Special Lecture
on

Challenges in Developing Nutritionally Enhanced Stress Tolerant Germplasm

15 January, 2004
New Delhi

by

Dr. S.K. Vasal

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Progress Through Science

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INTRODUCTION

Plant breeding research has resulted into a succession of landmark achievements during the twentieth century. We have witnessed a series of agricultural revolutions beginning with hybrid corn revolution in the US and later in Europe, China and now expanding into several developing countries of Latin America and Asia. The list of crops deploying hybrid technology continues to expand covering even vegetable, horticultural and even self-pollinated crops like rice not amenable to hybrid research. Hybrid corn revolution was followed by green revolution in wheat and rice some thirty years ago. A demand driven livestock revolution is also underway in Asia contemplating demand for meat and animal products to double by 2020. We are currently in the midst of an exciting and perhaps most dramatic revolution of our times. There have been continuing increases in the area planted to transgenic crops. The countries in the forefront are USA, Canada, Argentina, Brazil and China and the principal transgenic crops are soybean, cotton, maize and canola. The adoption rates vary in different crops but are significantly higher in soybean compared to other crops. Two noteworthy traits in transgenic crops are herbicide tolerance and Bt insect resistance. During the seven-year period 1996 to 2002, the global area of transgenic crops increased from 1.7 million hectares to 58.7 million hectares in 2002. Accompanying gene revolution is also scientifically revealing and informative. Human genome is already mapped and some crop species like rice, maize and others will also soon be mapped. Hope with all this knowledge being generated at an accelerated pace we may realize the dream of hunger fighter, Dr Norman Borlaug to be able to transfer useful genes from one crop species to another for genetic resistance to biotic and abiotic stresses and enhanced nutritional quality traits. Very often he cites examples of transferring rust resistance from rice to wheat and some specified proteins as gliadin and glutelins from wheat to other species as maize and others. Recently Egyptian scientists have successfully transferred drought tolerance from barley to wheat. Examples of this kind will certainly help plant scientists to tackle complex and difficult problems in an effective, efficient, and cost effective manner with a greatly reduced time span.

As we enter into 21st century, world continues to face numerous problems and challenges. One-fifth of the world population still lives in poverty. At least 800 million people go hungry every day. An estimated 840 million are under- or malnourished, an alarming number 2 billions suffer from micro-nutrient deficiency of Zn, Fe, and vitamin A, and some devastating diseases–HIV/AIDS virus (38.4 millions) and trachoma causing night blindness (150 million) affect drastically individuals productivity, natural resources continue to be degraded and demand for water is growing steadily by all users including agriculture. A few driving forces will continue to make the task of agricultural scientists difficult and cumbersome. Population growth has failed to stabilize and continues to grow despite all efforts by several governments. An estimated 73 million people are added every year and at this rate we expect a world population of 7.5 billion by 2025. Implication of this increase will be to stay engaged in agricultural research more seriously to feed additional 1.3 billion people. Increased consumption of livestock and animal products will pose additional challenges. The projections are that demand for meat will increase from the present level of 198 million tonnes to 303 million tonnes by 2020 (Delgado *et al.*, 1999). This figure reflects an increase in demand of poultry by 85 per cent, beef 50 per cent and pig meat 45 per cent. The demand of cereals to raise livestock will jump to a new high and is projected to double by 2020. Water requirement for livestock production will also increase by 62 per cent over the 1995 levels. Growing water demand by all users will make it a scarce and precious resource. The current water usage of 3906 cubic kms is projected to increase at least by 50 per cent and it is likely that increased share to agriculture or irrigation will be no more than 4 per cent. As we are all well aware that agriculture is the main user of water and in the last century alone there was almost five-fold increase to a total of 250 million hectares irrigated worldwide.

In my talk I intend to discuss some breeding objectives which are important but certainly complex and difficult. The first objective is to affect nutritional improvements in crops and the second is to build-up tolerance/resistance to biotic and abiotic stresses. Perhaps more challenging will be when nutritionally enhanced germplasm is available and it needs to be improved for stress traits which are lacking in this germplasm. In respect of nutritional improvements, one may think of several traits which are worthy of alteration to reflect enhancement in grain quality. To mention a few will include oil content, protein content, protein quality, mineral content, vitamins, anti-nutritional factors and perhaps many more. Challenges and opportunities for affecting a change in one or more traits will vary in different crops. Goals should therefore be pursued carefully as to what is practical and achievable without adversely affecting yield. Resistance to biotic stresses is an important breeding objective in practically all the crops. The nature of diseases could be fungal, bacterial, viral, nematodes, insect pests and in some situations even parasitic weeds. In general it is fair to say that good progress has been made with respect to most of the prevailing diseases in every crop. Challenges, however, remain for some diseases that are difficult and complex and for which artificial inoculation techniques are not fully perfected. Insect resistance work had been more difficult despite efficient mass rearing facilities and infestation techniques. Nevertheless, encouraging progress has been made with respect to some species of insects. Transfer of resistance to other genetic backgrounds also had been difficult because of low heritability of the trait and faulty methodology used. Work on parasitic weeds is limited but lot of work and resources have been used to improve tolerance to striga in Africa particularly at IITA in Nigeria. The work on abiotic stresses is regarded as important and highly prioritized but unfortunately resources devoted and work underway does not match the scale of problem(s) and losses incurred every year. Only a few institutions have taken this work seriously and made advances in perfecting techniques and identifying secondary traits that could aid in building tolerance or resistance to a particular stress. Abiotic stress traits of interest have been drought, low-N, water logging, acid soil tolerance, heat and high temperature, cold tolerance, high velocity winds, mineral and micro-nutrient deficiencies, high and low PH and soil toxicities and others. The focus of my presentation will be on cereal grain crops as attempts have been made to improve nutritional traits and some experience is already available worldwide. Also the emphasis will be on protein related traits using cyclic improvement procedures or known mutants that have been identified and found useful. My presentation will be in three main sections. The first section will cover protein-related nutritional aspects in different crops and then provide a detailed discussion on success story of Quality Protein Maize (QPM). The second section is devoted to in-depth discussion on abiotic stresses in general and drought and low-N in particular. Examples and experience cited will be on maize, based primarily on CIMMYT'S work at headquarters and in the regional programmes located worldwide. The last section will discuss breeding options, strategies and changing tactics in combining stress tolerant/resistant traits in QPM where lot of germplam has been developed and available for everyone's use.

