GENETIC VARIABILITY, STABILITY AND INHERITANCE OF GRAIN IRON AND ZINC CONTENT IN PEARL MILLET (*Pennisetum glaucum* (L.) R. Br.)

Thesis submitted in partial fulfilment of the requirements for the award of degree of

Doctor of Philosophy in Plant Breeding and Genetics

to the Tamil Nadu Agricultural University, Coimbatore.

By

G. VELU

I.D. No. 02-810-007

CENTRE FOR PLANT BREEDING AND GENETICS

TAMIL NADU AGRICULTURAL UNIVERSITY

COIMBATORE - 641003

2006

CERTIFICATE

This is to certify that the thesis entitled, **"GENETIC VARIABILITY, STABILITY AND INHERITANCE OF GRAIN IRON AND ZINC CONTENT IN PEARL MILLET (*Pennisetum glaucum* (L.) R. Br.)"** submitted in partial fulfillment of the requirements for the award of the degree of **Doctor of Philosophy** in **Plant Breeding and Genetics** to the Tamil Nadu Agricultural University, Coimbatore, is a record of *bonafide* research work carried out by **Mr. G. VELU** under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, diploma or other similar titles or prizes and part of thesis work had been published in scientific journals, copy of the same has been included in the thesis.

Place: Coimbatore
Date : 31.07.06

Dr. V. MURALIDHARAN
Chairman

Approved by

Chairman : **Dr. V. MURALIDHARAN**

Co-Chairman : **Dr. K.N. RAI**
ICRISAT

Members : **Dr. T.S. RAVEENDRAN**

Dr. G. UMAPATHY

Dr. M. JAYAPRAGASAM

Date: 24.04.07

External Examiner

ACKNOWLEDGEMENT

It gives me immense pleasure to express a great sense of gratitude to the Chairman of my Advisory committee, **Dr. V. Muralidharan**, Director, Tamil Nadu Rice Research Institute, TNAU, Aduthurai for his kind encouragement, guidance and unceasing support during the course of work.

I eulogize the genuine cooperation and impetus offered to me by Co-chairman of my Advisory committee, **Dr. K. N. Rai**, Principal Scientist, Pearl millet Breeding, ICRISAT, Patancheru and, for providing excellent research facilities, meticulous guidance and constructive criticism throughout the period of this investigation and preparation of the manuscript.

I am also very thankful to other members of my advisory committee, **Dr. T. S. Raveendran**, Director, Centre for Plant Breeding and Genetics, Dr. G Umapathy, Professor, Department of Cotton and Dr. M. Jayapragasam, Professor, Biochemistry for constructive suggestions and guidance for completion of this endeavor.

I wish to express my sincere and wholehearted gratitude to **Dr. T. S. Raveendran**, Director, Centre for Plant Breeding and Genetics for his continuous encouragement and great support to carry out my doctoral research at ICRISAT. I would also like to thank the Dean PGS, TNAU for his kind authorization and full support for carrying out my research work at ICRISAT.

I would like to express my heartfelt and profound gratitude to **Dr. V.N. Kulkarni**, Visiting Scientist (Pearl millet Breeding) for his able guidance, unfailing help and constant encouragement during the course of achieving the final shape of this thesis. I am also thankful to Dr. R.P. Thakur, Principle Scientist, Cereal pathology for his helpful suggestions.

I am also very thankful to Mrs. K. Sumalini and Mr. A. K. Singh (Scientific officers, PMB) for their kind help, which enabled me to accomplish my doctoral research with ease. I wish to acknowledge and express my thanks to Mr. Prashant (Scientific Officer, Statistics unit), ICRISAT, for his help rendered during analyses.

Assistance rendered by the technical staff especially Mr. Ahmeddudin, Mr. A. C. Reddy, Mr. Dasarath Singh, Mrs. Saradha and all ICRISAT, PMB staffs who helped me in completing my fieldwork efficiently, expediently and smoothly, is also acknowledged. I thank Mr. S.B. Stanley, Senior Secretary (PMB) for his kind and prompt co-operation.

Special thanks are extended to Dr K. L. Sahrawat, (Scientist) and Mr. P.V. Rao Central Analytical Services Laboratory, ICRISAT and Dr. T. Longvah, Deputy Director, National Institute of Nutrition, Hyderabad for their timely analysis and help.

Special thanks to Library staff, Learning Systems Unit for their excellent assistance during my research work at ICRISAT. I also thank Housing and Food services staff for making my stay at ICRISAT comfortable and enjoyable.

I take this opportunity to thank Dr. V. Balaji, Head Learning Systems Unit, **Dr. C.L.L. Gowda**, Program Leader, Global Theme-Crop Improvement, ICRISAT for their kind help and co-operation to work in ICRISAT.

