sima/Saza) and for making unleavened bread. The malted food is used as a weaning food and is used for making fermented beverage. In Ethiopia, ''Injera'' is made often from a mixture of teff and finger millet grain flour. (Chrispus and Oduori, 2005). In Africa, finger millet is used in the manufacture of alcoholic or nonalcoholic beverages after malting. The flour is often made into porridge or roasted in banana leaves or maize husks after slight wetting. Flour is pounded with bananas, made into flat sheets, and fried or baked (de Wet, 2006).

Kurien and Desikachar (1962) equilibrated moistened ragi for 2 h prior to grinding. The resultant flour was sieved between each operation. Steaming of the moistened grain prior to grinding resulted in a maximum

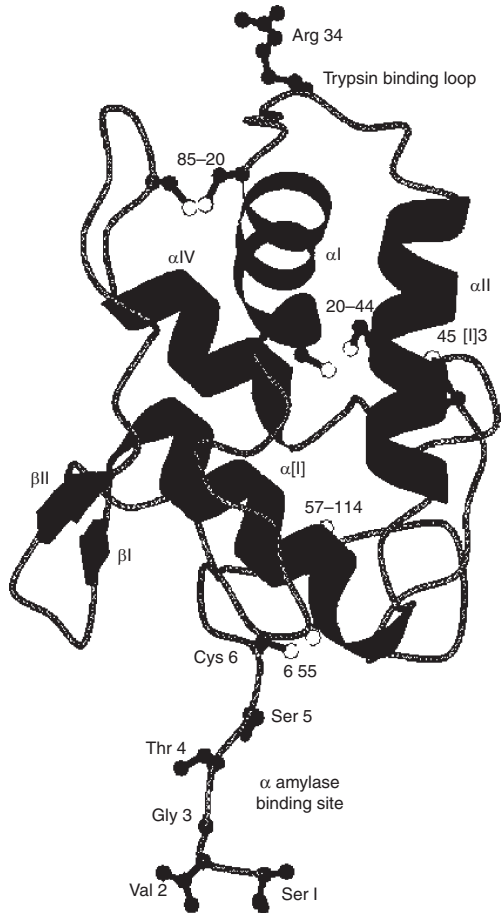


**FIGURE 6.9** Depiction of the two heads of the ragi double headed α-amylase and trypsin inhibitor. (Gourinath *et al.*, 2000. Reproduced with permission from the IUCr).

yield of flour. These workers suggested the use of wet milling to increase the yield of flour. Malleshi and Desikachar (1981a) used ''a short period of moist conditioning of the grain'' prior to grinding in a laboratory roller mill, a hammer mill, and a plate grinder. A 0.32 screen was installed when the hammer mill was used and a 200-mesh British Standard Sieve was used in conjunction with the plate mill. A 65% yield of white flour was obtained with either the hammer mill or the plate grinder. The roller mill flour offered a lower yield. Shankara *et al.* (1985) fixed a set of sieves to the same motor as the plate grinder and the flour was thus refined just after grinding. Most of the endosperm was recovered as the flour was passed through 60-mesh BSS sieves. Katti *et al.* (2008) reported the properties of the flour made from ragi in five different pulverizers *viz.* plate, emery,