PROTEIN-RELATED NUTRITIONAL IMPROVEMENT EFFORTS AND SOME ACHIEVEMENTS

Cereals play an important role in world agriculture. They contribute significantly to global food pool in achieving food and nutritional security. Considering area sown and annual production volume, they occupy an important position in world economy and trade as food, feed and industrial grain crops. In 2000, area harvested was 675 million hectares and production of 2059.8 million tonnes with an average yield of 3049 kilograms per hectare (Table 1). As can be seen, wheat, rice and maize are of prime importance but area and production from other crops such as barley, sorghum, oats, rye and millets are also quite significant. It may be noted that maize has great potential of yielding more per unit of land than other cereals. In Asia area devoted to cereals was 301.8 million hectares with a production volume of 938.8 million tonnes. This is almost 50 per cent of total world cereal production. Rice is the most important crop in Asia occupying almost half of the cereal area with a production of paddy rice touching 540 million tonnes. The other two important crops are wheat and maize, which rank second and third respectively. Other crops of importance with significant area are barley, sorghum and millets. Oats and Rye are also grown but area is quite small, less than one million hectare annually to each crop.

Table 1. World Cereal Statistics, Area, Yield and Production in 2000

Crop	Area (Million ha)	Yield (kg/ha)	Production (Million tonnes)
Cereals	675.631	3049	2059.8
Wheat	215.180	2706	582.2
Rice (paddy)	153.458	3863	592.0
Coarse grains	306.996	2882	884.7
Barley	55.698	2440	135.9
Maize	137.549	4336	596.4
Rye	9.896	2075	20.5
Oats	14.416	1811	26.1
Millets	36.161	752	27.2
Sorghum	42.805	1391	59.5

Source: FAO

Some of the cereal crops particularly rice, wheat and to some extent maize, sorghum and millets are consumed by humans as staple food to meet energy and protein requirements. Feed use of cereals in Asia is more in some countries than others but at least 158 million tonnes were used in 2000 for livestock. Food and feed use of cereals will be greatly prioritized in future in view of projected world population growth of eighty million people every year. Unfortunately much of the increase in population will take place in the developing countries mostly concentrated in South Asia. It is expected that demand for food and meat products will increase dramatically in the next two decades. A demand driven livestock revolution is underway in Asia and it is very likely that demand for meat and other animal products may almost double by 2020. This in turn will increase demand of cereals for feeding livestock. The demand for some cereals such as maize will increase more rapidly and will perhaps overtake demand for rice and wheat in the next two decades.

Cereal proteins vary in protein content but in general are of poor quality because of lack of balance in amino acid composition. Breeding for improved amino acid composition has been attempted in some crops and commercially exploitable high lysine varieties are now available at least in maize.

Protein Related Nutritional Characteristics of Cereal Grains

The crude protein content varies in different crops (Table 2). Rice is quite low in protein content (7 per cent). Intermediate levels of 9-10 per cent are encountered in maize, sorghum and barley. Wheat, oats and triticale exhibit high protein content of 12 per cent and more. In general, high protein content is inversely correlated with yield. In wheat and oats, however, high protein lines with good yielding ability are available. As far as protein quality is concerned, unfortunately all

Table 2. Protein and Lysine Content of Cereal Crops

Crop	Protein Content (%)	Lysine in protein (%)
Maize	8 – 11	1.8 – 2.0
Wheat	11 – 14	2.5 – 3.2
Rice	7 – 9	3.5 – 4.0
Barley	8 – 11	2.9 – 3.2
Oats	12 – 14	3.8 – 4.0
Sorghum	9 – 11	2.0 – 2.8

cereals are deficient primarily in lysine with a secondary deficiency in threonine or tryptophan (Table 3). The poor quality of proteins is attributed to high concentration of prolamin storage protein fraction in cereals. This particular fraction is practically negligible or devoid of lysine. The high level of this fraction is the sole cause of poor protein quality in cereals. The prolamin contents of major cereals fall into three distinct classes or groups (Table 4). High prolamin group constitutes 50-60 per cent of protein, as is the case in maize and sorghum, intermediate 30-40 per cent as in barley and wheat and low group with only 5-10 per cent as in rice and oats. The protein quality of cereals, like protein quantity, is inversely related to the prolamin content. Those groups of cereals like rice and oat, which have low prolamin content, thus exhibit superior protein quality. It may be pointed out that prolamin is one of the four protein fractions which make up the cereal protein. The other three fractions are albumins, globulins and glutelins which are soluble in water, saline solution, and alkali solution, respectively. The prolamins being soluble in alcohol solution are rich in proline and glutamine but are low in basic amino acids including lysine. Osborne and Mandel (1914) showed

Table 3. Limiting Amino Acids in Cereal Protein

Cereal	1st limiting	2nd limiting
Rice	Lysine	Threonine
Wheat	Lysine	Threonine
Maize	Lysine	Tryptophan
Sorghum	Lysine	Threonine
Millet	Lysine	Threonine
Tiff	Lysine	Threonine