I am greatly indebted to the help rendered by Dr. N. Manivannan, Associate professor, Dept. of Oilseeds and all the staff members of Centre for Plant Breeding and Genetics, Coimbatore.

I gratefully acknowledge **HarvestPlus (CGIAR)** challenge program for providing funds and Research Scholarship through ICRISAT to carry out my doctoral research successfully.

(G. Velu)

ABSTRACT

ABSTRACT

GENETIC VARIABILITY, STABILITY AND INHERITANCE OF GRAIN IRON AND ZINC CONTENT IN PEARL MILLET (*Pennisetum glaucum* (L.) R. Br.)

By

G. VELU

Degree : **Doctor of Philosophy**
(Plant Breeding and Genetics)

Chairman : **Dr. V. Muralidharan**
Director
Tamil Nadu Rice Research Institute
Tamil Nadu Agricultural University
Aduthurai - 612101, Tamil Nadu

2006

The present study was undertaken to determine the magnitude of genetic variability for grain iron (Fe) and zinc (Zn) among a diverse range of breeding lines, improved populations (among and within populations) and germplasm accessions; to assess the stability across different environments; to examine the relationships between them and with days to 50% flower and 1000-grain mass; and to determine the nature of inheritance and heritability.

Over two-fold variation was found for both grain Fe (30.1- 75.7 mg kg^{-1}) and Zn content (24.5-64.8 mg kg^{-1}) with medium to high broad-sense heritability in different classes of breeding materials and improved populations (42.0- 79.9 mg kg^{-1} Fe and 24.2-51.7 mg kg^{-1} Zn), indicating large genetic variability for the improvement of grain

Fe and Zn content and the scope for effective selection. The highest levels of Fe and Zn content were observed in well-adapted commercial varieties and in the parental lines of released hybrids, which had large *iniadi* germplasm base in their parentage, suggesting the possibility of making immediate impact on the nutritional security. Large within-population variability of over two-fold for grain Fe (40.9–118.9 mg kg^{-1}) and Zn (31.8–82.7 mg kg^{-1}) content was detected in progenies derived from two open-pollinated varieties (AIMP 92901 and GB 8735), indicating possibility of selection within-population and the prospects of enhancing grain Fe and Zn levels by recurrent selection. The difference between the summer and rainy season for grain Fe and Zn content among the entries was largely due to the soil Fe and Zn content of the fields used in the experiments.

The positive and highly significant correlation between Fe and Zn content in all the experiments, indicated the possibility of simultaneous genetic improvement for the elevated levels of both micronutrients. Significant positive correlations of 1000-grain mass and negative correlation of days to 50% flower with Fe and Zn content suggested good prospects of combining high Fe and Zn with farmers-preferred traits such as large seed size and early maturity.

Significant differences existed among the entries and environments with respect to all the four traits. Differential response of entries towards varying environments was evident as the genotype × environment interaction for grain Fe and Zn content and, days to 50% flowering was significant. Such statistical interactions were of non-cross over types as there was highly significant positive correlations of grain Fe and Zn among seasons. The high Fe and Zn content seed parents 863B, 843B and ICMB 88004 were

identified which were also stable across environments, and thus could be used as good sources for further genetic improvement.

The genetic component analysis indicated absence of epistasis for all traits. The Wr-Vr graph revealed presence of partial dominance for grain Fe and Zn content, 1000-grain mass and over-dominance for days to 50% flowering. The predictability ratio measured by $2\sigma^2gca/(2\sigma^2gca + \sigma^2sca)$ was around unity for both grain Fe and Zn content, implying preponderance of additive gene action. Also, there was highly significant positive correlation between the mid-parental value and hybrid performance, and no correlation between mid-parent value and mid-parent heterosis for both Fe and Zn showed an additional indication of the predominant role of additive gene action for these traits. The high grain Fe and Zn content in parents were governed by recessive alleles with increasing effects and the low content was due to excess of dominant alleles with decreasing effects. The grain Fe and Zn content in parents were correlated with their *gca* effects. The average heterosis was negative for grain Fe and negligible for grain Zn content. In general, this study suggested the effectiveness of pedigree/recurrent/progeny selection or backcross breeding to develop lines with increased levels of grain Fe and Zn content.