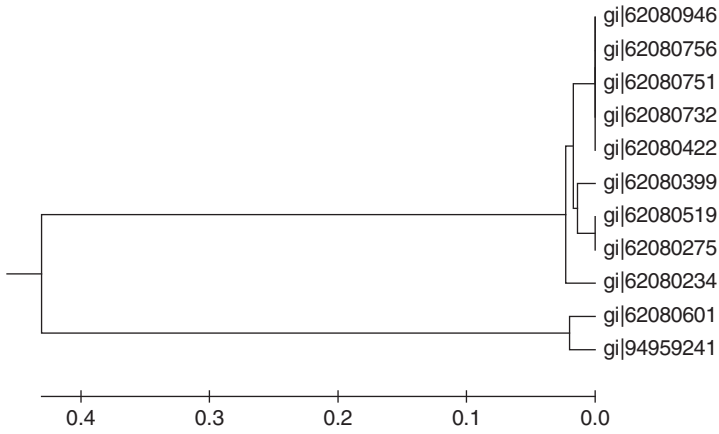


**FIGURE 6.10** Divergences in sequence of some ragi EST clones from that of an authentic Bowman Birk inhibitor (gi|94959241) using the Maximum Composite Likelihood Pairwise distance calculation and Mega4 (Tamura *et al.*, 2007).

hammer, pin, and roller flour mills. Fifty-three percent of the particles produced in an Emery mill were below 63 μm in size and was the finest flour encountered. The d*E* values or differences of reflection value between the white body (barium sulfate) and that of the sample were calculated. The d*E* was lowest for flour made with the Emery mill and highest with flour made from the roller mill. The highest level of damaged starch was with that from the roller flour mill followed by that made with the Emery, plate, pin, and hammer mills in decreasing order. The roller mill flour also had the lowest peak viscosity and hot paste viscosity and the highest cold paste viscosity among the flours tested. It was suggested that flours from different mills would be suitable for different food products depending on the texture needed in the final product.

Hadimani and Malleshi (1993) compared various properties of flour from ragi grain with flour from six other millets. The pasting behavior of ragi, pearl millet (*Penisetum thypoideum*), and foxtail millet (*S. italica*) as seen on the Brabender viscoamylogram showed low peak and setback viscosities. High peak viscosities and very high setbacks were characteristic of the pasting behavior of bran yard, little proso, and kodo millet flours. Malleshi *et al.* (2004) first optimized the roller clearance to obtain the clearest demarcation between the bran and flour fractions. The protein, fat, and ash contents were higher for the ragi flour than for the bran fractions when milled through either of the two different roller mills that they used: a quadramat junior or ERS. Finger millet flour contained smaller particles than flour made from sorghum and pearl millet attributable to the floury nature of the finger millet endosperm.

Rao *et al.* (2004) reported an effect of water-insoluble hemicelluloses on the gelatinization temperature but no effect on farinograph characteristics and suggested that they be used only as foam stabilizers. Rao *et al.* (2007) studied the effect of the addition of water-soluble and -insoluble non-starchy polysaccharides from unmalted and malted ragi to wheat for dough-forming characteristics and pasting behavior using a Brabender viscoamylograph. The viscosity of hemicellulose B from native ragi was higher than that isolated from malted ragi. The water-soluble fraction derived from malted ragi affected the Brabender Farinograph and viscoamylograph curves more than did any other fraction. There was an improvement in loaf volume, specific loaf volume, and decreased firmness. The authors concluded that it was possible to add these polysaccharides from ragi to wheat to provide improved functional properties.

''Ragi contains a high percentage of starch, but the digestibility and biological value of its proteins are superior to low-grade rice'' (Gangulee, 1939). Supplementing ragi with peanut flour and lysine increased the protein efficiency ratio (PER) to 2.98 almost on par with that of skim milk. Such a combination was recommended for use as weaning food (Daniel *et al.*, 1967). PER, nitrogen retention, and serum protein were higher in rats fed more ragi husk prepared using a wet grinding procedure and glucoamylase (Kanchana and Shruplekar, 1983).

Doraiswamy *et al.* (1969) fed boys between 6 and 12 years old, 350 g of ragi daily in three meals (''cooked and served as round balls at the three meals'') for 6 months. The ragi was supplemented with jaggery, or jaggery sugar and lysine, or jaggery and Lucerne leaf protein or sesame. Weight, height gain, hemoglobin, and RBC count was highest in children fed a diet of ragi supplemented with leaf protein (20 children in each group). The apparent protein digestibility (%) after 3 months into the trial was 55.1, 60.2, 66.0, 64.4 (six boys in each group), respectively.