Table 4. Prolamin Content of Major Cereals

Crop	Prolamin Fraction	Prolamin Group	Percent of Total Protein
Maize	Zein	High	50 – 60%
Sorghum	Kafarin	High	50 – 60%
Barley	Hordein	Intermediate	30 – 40%
Rye	Secalin	High	60%
Wheat	Gliadin	Intermediate	30 – 40%
Oats	Avenin	Low	10 – 12%
Rice	Prolamin	Low	5 – 10%

that rats of all ages went into rapid decline and eventually died if placed on a diet in which zein was the sole source of dietary protein. The prolamin fraction is named differently as is zein in maize, gliadin in wheat, kafarin in sorghum, hordein in barley, and avenin in oats. As indicated earlier, both oat and rice have good protein quality owing to low levels of prolamin. Despite high lysine in these two cereals compared to others, lysine is still the first limiting amino acid. Proteins from both these cereals have higher biological value relative to other cereal proteins. It is further interesting to point out that high protein content in oat does not adversely affect the biological value of protein.

Breeding Efforts for Improving Protein Quality in Cereals

People in the developing countries particularly in Asia consume cereal grains as staple food and derive their calories and protein requirements from such cereals. Nutritional improvement in such cereals through plant breeding efforts have been under active considerations for the past several decades but concrete breeding efforts could not be taken up in the absence of specific genes for such traits. Altering amino acid profile of cereal proteins and making them more balanced will impact hundreds of millions of people without altering their food habits and preferences.

Maize

To start with germplasm accessions were screened for genetic variability for lysine content. Variation was observed in maize but differences were rather small. It would have needed many years to elevate levels sufficiently high to make protein profile reasonably balanced in manifesting superior biological value. Despite increasing realization, the protein quality thus remained more of a concern but with no immediate solutions in sight as no good breeding options were available that could be deployed at that time to affect improvement. A beginning in genetic manipulation of protein quality began with the discovery of high lysine mutant opaque-2 (o2) (Mertz *et al.*, 1964) and a year later another mutant floury-2 (Nelson *et al.*, 1965) was discovered by Purdue University researchers. These exciting discoveries generated a lot of enthusiasm and hopes, and paved the way for improving protein quality in maize. It may be of interest to mention that these mutant alleles changed protein quality of endosperm and not that of germ. These mutants were able to alter amino acid profile of maize endosperm protein resulting in two-fold increase in the levels of lysine and tryptophan compared to normal genotypes. The phenotype of the mutants was easily recognizable from their soft chalky appearance. Alterations were noticed in other amino acids as well. An increase was observed for amino acids such as histidine, arginine, aspartic acid and glycine and a decrease in glutamic acid, alanine and leucine. Leucine, isoleucine ratio was improved and became better balanced, which in turn is considered beneficial as it helps to liberate more tryptophan for more niacin biosynthesis and thus help to combat pellagra. These mutants bring about improvements in lysine and tryptophan by suppressing lysine-deficient zein fraction without altering contribution of other fractions. A reduction in zein fraction causes proportional elevation of other fractions rich in lysine thus resulting in elevation of these two amino acids in protein, but not on an absolute basis of per unit of endosperm in the grain. Search was continued for new and better mutants, however, ones found (o7, o6, fl3) were in no way better than opaque-2. Breeding efforts were thus initially concentrated on opaque-2 and floury-2. Floury-2 did not hold its promise and thus was also dropped in early seventy's. High quality protein materials developed using o2 did not show competitive performance to their normal counterparts. They suffered from a number of problems including lower grain yield, unacceptable soft chalky endosperm, slower drying, more vulnerable to ear rot pathogens and to stored grain pests. These agronomic deficiencies were serious enough to cause decline in interest and even completely abandoning these efforts in many programmes. Only a few institutions such as CIMMYT, Purdue University, Crows Hybrid Seed Company in Milford, Illinois, and University of Natal in South Africa continued sustained efforts choosing different options to develop normal looking agronomically acceptable varieties and hybrids. Success of approach deployed at CIMMYT and germplasm developed will be described in detail in later section.

Barley

Discoveries of nutritionally superior mutant alleles o2 and fl2 in maize stimulated interest in other cereal crops. Screening efforts to identify similar type of mutant alleles as in maize were initiated at Sweden and Denmark. A high-lysine gene (Hily) was identified from the Hiproly source (Munck *et al.*, 1970) and another gene Riso 1508 was identified in Denmark (Doll 1971, 1983, Ingverson *et al.*, 1973). The latter mutant showed simple recessive inheritance and 40 per cent increase in lysine content. Both mutants suffered from agronomic defects. There was reduction in seed size and also reduction in yield. In feeding trials, Ris 1508 or Hily Hiproly barley produced optimal growth of pigs without addition of protein or amino acid supplements. It may be added that normal barleys are intermediate between maize, sorghum on one hand and rice, oats on the other. Again because of agronomic problems, widespread efforts in improving protein quality did not find excitement.

Sorghum

Thousands of accessions were screened for high lysine mutants in sorghum. Two mutants 15-11167 and 15-11758 were identified from Ethiopian world sorghum collections (Singh and Axtell, 1973). Later an induced mutant P721 was reported (Mohan and Axtell, 1975). The mutant allele P721 appeared to be partially dominant and showed 60 per cent increase in lysine over the normal. The lysine in normal was 2.11 per cent as against 2.88 per cent in high lysine. P721 had soft phenotype and had reduced yield. It behaved different in genetic backgrounds and only in a few, yield appeared to be satisfactory. Converted materials using this gene had poor acceptance because of soft kernels. Modified vitreous types have also been encountered (Ejeta 1979) but work was not pursued rigorously. Ethiopian high lysine sorghums are proposed to be used as weaning food pending conformation of the fact that digestibility is acceptable.