The simple, rapid and cost-effective Prussian blue staining method for qualitative assessment of grain Fe was efficient in distinguishing the entries with high and low Fe content, that could be used for discarding entries with low Fe content while screening large number of germplasm accessions and breeding lines.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

INTRODUCTION

CHAPTER 1

INTRODUCTION

Dietary deficiency of some of the important micronutrients such as iron (Fe) and zinc (Zn) has been reported to be a food-related primary health problem affecting nearly half of the world's population (Welch and Graham, 2002; WHO, 2002). This problem is particularly serious in developing and under-developed countries of Africa and Asia, where most of the people depend on cereal and legume-based diets, and have limited access to meat, fruits and vegetables, which are generally rich sources of these micronutrients (Sandstead, 1991; Gibson, 1994). Based on levels of blood haemoglobin (Hb) that reflects Iron Deficiency Anaemia (IDA), Fe deficiency is the most prevalent nutritional deficiency in the world with the recent estimates of 3.5 billion people in developing countries being Fe deficient. The magnitude of micronutrient (Fe and Zn) deficiency is particularly alarming among children, women of reproductive age, and pregnant and lactating women (Sharma, 2003). In India, about 80% of the pregnant women, 52% of the non-pregnant women and 74% of the children (6-35 months) suffer from IDA (Chakravarty and Ghosh, 2000). Fe deficiency is often accompanied by Zn deficiency as both of these nutrients are derived from similar sources in the diet (Welch, 2001). An estimated 49% of the world population is at risk due to low Zn intake (International Zinc Association, 2000; FAO, 2003).

Fe deficiency leading to nutritional anaemia, has many negative impacts on human health and well being, such as decreasing work capacity and slowing the cognitive development in Fe-deficient children (Hercberg *et al.*, 1987). Zn is an essential trace element with wide public health and clinical significance. It affects many physiological functions, including wound healing, growth and development, and neurophysiological well being (Sandstead, 1994). The recommended daily allowance (RDA) of both Fe and Zn is 12-15 mg for adults and 10 mg for children (FAO/WHO, 2000).

Human nutritionists have focused on supplementation, fortification and dietary diversification to address micronutrient deficiencies. Fortified foods and food supplements do not reach all those affected in the developing countries, because of weak market infrastructure and also because these products have high recurring costs (Timmer, 2003). Sustainable solutions to the micronutrient problem in these countries can be developed through agricultural approaches. One such approach is crop diversification, and the other one is to enhance the levels of micronutrients in major staple food crops through plant breeding strategies i.e., 'biofortification' of staple food crops. The latter approach involves the development of new varieties of staple food crops that are selectively bred to enhance specific nutritional qualities, such as high levels of biologically available Fe and Zn. Research has demonstrated that large genetic variability for micronutrients are available within the genomes of major staple crops that could allow for substantial increases in grain Fe and Zn content through genetic enhancement (Welch, 2001).

Exploiting genetic variation in crop plants for micronutrient content is one of the most powerful tools to change the nutrient balance of a given diet on a large scale. The fundamental assumption in proposing a plant breeding approach to address micronutrient malnutrition is that these traits must be delivered in high-yielding cultivars. The Consultative Group on International Agricultural Research (CGIAR) has been investigating the genetic potential to increase bioavailable Fe and Zn in staple food crops. Among the cereal crops, these include rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), sorghum (*Sorghum bicolor* (L.) Moench) and pearl millet (*Pennisetum glaucum* (L.) R. Br.). Many people in developing countries subsist on cereal-based diets low in Fe and Zn. In the arid and semi-arid tropical regions of Asia and Africa, these are based on sorghum and pearl millet. It is thought that increasing Fe and Zn concentrations in these crops could increase the dietary intake of Fe and Zn in these regions significantly. The immediate objectives of the HarvestPlus initiative of the CGIAR, are to determine the genetic variability and heritability of these mineral traits, their stability across diverse soil conditions and climatic zones, the number of genes

influencing these traits, the feasibility of breeding for their increased concentrations simultaneously in edible tissues without affecting yields or other quality characteristics, and finally the bioavailability of these micronutrients contained in the edible parts of the plant (Bouis, 2003). An increase in mineral concentrations in edible parts might be a consequence of slower growth, reduced yield, low harvest index or smaller seeds. Thus, in addition to mineral concentration (expressed as mg kg^{-1} dry weight of edible parts), measurements of mineral content (expressed as mg per seed or plant) are also crucial for understanding the effects of genetic and environmental factors on the biofortification of crops.

A survey of rice accessions indicated a four-fold variation in grain Zn and Fe concentrations (Gregario *et al.*, 2000); similar variation was found in diverse wheat accessions, although Fe and Zn concentrations were generally lower and showed less genetic variability among cultivated tetraploid and hexaploid varieties than among wild diploid (*Triticum boeoticum*, *Triticum monococcum*) and tetraploid (*Triticum dicoccoides*) wheats and wild relatives (*Aegilops tauschii*) of wheat (Monasterio and Graham, 2000). A somewhat greater variation in kernel Fe and Zn concentrations among the accessions had been indicated in initial surveys of maize germplasm (Banziger and Long, 2000), but this was not substantiated in later studies at specific locations. Nevertheless, there were accessions in all three cereals that produced grains with Fe and Zn concentrations that were at least double the most widely grown varieties. Furthermore, Fe and Zn concentrations in grain was positively correlated in these cereals, and were relatively less affected by the environments, implying that increased grain Fe and Zn concentrations can be combined with improved agronomic traits (Graham *et al.*, 2001). Thus, breeding for biofortification of Fe and Zn in cereals is feasible. In addition, increasing the micronutrient concentrations in staple food crops will act as an incentive to farmers by means of increasing the crop productivity when these seeds are sown in micronutrient-poor soils with greater seedling vigour (Welch, 1999).

