PROSPECTS FOR IMPROVING SORGHUM GRAIN QUALITY

J L Forsyth[1], M M O'Kennedy[2], A Grootboom[2] and P R Shewry[1]

[1]IACR-Long Ashton Research Station, Department of Agricultural Sciences, University of Bristol, Long Ashton, Bristol, BS41 9AF, United Kingdom,
E-mail: peter.shewry@bbsrc.ac.uk

[2]CSIR Food, Biological and Chemical Technologies (Bio/Chemtek), P O Box 395, Pretoria, 0001, South Africa,
E-mail: mokennedy@csir.co.za

Grains of most cereal species, like wheat, maize and sorghum, which represent the world's largest providers of food and consequently important economical commodities, contain inadequate levels of some essential amino acids, particularly lysine, threonine, tryptophan and methionine. These deficiencies arise from the amino acid composition of the grain storage proteins, called prolamins, which account for up to 80% of the total grain proteins. In sorghum these deficiencies are also exacerbated by cooking, which reduces sorghum protein digestibility. Similarly, binding of tannins to proteins can also reduce digestibility in high tannin lines. Our ability to improve the nutritional quality (defined as the content of essential amino acids) of sorghum grain protein by classical plant breeding is limited by the low level of variation in the gene pool available for crossing. Genetic engineering offers an opportunity to overcome this limitation by introducing wild type or mutant genes from other organisms. We will therefore briefly review this topic, initially discussing the availability of suitable genes and then the current status of the technology for genetic engineering of sorghum.

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench formerly *S. vulgare*) is an important cereal grain used as food and animal fodder. Sorghum is considered a primary staple food crop in the semi-arid tropics of Asia, Africa and South America and cultivated in dry areas with low soil moisture that are not suitable for maize. Grains of most cereal species, like wheat, maize and sorghum, which represent the world's largest providers of food and consequently important economical commodities, contain inadequate levels of some essential amino acids, particularly lysine, threonine, tryptophan and methionine. Wide variability has been observed in the essential amino acid composition of sorghum protein, probably because the crop is grown under diverse agroclimatic conditions which affect the grain composition (FAO, 1995). Lysine content was reported to vary from 71 to 212 mg per gram of nitrogen and the corresponding chemical score varied from 21 to 62. The methionine and tryptophan content on average are 87 and 63 mg per gram of nitrogen (FAO, 1995; http://www.fao.org/docrep/T0818E/T0818E0d.htm). These deficiencies arise from the amino acid composition of the grain storage proteins, called kafirins, which account for up to 80% of the total grain proteins[1]. These deficiencies are also exacerbated by cooking, which reduces sorghum protein digestibility[2]. Similarly, binding of tannins to proteins can also reduce digestibility in high tannin lines[2].

Our ability to improve the nutritional quality (defined as the content of essential amino acids) of sorghum grain protein by classical plant breeding is limited by the low level of variation in the gene pool available for crossing. Thus, only two mutant high lysine genes are currently available, the spontaneous mutant gene *hl* which was initially identified in an Ethiopian line[3] and the *P721 opaque* gene which was induced with ethyl methane sulphonate (EMS)[4]. Both of these lines can be defined as "low prolamin" mutants with pleiotropic effects on other grain characteristics. Hence it has proved difficult to incorporate the high lysine phenotype into varieties with high yield and acceptable agronomic performance and processing properties.

Genetic engineering offers an opportunity to overcome this limitation by introducing wild type or mutant genes from other organisms. We will therefore briefly review this topic, initially discussing the availability of suitable genes and then the current status of the technology for genetic engineering of sorghum.

Gene for high value proteins

Munck *et al.*[5] described the identification of a high lysine barley line called Hiproly, as a spontaneous mutant in the world barley collection. Subsequent studies showed that this differed from almost all the other high lysine cereal mutants in that the effect did not result from a decrease in the proportion of lysine-poor prolamins (with associated pleitropic effects on yield) but from increases in the proportions of four specific lysine-rich proteins[6]. These are the chymotrypsin inhibitors CI-1 (9.5 g% lys) and CI-2 (11.5 g% lys), beta-amylase (5.0 g% lys) and protein Z (a serpin proteinase inhibitor) (7.1 g% lys)[7]. Of these, CI-2 has received most attention, being used widely as a model protein to study folding pathways and structure/function relationships.