Rice

Milled rice is low in protein concentration (7 per cent). It contributes 40-80 per cent of the calories and at least 40 per cent of the protein in the Asian diets. Rice has good quality protein despite poor protein concentration. Lot of work has been done for five decades at IRRI to improve protein content and quality in rice. The Researchers concluded after many years of work that there is little hope and prospect of further improving the lysine concentration in rice protein (Coffman and Juliano, 1979). Improvement for protein concentration appeared to be a good possibility, but results so far have been disappointing as witnessed by the lack of high protein rice cultivars.

Rice protein consists mostly of glutelin (80 per cent), prolamin (<5 per cent), albumin (5 per cent) and globulin (10 per cent). It is of interest to point out that albumin and globulin are concentrated in the aleurone layers. The lysine content of different fractions is glutelin (3.47 per cent lysine), albumin (4.92 per cent lysine), globulin (2.56

per cent lysine) and prolamin (0.51 per cent lysine). Bran and embryo proteins are mainly albumin proteins and are rich in lysine.

Rice has more lysine and better biological value compared to other cereals (Coffman and Juliano 1979; Khush and Juliano 1984; Tanaka 1983; Frey 1977).

Oat

It ranks fifth in the total production following wheat, rice, corn and barley. It is mainly used for animal feed. Oat protein has good protein concentration and has excellent balance of amino acids (Robbins *et al.*, 1971). Its protein quality and biological value is maintained even at higher protein concentrations. Genetic enhancement and manipulation for higher protein content is possible and commercial cultivars having 20 per cent protein have been developed (Briggle 1971). High yield has no adverse effect on protein content. A few high protein cultivars – Dal, Goodland, Marathan and Wright developed in Wisconsin have 2-3 per cent increase in oat protein.

Wheat

Wheat is chiefly used as food and its use as feed is less important. Surpluses are sometimes fed to livestock. Despite extensive research efforts, the high lysine mutants have not been encountered. There are better prospects of increasing protein content and lines exceeding 12 per cent have been isolated. From by products of wheat milling, as much as 28 per cent of the wheat grains mainly bran and shorts find their way into mixed livestock feeds.

Triticale and Rye

Mostly used as feed for livestock, Triticale has improved protein content and quality and so continues to generate optimism as a potential feed source.

Quality Protein Maize Success Story

As pointed out earlier, CIMMYT scientists used opaque-2 gene as other genes did not offer any advantage over the opaque-2 gene. In the beginning emphasis was on developing soft endosperm cultivars. As agronomic problems pointed out earlier became obvious several different options were tried which could result in acceptable quality protein maize germplasm. These approaches are described in several CIMMYT publications and journal articles (Byarnason and Vasal, 1992; Vasal *et al.*, 1984; Vasal *et al.*, 1980; Vasal *et al.*, 1979; Vasal

1994; Vasal 2000). Only one approach appeared promising which could resolve all problems confronting soft opaques and result in high-quality protein materials with acceptable yield performance, kernel phenotype and least vulnerable to ear rots and stored grain pests. The approach involved use of two genetic systems involving the opaque-2 gene and the genetic modifiers of opaque-2 locus. Using this approach, the initial emphasis was on developing hard endosperm opaque-2 donor stocks. Subsequently these donor stocks were used to convert normal maize materials to hard endosperm opaque-2. In addition several broad-based gene pools were formed. By late 1978, a huge volume of quality protein maize germplasm was developed with normal looking kernel phenotype. Merging and reorganization was attempted at this point to form a fixed number of pools and populations for systematic handling and improvement (Vasal 1994, 2000). In all 10 populations (populations 61, 62, 63, 64, 65, 66, 67, 68, 69, 70) and 13 QPM pools (pools 15, 17, 18, 23, 24, 25, 26, 27, 29, 31, 32, 33, 34) resulted from this effort. In addition two- high oil and one sugary2-opaque2 composite were developed. Problems were overcome and progress was attained in most traits deficient in original soft opaque-2 materials. In mid-1980s QPM hybrid effort was initiated. International testing of QPM varieties and hybrids has been extensively done and the results have been extremely encouraging. Several countries have identified varieties or hybrids which are competitive and are either equal or better than the best normal checks included in the trials (Table 5). Also, during mid 1990s, 55 QPM

Table 5. Superior White QPM Hybrids Tested Across Fifteen Locations during 1998

Pedigree	Yield t/ha	Ear rot (%)	Tryptophan (%)	Ear Modification	Silking (Days)	Plt ht (cm)
CML142xCML146	6.48	3.7	0.096	2.0	55	242
CML159xCML144	6.39	4.3	0.100	1.6	56	230
(CLQ6203xCML150) CML176	6.28	5.7	0.088	2.1	55	239
CML145xCML144	5.81	5.8	0.840	2.0	54	241
CML158xCML144	5.59	7.1	0.103	1.3	55	228
CML146xCML150	5.48	8.1	0.084	3.6	56	222
POZA RICA 8763 TLWD	5.34	12.0	0.095	2.8	54	230
Normal Hybrid check	5.58	9.5	0.070	2.0	56	228

Local checks: HB-83, CB-HS-5G, H-59, XM7712, GUAYOPE inbreds (CMLs 140-194) were announced and made available to public and private sector. Recently four more inbreds (CMLs 490-493) have been released. In the past four years at least 22 countries have released QPM materials including China, India and Vietnam (Table 6). Successful field days were conducted in most of the countries releasing the hybrids. In many instances, high-ranking politicians attended the ceremonies. There is enthusiasm and hope of covering more area under QPM in the coming years.