Malleshi and Desikachar (1981b) reported that the bulk volume of puffed ragi in 14 varieties tested varied from 3.9 to 7.7 ml/g. Baskaran *et al.* (1999) tested the acceptability of supplementary foods prepared from popped grains, defatted soy, vegetable fat, vitamins, and minerals in 45 lactating and pregnant women and in a thousand school children. Popped bengal gram was added to some formulations. The grains tested were wheat, ragi, bajra, and sorghum. Wheat-based foods were the most preferred followed by that of ragi. Foods prepared from bajra and sorghum were least liked. Baskaran *et al.* (2001) noted a PER of 2.8–2.9 for rats fed ragi, puffed bengal flour, and defatted toasted soy flour or only ragi and defatted toasted soy flour (comparable to a PER of 3.0 obtained from rats fed a skim milk protein diet). The net protein utilization (NPU) and body weight gain was slightly lower for the diets incorporating popped bengal gram flour along with soy than those that contained only ragi and soy flour. The NPU for these foods ranged between 62 and 68 (65–66 for

ragi-based foods), while it was 73.5 when rats were fed a diet with skimmed milk protein as the source of protein. Baskaran *et al.* (2000a) recommend that supplementary foods made from wheat (*Triticum vulgare*), ragi (*E. coracana*), bajra (*Pennisetum americanum*), or sorghum (*Sorghum bicolor*) blended with soy (*Glycine max*) or bengal gram (*Cicer arietinum*) be maintained at about 10% (ERH 68–70%) to prevent mold growth in them. The use of high-density polyethylene and polypropylene was recommended as packing material.

Sastri (1939) mentions that the popular use of ragi for malting began in India prior to 1917. A method for the production of ragi malt extracts is described therein. Ragi malt extracts are prepared by 40 h of steeping, germination for 5–6 days, kilning for 24 h, and curing at 95 °C for 30 min. The best malt extracts were obtained by percolating water at 70 °C through broken grain, followed by filtration and concentration to a honey-colored product. Chandrasekhara and Swaminathan (1953a) standardized conditions for the preparation of malt extracts after mashing germinated ragi and wheat followed by filtration and concentration of the extracts. Pradeep *et al.* (2010) obtained 15.6% alcohol (v/v) using a medium that contained more than 25% reducing sugar derived from ragi starch, peptone, yeast extract, glycine, ammonium sulphate, and magnesium sulphate after 72 h of fermentation. Supplementation of medium also increased cell viability. Addition of glycine increased production of fusel oil.

Malleshi and Desikachar (1979) reported variations in amylase activity from 75 to 199 units among nine ragi varieties germinated for 3 days. The paste viscosity of gruels prepared from these germinated ragi samples varied from about 48 to 18 cP. When the amylase activity is greater, the hot paste viscosity is less. The white ragi (WB1) germinated poorly and had the lowest amylase activity. Malleshi and Desikachar (1982) measured the activity of α-amylase extracted from flours of germinating ragi, pearl millet, sorghum, maize, and Italian millet. The activity of α-amylase elaborated by ragi was the highest. They optimized the time and temperature of steeping and germination of ragi and the temperature and duration of kilning to reduce the loss of grain material due to leaching metabolism and the formation of sprouts while allowing for the maximum development of α-amylase activity.

Germination of finger millet at 15–20 °C for 4–5 days yields malt with high enzyme activity; this followed by drying to 12% moisture and kilning at 70 °C for 45 min caused the least reduction in activity of α-amylase and in availability of lysine Malleshi and Desikachar (1986a). Malleshi and Desikachar (1986b), after studying the malting characteristics of 11 cereals and millets, concluded that ragi produced adequate amounts of α-amylase during germination and malt with ''agreeable flavor and acceptable taste.'' Wheat was found to be comparable.