The major form of CI-2 comprises 83 amino acid residues including 7 lysines (i.e. 8.4 mol%). The three-dimensional structure shows a four stranded beta-sheet with a single

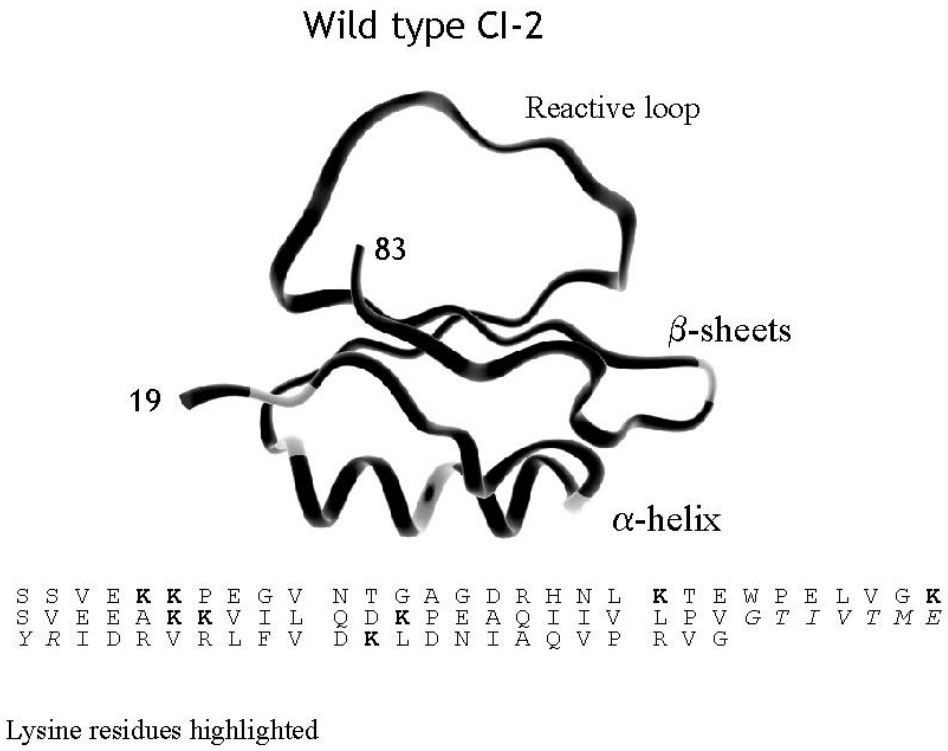

Figure 1. The three dimensional structure and sequence of the lysine-rich barley protein CI-2. Residues 1-19 are unstructured and therefore not shown. Lysine residues at positions 21, 36, 37, 43 and 72 are shown in light grey.
(Figure kindly provided by Dr. Frederic Beaudoin).

alpha-helix and reactive loop (Fig. 1), with the *N*-terminal 19 residues of the protein being unstructured. Roesler and Rao[8] have designed a number of mutant forms of CI-2 and shown that one form containing 20 mol% lysine (BHL4) had high stability *in vitro* indicating that it could be suitable for expression in transgenic plants.

We have also designed a series of mutant forms of CI-2, with additional lysine residues inserted into the loop or as a "hairpin" structure. The stability of the proteins expressed in *E. coli* is currently being assessed.

The use of a further high lysine barley seed protein, hordothionin, is discussed below. A number of methionine-rich proteins have been characterized, with most falling into two classes. Methionine-rich 2S albumin storage proteins occur in Brazil nut (26 mol% Met) and sunflower (16 mol% Met) and have been expressed in transgenic plants to increase seed methionine by up to 30% in seeds of tobacco and oilseed rape, by three-fold in *Vicia narbonensis* (using the Brazil nut protein)[9-11] and by 94% in

lupin (using the sunflower protein[12]). However, in the latter case, the total seed sulphur content was not increased and compensatory decreases in cysteine and methionine were observed. This indicates that increasing the transport of sulphur to the grain may be a requisite for increasing total grain methionine content. However, a further and more serious consideration is that both the Brazil nut and sunflower proteins are allergens[13,14], a property shared by a number of other 2S albumins[14]. Hence it is unlikely that proteins of this group will be used to engineer crops to be consumed by humans.

The second major group of methionine-rich proteins comprises the beta-and delta-prolamins of maize (zeins), sorghum (kafirins) and related species. These are discussed in a preceding chapter (Shewry and Halford, 2003, preceding chapter) and will not be discussed further here.