Table 6. Recent Releases in Asia involving CIMMYT Germplasm

Name	Institutions/Country	CIMMYT Germplasm Involved
Shaktiman – 1	DMR, India	(CML 142, CML 150)
Shaktiman – 2	DMR, India	(CML 176, CML 186)
HQ 2000	NMRI, Vietnam	(CML 161, CML 165)
Yun Yao 19	Yunnan, China	(CML 140)
Yun You 167	Yunnan, China	(CML 194)
Qian 2609	Guizou, China	(CML 171)
Lu Dan 206	Shandong, China	(P70)
Lu Dan 207	Shandong, China	(P70)
Lu Dan 807	Shandong, China	(P70)
Hybrid 2075	Sichuan, China	(CIMMYT QPM Populations)
Zhongdan 9409	CAAS, China	(Pool 33 QPM)

Feed use of cereals has been steadily increasing. On worldwide basis, roughly one-third of cereal grain crops are used for feeding livestock. The feed use of cereals in Asia totaled 158.1 million tonnes. China was the largest user (103 million tonnes) followed by other countries in order of their use, Japan (15.9 millions tonnes), South Korea (7.6 million tonnes), Taiwan (5.0 million tonnes) and India (8.0 million tonnes). Maize use as feed is quite large in Asia and perhaps exceeds 50 per cent of total production.

The expanded demand for meat and other animal products has witnessed unprecedented growth. In the next two decades the growth is likely to continue at the rate of 3.3 percent per year. The demand for feed will thus rise rapidly and will have to be met by cereal(s) which have potential for increased productivity and possible improved nutritional value for better feed efficiency. Maize will certainly play a dominant role and QPM will have the added advantage being superior in protein quality and higher in feed efficiency.

DEALING ABIOTIC STRESSES – PAST EXPERIENCE AND SOME ACHIEVEMENTS

The importance of abiotic stresses and their ever-increasing global concern can not be underestimated. Losses occurring every year due to one reason or another are massive and invariably result in fluctuating production and market price. As to the kind of abiotic stresses, these are indeed numerous and are of varying nature. Most of the stresses are either environmental or related to problematic soils. The environmental stresses include drought, heat, high or low temperatures, air pollutants, high velocity winds, hail, and frost. The soil-related stresses may be due to mineral element deficiencies, toxicities, extremes in soil PH, low-N, and excess soil moisture. The discussion in this paper will, however, be limited to drought, low-N and water logging as CIMMYT and some NARs are addressing these problems. Environmental stresses generally are unpredictable both in time and severity. In contrast soil-related stresses are known with some scientific facts and their effect on production potential or losses can be estimated reasonably well. The stresses mentioned above are important globally and their awareness in recent years has increased as being the primary or major constraint to limiting maize production in the developing world. Systematic and well-focused research efforts in stress breeding research are rather limited as only a few institutes or programmes have the resources, manpower and scientific skills to conduct this work. The quest for abiotic stress-tolerant germplasm can make a massive impact in reducing and preventing losses worth billions of US dollars. Also as we stretch and move to more marginal lands, the need and demand for this germplasm will grow. The success in research and breeding efforts will depend to a large extent on genetic variation that can be encountered for these traits in either existing germplasm under improvement or what is conserved in several maize gene banks around the world including CIMMYT bank. Differences among and within species is also known to exist. Maize researchers are fortunate that genetic variation is present in maize for stress traits and can be exploited in superior performing genetic backgrounds (Vasal *et al.*, 1999; Banziger *et al.*, 2000). The genetics of resistance may be simple or complex and in some instances may be influenced by modifying gene complex. Specific genes controlling these traits are not known but perhaps exist and may be detected in the future. Only consciously-planned and well-executed studies will help us in this resolve. Simple genetic stress-tolerant systems will be of utmost help in environmental stresses as such stresses are puzzling, complex, solutions not simple and rapid progress not easily attainable. In dealing with such stresses, input of other disciplines is needed and highly critical. It is therefore imperative that concerted efforts of all maize researchers be directed in a coordinated and collective manner to achieve positive and rapid results and sometimes exciting breakthroughs. This will require a strong will and desire on part of everyone to demonstrate spirit of cooperation, good working relationship and openness to interact and be receptive to good ideas.

In quest for tolerant germplasm for each stress trait, it will perhaps be desirable to identify a few key traits or secondary traits that can facilitate selection process. It will be preferable if these traits can be visually observed, are highly heritable, less prone to mistakes in data recording and in either selection or rejection of the material in the field. Excellent examples already exist in this regard. In maize, anthesis-silking interval (ASI) is considered a good secondary trait to facilitate selection for drought tolerance (Edmeades *et al*., 2000). It is also an important trait for other stresses. Because of the separation of male and female inflorescences in the maize reproductive system, ASI acts as a weak link in the maize plant. A large ASI could result in a barren plant or an ear with poor seed set and yield. ASI is particularly critical at flowering and can be completely knocked down by more than one influencing factor. Inbreeding, high density and pre-flowering biotic stresses can adversely affect ASI. It could thus be an important tool in drought work. Ear number and ear aspect rating as judged by seed set are additional traits that could facilitate drought work. Similarly extent of brace or prop roots above the soil may serve as useful criteria for discriminating among susceptible and resistant genotypes for root lodging and water logging. Shades of green coloration reflecting chlorophyll content may appear to be a useful tool in selecting low-N tolerant genotypes.

In stress breeding, the choice of germplasm is not only important but highly critical.It is a key to rapid success in providing superior germplasm in the hands of users as quickly as possible. Generally high performing maize populations with sufficient genetic variation for the trait in question are selected. Preference may also be given to those populations that have some level of tolerance for the stress trait.

Other aspects or features worth considering will be inbreeding tolerance behaviour, heterosis expressed, and good combining ability if such populations are to serve as dual purpose materials for extracting OPVs and hybrids. In the absence of such materials, new populations and synthetics can be formed using a selected fraction of pre-screened lines for this trait.