Malleshi and Klopfenstein (1998a,b) compared the nutrient and amino acid composition of flours prepared from malted barley, sorghum, pearl millet, and ragi. The yield of malted flour was 77.5% from ragi and 86.0% for sorghum with a drop in protein, fat, ash, and fiber. The content of methionine, phenylalanine, and lysine was higher in flour prepared from malted finger millet than in similar flours derived from sorghum and pearl millet. Brandtzaeg *et al.* (1981) used the process of heating a slurry of malted ragi and green gram to produce gruels of lowered viscosity than those of many proprietary weaning foods then in the market. The lowering of viscosity allowed more nutrients to be fed per feed than that which could be delivered by the proprietary weaning foods. The ragi was germinated for 48 h while the green gram was germinated for 24 h. Ragi was decorticated using a moist conditioning and grinding technique developed previously (Malleshi and Desikachar, 1981a,b). *Eleusine* was chosen from four other grains tested as it elaborated α-amylase in larger amounts and because the flour prepared from it was not bitter as was the case with *Pennisetum*. The protein content and ether extractives (lipid) decreased in germinated ragi endosperm from 8.2% to 6.8% and 1.8% to 1.4%, respectively. The calcium level dropped from 372 to 310 mg%, total phosphorus from 215 to 163 mg%, and phytate phosphorus from 72 to 42 mg%. The PER obtained from rats fed ragi malt was the same as that of diets prepared from unmalted ragi. Rao (1994) noted that the PER obtained from rats fed diets containing malted and ungerminated white ragi was higher than that in rats fed diets from malted and ungerminated brown ragi.

Malleshi and Desikachar (1986c) noted that there was no improvement in the PER of diets made from malted ragi, pearl millet, or foxtail millet. The PER of rats fed a weaning food prepared from ragi and green gram was 2.2 compared to 2.3 and 3.2 for proprietary weaning food and food containing skim milk. The NPU for the three diets were 51.6, 62.0, and 83.4; nitrogen retention was 48.6%, 56.1%, and 69.4%; the biological value was 73.8%, 79.2%, and 85.0%; and the true digestibility was 82.8%, 79.2%, and 91.9%. Thus though the extent of nitrogen retention was lower in rats fed malted weaning food, the true digestibility was comparable while similar parameters were highest in rats fed skim milk powder (Malleshi *et al.*, 1986c).

Sorghum, pearl millet, and ragi flours (60%) were blended with green gram flour (30%) and nonfat dry milk and extruded in a Brabender single screw extruder. There were indications that ragi starch was modified more during extrusion than were sorghum or pearl millet starches. The PER of the extruded finger millet food was 2.55, the biological value was 89.3%, the true digestibility was 79.65%, and NPU was 71.0 (Malleshi *et al.*, 1996).

Malleshi *et al.* (1989) noted that, under ambient conditions (27 °C and 65%RH), the shelf life of the malted ragi and green gram weaning food was 3 months and was reduced to 2 months under accelerated storage

conditions (38 °C, 92%RH). The shelf life of the product could be increased with the use of a laminated packaging material. During storage, there was a slow increase in the amount of free acids released at moisture contents above 10%. There was a fall in α-amylase activity and an increase in paste viscosity during storage.