The third amino acid which is deficient in sorghum is tryptophan. Tryptophan-rich proteins do not occur widely but the puroindolines of wheat contain short motifs with three or five tryptophan residues[16]. These proteins are associated with grain softness in wheat and related cereals[17] so it is possible that their expression in sorghum could result in effects on grain texture.

Genetic engineering of sorghum

Biotechnology, in recent years, has provided a powerful means of genetically enhancing various previously recalcitrant monocotyledonous cereal crops. Five basic tools of technology have been developed for sorghum improvement: (1) *in vitro* protocols for efficient plant regeneration; (2) molecular markers; (3) gene identification and cloning; (4) genetic engineering and gene transfer technology to integrate desirable traits into the sorghum genomes; and (5) genomics and germplasm databases[18].

The production of transgenic sorghum plants via particle bombardment of immature zygotic embryos[19,20,21], immature inflorescences[22] and shoot tips[20], introducing mainly reporter and selectable marker genes, has been reported in the last decade. In addition, during the year 2000, transgenic sorghum plants were produced via *Agrobacterium*-mediated transformation using immature zygotic embryos as explant[23]. Recently, a functional gene which codes for a feedback insensitive dihydropicolinate synthase, the first enzyme of the lysine-specific pathway, was introduced into the genome of sorghum with the goal of producing transgenic sorghum plants with increased lysine content[20]. Zhao and co-workers[24] transformed grain sorghum with the high lysine analog (HT12 protein) of *Hordeum vulgare* alpha-hordothionin protein, which contains 44-residues including 12 lysine residues (27%). The coding sequence for this HT12 protein was under the control of the 27 kDa maize gamma zein promoter and terminator. Three copies of this cassette were linked together within one T-DNA borders. This vector was co-transformed with the selectable marker gene *bar* controlled by the maize ubiquitin promoter and pinII terminator. The authors[24] shown a 50% increase in lysine in the transgenic sorghum compared to the untransformed sorghum when screened with western blot and ELISA analysis. Similar to this approach, our strategy is to produce transgenic sorghum plants with elevated lysine and methionine contents by the introduction of genes encoding the methionine-rich maize beta-zein and the lysine-rich barley chymotrypsin inhibitor CI-2 proteins, in order to genetically enhance the nutritional quality of grain sorghum (O'Kennedy *et*

al., 2003 preceding chapter). Biolistic and *Agrobacterium*-mediated transformation protocols for selected lines have therefore been established to provide the technological basis for improvement of the nutritional quality and tolerance to, biotic and abiotic stress of grain sorghum.

REFERENCES

1. Taylor, J.R.N., Schossler, L. and Van der Walt, W.H. Fractionation of proteins from low-tannin sorghum grain. *Journal of Agricultural and Food Chemistry* **32** (1984) 149-154.

2. Taylor, J.R.N. and Belton, P.S. Sorghum. In 'Pseudocereals and Less Common Cereals' (P.S. Belton and J.R.N. Taylor, eds) Springer (2002) pp. 25-91.

3. Singh, R. and Axtell, J.D. High lysine mutant gene *(h1)* that improves protein quality and biological value of grain sorghum. *Crop Science* **3** (1973) 535.

4. Axtell, J.D., Van Scoyoc, S.W. Christensen, P.J. and Ejeta, G. Current status of protein quality improvement in grain sorghum. In 'Seed Protein Improvement in Cereals and Grain Legumes' IAEA, Vienna (1979) pp. 357.

5. Munck, L., Karlsson, K.E., Hagberg, A. and Eggum, B.O. Gene for improved nutritional value in barley seed proteins. *Science* **168** (1970) 985.

6. Bright, S.W.J. and Shewry, P.R. Improvement of protein quality in cereals. *CRC Critical Reviews in Plant Sciences* **1** (1983) 49-93.

7. Hejgaard, J. and Boisen, S. High lysine proteins in Hiproly barley breeding: identification, nutritional significance and new screening methods. *Hereditas* **93** (1980) 311.

8. Roesler, K.R. and Rao, A.G. Conformation and stability of barley chymotrypsin inhibitor-2 (CI-2) mutants containing multiple lysine substitutions. *Protein Engineering* **12** (1999) 967-973.

9. Altenbach, S.B., Pearson, K.W., Meecker, G., Staraci, L.C. and Sun, S.S.M. Enhancement of the methionine content of seed proteins by the expression of a chimeric gene encoding a methionine-rich protein in transgenic plants. *Plant Molecular Biology* **13** (1989) 513-522.