Breeding methodologies for stress work should be relevant and appropriate for improvement in stress trait and to the needs of the germplasm products. Both direct and indirect breeding options and alternatives can be deployed considering the robustness of population and hybrid research activities (Edmeades *et al.*, 1998; Vasal *et al.*, 1997). Alternate strategies embedded in inbreeding and high densities should as well be considered as important tools either as separate tactics or as part of on-going recurrent selection programmes and inbred line development efforts. Field conditions for stress work need to be relevant for that stress. For low-N work, fields ought to be deprived of residual nitrogen by sequential maize plantings season after season without nitrogen application. Deployment of additional tactics to create nitrogen-stressed conditions should also be considered. For drought a rain free season is a must to control and manage stress levels. In water logging work, it should be possible to hold water in the field for a specified period before draining it out. Aside from creating stress field conditions, the management of stress levels in the field is equally important. Experience and expertise will be necessary to create right stress levels for different treatments. If materials are to perform well under stress and non-stress conditions, the success of the selection programme will depend very much on evaluating genotypes/families under no stress, intermediate stress, and severe stress conditions. Given the stress trait, the stress levels can be further augmented by using higher densities. The heritability of stress trait under improvement needs to be improved. This is possible by using good field conditions, managing well stress levels, better field designs, and types of progeny tested in recurrent selection programme. Use of selfed progenies and inbred testers in population improvement efforts is to be preferred to non-inbred progenies and populations as testers to help improve heritability of the trait. Of various breeding alternatives discussed (Vasal, *et al.*, 1997), one may choose either stratified mass selection or full-sib family selection or modified half-sib reciprocal recurrent selection depending on manpower, physical facilities, available testing sites and resources allocated to this activity.

Drought Tolerant Maize Germplasm

Drought tolerant source Michoacan 21 commonly referred to as "Latente" is known for several decades. Because of poor agronomic performance and other undesirable attributes, it has been difficult to exploit and transfer this trait to other genetic backgrounds. In the absence of specific genes controlling this trait, the research initiatives in improving this trait have been limited worldwide. An exploratory and modest effort was undertaken at CIMMYT in early 70s to improve drought tolerance of most productive and widely grown Tuxpeno population adapted to lowland conditions (Fischer *et al*., 1983). Recurrent full-sib family procedure was used to improve this material for drought tolerance using winter season at CIMMYT experimental station Tlaltizapan to evaluate families for drought at different water regimes or stress levels. Progress from eight cycles of selection was encouraging and demonstrated in a convincing manner that the performance of maize populations experiencing water deficits at flowering and during grain filling can be improved by recurrent selection at no cost to performance in well- watered conditions (Bolanos and Edmeades, 1993; Edmeades *et al.*, 1993). The selection resulted in a significant increase in grain yield of 108 kg per hectare per cycle at yield levels ranging from 1 to 8 tonnes per hectare. At a yield level of 2 tonnes per hectare, this represents a gain of 6.3 per cent per cycle. In a later study the gains averaged 90 kg per hectare per cycle across all sites at a mean yield level of 5.6 tonnes per hectare (Byrne *et al.*, 1995). Yield potential was increased in both well-watered and drought stress environments, however the gains for yield were higher for the drought selected population as compared to same population improved through international testing (gains of 1.6 per cent versus 1.2 per cent). Encouraged by above results, more populations were subjected to selection under drought. A shift in methodology from full-sib to selfed progeny selection was also made at this time. As testers were developed, test cross evaluation was also introduced to accumulate scientific information and to facilitate development and identification of hybrids. Varying numbers of cycles of selection have been completed in populations TS6, La Posta sequia, Pool 26 sequia, Pool 16 sequia, Pool 18 sequia, and newly formed populations DTP-1 and DTP-2. Evaluating progress from selection, gains under drought were significantly higher than under normal irrigation. More recent finding is considered important and exciting in showing that drought selections also perform well under low-N conditions (Banziger *et al.*, 2002). Today several good populations tolerant to drought are available with additional cycles of selection completed (TS6C3, La Posta sequia C3, Pool 18 sequia C4, Pool 26 sequia C3, Pool 16 C2, DTP-1C7, DTP-2 C5). Both white- and yellow-grained versions are now available in DTP-1 and DTP-2.

Recurrent selection procedures described above were quite effective in developing source drought tolerant populations. Intensive inbreeding efforts also have been underway in these populations to develop inbred lines. Recently a few drought tolerant lines have been released as CMLs (CMLs 339-344, 347, 348, 488, 489). Inbred line evaluation trials under drought of lines with no previous history of selection for drought have helped to identify a number of good drought tolerant lines (Vasal, *et al.*, 1997, 1999, 2000, 2001). Similarly in CIMMYT-ARMP, promising downy mildew resistant lines have been evaluated for drought, Low-N, and water logging. Interesting results have come out from such evaluations. Several late lines have yielded 3 tonnes and more under drought. Similarly a few of the promising early lines yielded 1.5 tonnes/ha and more. Hybrids tolerant to drought were also identified, though such hybrids were never previously selected for drought. Information and results discussed above present convincing evidence of effectiveness of both approaches in drought work.