Basappa *et al.* (1997) studied extensively the nutritive value of *chhang*, an alcoholic beverage made from finger millet, in the sub himalayan region. Ragi grains are cooked in water till grains swell and split. Starter cultures grown on rice flour (*phab* is the local name) are then added and fermentation allowed to proceed for 6 days. These workers used both traditional starter cultures and one made from *Lactobacillus* sp., *E. jibuligera* (an yeast that secretes amylolytic enzymes) and *S. cerevisiae* isolated from *phab*. Carbohydrate content halved after fermentation (78% to about 35%), while protein content increased from 7% to 10%. The average alcohol content was about 15% (g%). The fermented product contained 2–2.7% acid. There was substantial increase in the levels of riboflavin, pantothenic acid, niacin, and folic acid. Vitamin B12 was found only in the fermented material. It was suggested that the beverage could meet a substantial portion of nutrient requirements. The *chhang* contained more free amino acids than did unfermented flour extracts. The concentration of most essential amino acids increased during fermentation while that of Proline decreased. Varadaraj and Horigane (1998) reported that sprouting was faster at 25 °C than at 18 °C with germinating ragi. Sprouting was accompanied by a fall in prolamin content and concentration while a rise in the amount of globuins was noted. Proteinase and α-amylase activity increased with temperature and days of germination. Soluble sugars increased with time of germination. The count of mesophilic bacteria was the same for raw as well as for germinated grains while that of lactic acid bacteria (LAB) increased during sprouting and remained constant thereafter. The predominant bacteria from germinating ragi were the LAB. Antony *et al.* (1996) noted an increase in lactic and acetic acids during natural fermentation of ragi slurries. Reducing sugar content decreased initially and then increased as fermentation proceeded. Release of Xylose was noted. Lipid content decreased by about 43%. The predominant bacteria associated with the fermentation process were Gram-positive rods and cocci and presumed to be LAB. Antony and Chandra (1997) measured changes in ragi flour undergoing autofermentation. The pH dropped from pH 6.4 to 4.3 after 48 h, while the content of acetic and lactic acid increased. There was a substantial reduction in the total lipid content. The microbial load increased—primarily Gram positive cocci with no yeast. The use of a fermented slurry as the starter innoculum reduced the fermentation time. Antony and Chandra (1998) noted a decrease in phenolic and trypsin inhibitory activity in ragi batter fermented for 24 h with concomitant increase in protein digestibility and mineral availability.

Mugocha *et al.* (2000) reported the standardization of a traditional beverage made from fermented finger millet and milk: *Mageu*. They mixed in different proportions skim milk powder reconstituted in water and gruel made from finger millet, prior to fermentation with *Lactobacillus* cultures adapted from cereal fermentations (*Lactobacillus delbrueckii*, *Lactobacillus mesenteroides*, *Lactobacillus curvatus*, and *Enterococcus durans*) or those used commercially for the production of yoghurt (*L. delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*). The pH was brought down by the cereal cultures, but not by the milk-based ones. A thick gel was obtained with the milk-adapted cultures and the consistency of the fermented gruel milk mixture was best with one of the milk cultures incubated at 45 °C and stored at 7 °C.

The possibility of making gluten-free beverages from millets was explored in the review by Taylor and Emmambux (2008).

Finger millet (*E. coracana*) and *Phaselous vulgaris* were soaked, germinated for 48 h, dried, powdered, cooked, and fermented using *Lactobacillus salivarus* and then dried again. Mango puree and roasted peanut powder were added to the food. A viscosity of 1.0 Pa was set as the upper limit of viscosity for food fed to children. This viscosity was attained when 10% of the unprocessed ingredients were used in the preparation of food while it could be achieved with processed ingredients when 33% (w/v) was used. The availability of energy thus increased about 3.4 times. Decreases in phytate during processing may have resulted in the increased availability of calcium, zinc, and iron. Tannin was not detectable after processing. Adequate protein and energy could be delivered when fed to children below the age of 2 years thrice daily (Mbithi-Mwikya *et al.*, 2002). Tripathi and Platel (2010) studied bioavailability of externally added zinc (Zinc Oxide or Zinc Stearate @ 50 mg zinc per kg ragi flour) in finger millet flour during different periods of time till 60 days using an ''*in vitro* simulated gastrointestinal digestion procedure'' involving measurement of zinc coming out of dialysis bags containing the flour treated with pesin and acid and then with neutralized bile salts along with pancreatin. ''The bioaccessible zinc content in the unfortified finger millet flour was 0.18 mg/100 g, while that in the flours fortified with zinc oxide and zinc stearate was 0.25 and 0.49 mg/100 g.'' There was a slight decrease in bioavailability on storage of flour or when made into roti or dumpling. Supplementation with zinc did not affect free fatty acid levels in flour during storage. Addition of EDTA doubled the availability of zinc.