10. Altenbach, S.B., Kuo, C.-C., Staraci, L.C., Pearson, K.W., Wainwright, C., Georgesen, A. and Townsend, J. Accumulation of a Brazil nut albumin in seeds of transgenic canola results in enhanced levels of seed protein methionine. *Plant Moelcular Biology* **18** (1992) 235-245.

11. Saalbach, I., Waddell, D., Pickardt, T., Schieder, O. and Muntz, K. Stable expression of the sulphur-rich 2S albumin gene in transgenic *Vicia narbonensis* increases the methionine content of seeds. *Journal of Plant Physiology* **145** (1995) 674-681.

12. Molvig, L., Tabe, L.M., Eggum, B.O., Moore, A.E., Craig, S., Spencer, D. and Higgins, T.J.V. Enhanced methionine levels and increased nutritive value of seeds of transgenic lupins (*Lupinus angustifolius* L.) expressing a sunflower seed albumin gene. *Proceedings of the National Academy of Sciences USA* **94** (1997) 8393-8398.

13. Melo, V.M.M., Xavier-Filho, J., Lima, M.S. and Prouvost-Danon, A. Allergenicity and tolerance to proteins from Brazil nut (*Bertholletia excelsa* H.B.K.). *Food and Agricultural Immunology* **6** (1994) 185-195.

14. Kelly, J.D. and Hefle, S.L. 2S methionine-rich protein (SSA) from sunflower seed is an IgE-binding protein. *Allergy* **55** (2000) 556-559.

15. Monsalve, R.I., Villalba, M., Rico, M., Shewry, P.R. and Rodriguez, R. The 2S albumin proteins. In ' Plant Food Protein Allergens'. (E.N.C. Mills and P.R. Shewry, eds) Blackwell Science Ltd. (2003) In press.

16. Gautier, M.-F., Aleman, M.-E.,Guiro, A., Marion, D. and Joudrier, P. *Triticum aestivum* puroindolines, two basic cystine-rich seed proteins: cDNA sequence analysis and developmental gene expression. *Plant Molecular Biology* **25** (1994) 43-57.

17. Giroux, M.J. and Morris, C.F. Wheat grain hardness results from highly conserved mutations in the friabilin components puroindoline a and b. *Proceedings of the National Academy of Science, USA.* **95** (1998) 6262-6266.

18. Maqbool, S.B., Devi, P. and Stricklen, M.B. 2001. Biotechnology: Genetic improvement of sorghum (Sorghum bicolour (L.) Moench). In vitro Cell Dev. Biol. – Plant 37:504-515.

19. Casas, A.M., Kononowicz A.K., Zehr U.B., Tomes D.T., Axtell J.D., Butler L.G., Bressan R.A. & Hasegawa P.M. Transgenic sorghum plants via microprojectile bombardment. *Proc. Natl. Acad. Sci. USA*. 90 (1993): 11212-11216.

20. Tadesse, Y.S. 1999-2000 Ph.D. thesis. Genetic transformation of sorghum (*Sorghum bicolor* (L.) Moench) towards improving nutritional quality. Vrije Universiteit Brussel Instituut Voor Moleculaire Biologie, faculteit Wetenschappen, Belgium.

21.Emani, C., Sunilkumar, G. and Rathore, K.S. Transgene silencing and reactivation in sorghum. *Plant Science* 162 (2) (2002): 181-192.

22. Casas, A.M., Kononowicz, A.K., Haan, T.G., Zhang, L., Tomes, D.T., Bressan, R.A. and Hasegawa, P.M. Transgenic sorghum plants obtained after microprojectile bombardment of immature inflorescences. *In vitro Cell Development Biology* 33 (1997): 92-100.

23. Zhao, Z-Y., Cai, T., Tagliani, L., Wang, N., Pang, H., Rudert, M., Schroeder, S., Hondred, D, Seltzer, J. and Pierce, D. Agrobacterium-mediated sorghum transformation. Plant *Molecular Biology* 44 (2000): 789-798.

24. Zhao, Z-Y., Glassman, K., Sewalt, V., Wang, N., Miller, M., Chang, S., Thompson, T., Catron, S., Wu, E., Bidley, D., Kedebe, Y. and Jung R. In "Nutritional improved transgenic sorghum", proceedings of the 10th IAPTC&B Congress, (I.K. Vasil., ed.), June 23-28, 2002 . Orlando, Florida, USA. Kluwer Academic Press ISBN 1-4020-1126-1.