Low-N Tolerant Maize Germplasm

Research efforts had been underway at CIMMYT to improve and develop source populations tolerant to low-N conditions using recurrent selection methods. Two sets of materials, the population across 8328 and Pool 16 were chosen to affect improvement for this trait. Six cycles of selection have been completed in across 8328BN. Evaluations after five cycles under both high and low-N showed gains of 137kg/ha (2.3 percent per cycle) under the former conditions and of 75kg/ha (2.8percent per cycle) in the latter. The second material Pool 16 BN, is being improved for both drought and low-N. Some interesting findings have come to light from cycles of selection evaluation trials. It appears that drought tolerant selections also perform well under low-N conditions. Thus all drought tolerant materials TS6, La Posta sequia, Pool 26 sequia, and Pool18 sequia when evaluated under low-N conditions have shown considerable improvement to this trait as well. Gains observed under low-N were very similar to what had been reported for drought. As with drought, similar evidence has emerged from low-N work that improved tolerance to this trait by recurrent selection has in no way affected performance at normal N levels (Banziger *et al.*, 2002). The information on spill-over effects is significant and of great value to maize researchers engaged in stress work. Additional materials, Pool BN Precoz, Pool BN Tardio, are also being developed using bank accessions in their formation. Lines and hybrids tolerant to low-N with no previous history of selection for this trait have also been identified at CIMMYT headquarter and in CIMMYT-ARMP (Vasal, *et al.*, 1999, 2000, 2001).

DEVELOPMENT AND IMPROVEMENT OF ABIOTIC STRESS TOLERANT QPM GERMPLASM

Below I have attempted to describe and discuss a number of breeding options and strategies to develop abiotic stress tolerant QPM germplasm.

Adaptation versus Development

This is perhaps the simple and most cost effective and efficient approach. It will require selection for a particular stress in a specified stress environment for a few cycles. The QPM related traits affecting kernel phenotype due to high frequency of genetic modifier will remain unaffected and may show even slight progress if pressure is applied for modifiers during the selection process. This type of approach has been successfully used in China to improve photoperiod insensitivity of two CIMMYT QPM pools 33 and 34. Three cycles of bi-parental mass selection brought noticeable change in photoperiod insensitivity.

Improvement of Source Populations for Stress Traits

This approach has been tried in normal materials to improve drought, low-N and acid soil tolerance at CIMMYT headquarter and in CIMMYT's regional programmes in south America and Africa. Family-based selection schemes of full-sibs and selfed progenies were quite effective and resulted in measurable gains equal to or better than non-stress conditions. Since selection was based on family performance under stress and non-stress conditions, the improved populations performed well both under stress and non-stress conditions. From my own experience I could further add some important considerations in the choice of selection schemes with QPM materials. The schemes ought to be simple, cost effective, OPV and hybrid oriented, provide spin off products, possibility of early collaboration with partners, reduced time span per cycle and more importantly should have multiplier effects. Carefully chosen schemes or their modifications should work as well for the improvement of source QPM populations. The schemes should also place least burden on laboratory facilities.

Conversion of Normal Stress Tolerant Germplasm to QPM

This approach requires crossing normal stress tolerant germplasm to one or more QPM donors. The F1's will need to be advanced to F2. In this segregating generation, modified opaque-2 kernels are selected with great care taking into consideration that expected frequency of such kernels does not exceed 25 per cent. Selection of right kernels is highly critical to avoid mistakes in ensuing generations. The quality of modified kernels will dictate the next step, to advance further F2 to F3 or to go for the next backcross. For such conversion programmes, skilled and experienced QPM researchers are very much required. Decision to proceed with no backcross or additional backcross(es) will depend on breeder's judgement. Sometimes to accelerate progress and to accumulate modifiers, one may choose to backcross with non-recurrent parent.

Conversion of QPM to Stress Tolerant Using QPM Stress Tolerant Donors

This approach appears appropriate and desirable provided QPM stress tolerant donors have been developed and are available. Using this approach the possibility of making mistake is minimized, the frequency of modifiers not reduced and conversion time span is considerably reduced. As more QPM donors are available, one may choose the donor from the same heterotic grouping. Care should also be taken to choose donors that are adapted and are of the same maturity.

Inbred–hybrid Approach

From my own experience I have found this as an efficient and practical approach. It can be done as part of inbred-hybrid programme. It requires strong QPM inbred line development efforts and conducting periodic or systematic inbred line evaluation nurseries under specific abiotic stress conditions. Results have been encouraging in detecting tolerant lines for various stresses in normal maize inbred lines. Also as tolerant lines are detected, recycling among lines can be initiated to develop more diverse QPM lines. Stress manipulations can also be done during inbred line development stages. Use of high density is also recommended as a useful tool in stress breeding work as it creates competition for water and nutrients. Inbred–hybrid approach also has added advantage as it can be used as a single multi-pronged strategy for selecting simultaneously more than one stress.

REFERENCES

Banziger M., Edmeades G.O., Beck D.L. and Bellón M.R. 2000. Breeding for drought and nitrogen stress tolerance in maize: From Theory to Practice. Mexico, DF (Mexico): CIMMYT. 68 p.

Banziger M., G.O. Edmeades, and H.R. Lafitte. 2002. Physiological mechanisms contributing to the increased N stress tolerance of tropical maize selected for drought tolerance. *Field Crops Research* **75(2-3):** 223-233.

Bjarnason M. and S.K. Vasal. 1992. Breeding of quality protein maize (QPM), in Plant Breeding Rev., Janick, J., ed., **Vol. 9:** 181-216

Bolaños J., and G.O. Edmeades. 1993. Eight cycles of selection for drought tolerance in lowland tropical maize. I. Responses in grain yield, biomass, and radiation utilization. *Field Crops Research* **31:** 233-252.

Briggle L.W. 1971. Improving nutritional quality of oats through breeding. *Agron. Abstr.*, p. 53.

Byrne P.F., J. Bolaños, G.O. Edmeades, and D.L. Eaton 1995. Gains from selection under drought versus multilocation testing in related tropical maize population. *Crop Sci.* **35:** 63-69

Coffman W.R. and B.O. Jualiano, 1979. Seed protein improvement in rice: Status Report, pp. 261-75. In Proc. Symp. On Seed Protein Improv. In *Cereals and Grain Legumes*, Neuherberg, Fed. Repub. Ger, 4-8 Sept. 1978.