Desikachar (1972) observed that the setback of flours (greater tendency to retrograde) made from grains that had been steamed at atmospheric pressure and then steamed increased in comparison with flours made from native ragi. Flours prepared from either ragi steamed under pressure or steamed after soaking (parboiled) had a much lower peak and setback viscosity than those prepared from untreated ragi. Dumpling

(*Mudde*) or roti made from parboiled ragi was acceptable in texture and taste. The color of such products was darker than the usual and the use of white ragi was recommended. Shobana and Malleshi (2007) reported the results of their work on the decortication of parboiled ragi. The equilibrium moisture content of grains soaked at 70 °C was 41 ± 1%. Grains soaked at 70 °C were more suitable. Steaming the grains for 20 min at atmospheric pressure, 15 min at 1 kg/cm$^2$, 10 min at 2 kg/cm$^2$, 2.5 min at 3 kg/cm$^2$, 3 min at 4 kg/cm$^2$, and 2 min at 5 kg/cm$^2$ was required for disappearance of the white belly. The steamed material was dried in a cross flow drier at 40 and 50 °C. Grain dried at 40 °C was more pliable and could be decorticated. Of the many mills used for decortication, the use of a horizontal disc mill (wherein both discs are embedded with carborundum) was found effective. Size grading helped in uniform removal of bran. Protein, fat, calcium, and phosphorus contents of the decorticated millet were 6.3%, 0.9%, 0.18%, and 0.10%, representing a 22%, 40%, 43%, and 40% reduction in values obtained for the starting whole grain. The parboiled and dried grains could be cooked to softness in 5 min. It was proposed that this product could be used either directly or for flaking (expansion) and in other traditional preparations where ragi is used—''mudde (stiff Porridge), roti (unleavened bread), and ambali (gruel).'' There was very little enzyme-resistant starch that could be isolated from popped, roller dried, extruded, expanded, parboiled, and malted ragi (Mangalla *et al.*, 1998).

Ushakumari *et al.* (2007) developed a process for the preparation of expanded ragi. After parboiling and decortication, the grains were conditioned to 40% moisture, flattened in a roller flaker, and then toasted in salt maintained at 220–225 °C for 6 s. Shape factor (ratio of measurements on two peripendicular axes), the expansion ratio (ratio of the volume of expanded millet to that of the decorticated millet of equal weight), apparent bulk density (volume of known weight of expanded millet), and sensory characteristics were the criteria used. The optimum conditions to prepare a product with the highest expansion ratio was then determined.

Enzyme-resistant starch is the subject of the work published by Roopa and Premavalli (2008).

## X. GLYCEMIC INDEX

Ramananthan and Gopalan (1957) were the first to investigate a ''strong clinical impression that patients with *diabetes mellitus* tolerate ragi (*E. coracana*) better than they did rice.'' Glucose levels in the blood of six normal male subjects and two diabetics (one male and one female) were measured after consumption of meals made up of cooked rice, parboiled rice, wheat, ragi, rice starch, or ragi starch. Ragi flour and ragi starch gave

the lowest glycemic response. This was true for both normal and the two diabetic subjects. Ragi starch released less glucose into the blood than did rice starch while after *in vitro* enzymic digestion, the differences between the two starches disappeared. Patel *et al.* (1968) found no reduction of blood glucose levels when the diets of eight diabetic males (40–80 years of age) were changed from rice to one with ragi as the staple grain.

Geetha and Parvathi (1990) reported that supplementation of diets with ragi for a month resulted in a larger lowering of fasting and post prandial glucose levels than did supplementation with other millets. Kavitha and Prema (1995) fed 20 noninsulin-dependent diabetic men with isocaloric lunches made from rice, wheat, ragi, and tapioca. Wheat diets supported a lower glycemic response than those of ragi, rice, and tapioca (in that order). The gylcemic response to *rotis* and *dosas* made from raw and germinated ragi *rotis* was lower than that from glucose or rice *dosas* in six noninsulin-dependent men. The gylcemic response on consumption of ragi *rotis* was lower than when wheat or rice *rotis* were consumed (Lakshmi Kumari and Sumathi, 2002). Shobana *et al.* (2007) prepared foods from whole wheat, decorticated ragi, popped, and flaked (expanded) rice, and a mixture of bengal gram, green gram, and black gram flours. Spices including cumin, pepper, cinnamon, asafoetida, turmeric powder and tamarind powder, fenugreek, guar gum, amla, and gurmar (*Gymnema sylvestre*) were added to a total extent of 11%. Oil, skimmed milk powder, and vitamins and minerals were then added. The incremental area of the glucose curve for the test meal was investigated in five male and three female subjects and was expressed in relation to that of white bread (Glycemic Index). The glycemic index per gram of available carbohydrate was calculated (Glycemic Load). The average GI values for the wheat, ragi, flaked, and popped rice-based foods were 55.4 $\pm$ 9, 93.4 $\pm$ 7, 105 $\pm$ 6, and 109 $\pm$ 8, while the GL values were 28$\pm$2, 47 $\pm$ 3, 53 $\pm$ 5, and 55 $\pm$ 4, respectively. Thus, even after decortication, the glycemic index of ragi was lower than of the two rice products studied.

Mani *et al.* (1993) measured the glycemic index in noninsulin-dependent diabetes mellitus (NIDDM) patients. Six groups of six patients each were formed. Each group was fed one of six recipes tested: varagu (*Paspalum scorbiculatum*), varagu in combination with green gram dal (*Phaseolus aureus* Roxb.), varagu in combination with whole green gram, bajra (*P. typhoideum*), jowar (*Sorghum vulgare*), and ragi (*E. coracana*). The glycemic index was varagu (*P. scorbiculatum*) 68 $\pm$ 8; varagu + green gram dal (*P. scorbicultam* + *P. aureus* Roxb.) 78 $\pm$ 12; varagu + whole green gram 57 $\pm$ 6; bajra (*P. typhoideum*) 55 $\pm$ 13; jowar (*S. vulgare*) 77 $\pm$ 8; ragi (*E. coracana*) 104 $\pm$ 13. In their experience, ragi was not very effective in lowering glycemic index while varagu was most effective.

## XI. RESISTANCE TO HERBICIDE AND TRANSFORMATION

Eapen and George (1989) described a protocol for producing somatic embryos from ragi. Initially, callus was derived from caryopsis and isolated shoot tips in basal MS medium supplemented with picloram (4 mg/L) and kinetin (0.5 mg/L). The callus was transferred to lower levels of picloram to induce somatic embryos. The embryos germinated in a medium devoid of hormones. Shoot and root elongation took place in medium containing kinetin or zeatin at (1 mg/L) in combination with reduced levels of picloram (0.1 mg/L). Yemets *et al.* (2008) using a naturally mutated α-tubulin gene from *E. indica* providing resistance to the herbicide Trifluralin, TFL (2,6-dinitro-*N*,*N*-dipropyl-4-(trifluoromethyl) benzenamine) developed a biolistic method for the transformation of *E. coracana*.

In goose grass (*E. indica*), two alleles of α-tubulin 1 (each is the result of a single unique point mutation) have been described, which confer either an intermediate or high level of tolerance to a number of antimicrotubule herbicides, for example, dinitroanilines and phosphoroamidates (Anthony and Hussey, 1999; Yamamoto *et al.*, 1998; Yamamoto and Bird, 1999; Zeng and Baird, 1997). A positive transformation system may be developed using these herbicides.

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