Delgado C., M. Rosegrant, H. Steinfeld, S. Ehui, and C. Courbois.1999. The Next Food Revolution. Chapter 14. Livestock to 2020 IFPRI.

Doll H., and B. Koie. 1975. Evaluation of high lysine barley mutants. In breeding for seed protein improvement using nuclear techniques. IAEA, Vienna, pp.55-59.

Ejeta G. 1979. Selection for genetic modifiers that improve the opaque kernel phenotype of P-721 high lysine sorghum (*Sorghum bicolor* [L.] Moench). Ph.D. thesis, Purdue Univ., Lafayette, Ind., 1979.

Frey K.J. 1977. Proteins of oats. *Z. Pflanzenzucht.* **78:** 185-215.

Edmeades, G.O., J. Bolaños, M. Hernandez, and S. Bello. 1993. Causes for silk delay in a lowland tropical maize population. *Crop Science* **33(5):** 1029-1035.

Edmeades G.O., J. Bolanos, M. Banziger, J.M. Ribaut, J.W. White, M.P. Reynolds, and H.R. Lafitte. 1998. Improving crop yield under water deficits in the tropics. p. 437-451. In: V.L. Chopra *et al.* (eds.). *Crop Productivity and sustainability shaping the future.* Proc. 2nd Int. Crop. Sci Congress. Oxford and IBH. New Delhi.

Edmeades G.O., J. Bolanos, A. Eilings, J. M. Ribaut. M. Banziger, and M. E. Westgate. 2000. The role and regulation of antheis silking interval in maize. In *Physiology* *and Modeling Kernel Set in Maize*. CSSA Special Publication No. 29: p. 43-73.

Ingverson J., B. Koie, and H. Doll. 1973. Induced seed protein mutant of barley. *Experientia* **29:**1151-52.

Khush G.S., and B.O. Juliano. 1982. Status of rice varietal improvement for protein content at IRRI. P. 199-202, 1984. In Nuclear techniques for cereal grain protein improvement. Proc. Res. Coord. Meet, Vienna. IAEA, Vienna, 6-10 Dec. 1982.

Mertz E.T., L.S Bates, and O.E. Nelson. 1964. Mutant gene that changes protein composition and increases lysine content of maize endosperm, *Science*, 145, 279

Mohan D.P., and J.D. Axtell. 1975. Diethyl sulfate induced high lysine mutant in sorghum. Pap. Presented at Ninth Biennial Grain Sorghum Res. and Util. Conf., Lubbock, Tex., 4-6 Mar. 1975.

Munck L., K.E. Karlsson, and A. Hagberg. 1971. Selection and characterization of high protein lysine variety from the world barley collection. In Nilan, R. (ed.) Barley genetics II. Pullman, Wash., pp. 544-58, 1971.

Nelson O.E., E.T. Mertz, and L.S. Bates. 1965. Second mutant gene affecting the amino acid pattern of maize endosperm proteins, *Science*, **150**, 1469,

Robbins G.S., Y. Pomeranz, and L.W. Briggle. 1971. Amino acid composition of oat oats. *Agric. Food Chem.* **19:** 536-39.

Singh J., and H.K. Jain. 1977. Studies on assessing the nutritive value of opaque-2 maize. *Indian Agric. Res. Inst.*, New Delhi.

Singh R., and J.D. Axtell. 1973. High lysine mutant gene (hl) that improves protein quality and biological value of grain sorghum. *Crop Sci.* **13:** 535-39.

Vasal S.K., E.Villegas, and R. Bauer. 1979. Present status of breeding quality protein maize, in Seed protein improvement in Cereals and Grain Legumes, IAEA, Vienna, p. 127-150.

Vasal S.K., E. Villegas, M. Bjarnason, B. Gelaw, and P. Goertz. 1980. Genetic modifiers and breeding strategies in developing hard endosperm opaque-2 materials, in Improvement of Quality Traits of maize for Grain and Silage use, Pollmer, W.G. and Phipps, R.H., Eds., Nighoff, The Hague, p. 37-73.

Vasal S.K., E. Villegas, C.Y. Tang, J. Werder and M. Read. 1984. Combined use of two genetic systems in the development and improvement of quality protein maize, *Kulturpflanze*, 32: 171-184.

Vasal S.K. 1994. High quality protein corn. In: A.R. Hallauer (ed.), *Speciality corns*, CRC Press, Boca Raton, Fl. P. 80-121.

Vasal S.K., H.S. Cordova, D.L. Beck, and G.O. Edmeades. 1997. Choices among breeding procedures and strategies for developing stress tolerant maize. Germplasm In G.O. Edmeades *et al.* (ed) Proc. Developing Drought and Low Nitrogen Maize 25-29 Mar. 1996. CIMMYT, El Batan, México.

Vasal S.K. G. Srinavasan, H.S. Córdova, S. Pandey, D. Jeffers, D.J. Bergvinson, D.L. Beck.1999. Inbred line evaluation nurseries and their role in maize breeding at CIMMYT. *Maydica* **44(4):** 341-351.

Vasal S.K. Quality Protein Maize Story. 2000. *Food and Nutritional Bulletin*, **Vol. 21, No. 4:** 445-450.

Vasal S.K. 2000. High Quality Protein Corn. P.85-129. In: *Specialty Corns*. Hallauer (ed.). Florida (USA), CRC Press.

Vasal S.K., F. Gonzalez Ceniceros, and O. Balla. 2001. Research activities and some achievements of CIMMYT-ARMP. The 30th National Corn and Sorghum Research Conference, Bangkok (Thailand), 19-23 June, 2001. Bangkok, Thailand.

Vasal S.K. 2002. Quality protein maize: overcoming the hurdles. *Journal Crop* Production, **Vol. 6, No. 1/2**, pp. 193-227.