Cereals provide the most calories to humans and dominate the diets of most resource-poor people in the world. Pearl millet is the sixth most important cereal crop in the world, next to wheat, rice, maize, barley and sorghum and, it provides the cheapest source of energy, protein, Fe and Zn among all cereals (FAO and ICRISAT, 1996). It is grown on about 26 million ha in the arid and semi-arid tropical areas of Africa. In these areas, pearl millet is grown largely for human food, and indeed is the staple cereal of 90 million people who live in agro-climatic zones where production levels of pearl millet are severely constrained by heat, low and erratic rainfall, and poor soil fertility (FAO, 2000). Grain is always the principal object of cultivation. The nutritive value of pearl millet is higher than rice, sorghum and wheat (Uprety and Austin, 1972). Among the major pearl millet producing regions, per capita consumption is highest (92 kg $year^{-1}$) in the rural population in the western region of Rajasthan, followed by dry areas of Gujarat. The other major pearl millet consuming regions are inland central Maharashtra, western Maharashtra, northern Maharashtra, Saurashtra, the northern plains of Gujarat and northeastern Rajasthan. In these regions, pearl millet contributes to about 20-40% of the total energy and protein intake. Its contribution of micronutrients especially Fe and Zn is higher, varying from 30 to 50% of the intake of these micronutrients from cereals (Parthasarathy Rao *et al.*, 2006). Pearl millet grains provide a low-cost solution to combating malnutrition due to micronutrient deficiency. Also, it has additional health related advantages because of its higher levels of insoluble dietary fiber and more balanced amino acid profile. Thus, pearl millet with enhanced nutritional quality could contribute significantly to improving the nutritional value of the diets of people dependent on pearl millet as a major energy source.

The availability, assessment and exploitation of genetic variability is the main pre-requisite for any successful breeding program. Pearl millet has a vast reservoir of genetic variability for various qualitative and quantitative traits (Khairwal and Singh, 1999). Large genetic variation has been found for grain Fe, Zn and other minerals in pearl millet (Jambunathan and Subramanian, 1988), making selection of nutritionally superior breeding

materials possible. Being a highly cross-pollinated crop, pearl millet populations are highly heterozygous and heterogeneous, leading to within-population variability that can be used to enhance the Fe and Zn levels in these populations. The efficiency and choice of breeding program will depend on the pattern of inheritance and stability of the traits under study. The information available about genetic control and the stability of performance of these micronutrients in crops is very limited. Hence the 'HarvestPlus' biofortification challenge program on pearl millet research at the International Crops Research Institute for Semi-Arid Tropics (ICRISAT) aims at genetically enhancing the micronutrient content of pearl millet grain in agronomically superior varieties. As a part of this program, the present study was undertaken with the following specific objectives.

i. To determine the magnitude of genetic variability for grain Fe and Zn content in a diverse range of breeding materials such as inbred lines, populations and germplasm accessions to assess the levels to which their levels can be increased through breeding

ii. To assess the extent of intra-population variability for Fe and Zn and prospects of enhancing their levels through recurrent selection and

iii. To study the inheritance of grain Fe and Zn content and its implications in breeding open-pollinated varieties and improved parental lines of hybrids.

REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

2.1. PEARL MILLET AND ITS IMPORTANCE

Pearl millet is grown principally for grain in the hot arid and semi-arid areas of Africa and Asia. It is sown on approximately 14 million ha in Africa and 9.5 million ha in India, annually producing 10.5 and 6.5 million tons of grain, respectively. In terms of global production, pearl millet is the sixth most important cereal crop after wheat, rice, maize, barley, and sorghum (FAO and ICRISAT, 1996). Increasing the productivity of pearl millet to keep pace with the growing food demand of human and feed demand of livestock populations in the semi-arid tropics is a gigantic task requiring concerted efforts from national and international research and development organizations including both public-sector and private-sector agencies (Rai and Anand Kumar, 1994). More than 70 single-cross pearl millet hybrids released so far, occupy more than 60 percent of the major pearl millet areas of Gujarat, Maharashtra, Haryana, Uttar Pradesh, Rajasthan and Tamil Nadu, and have significantly contributed to enhanced productivity (875 kg ha^{-1}) (Bhatnagar, 2003).

Pearl millet grain has high nutritional value of protein, energy and minerals when compared to major cereals and coarse grains (Maiti and Bidinger, 1981). It supplies 80-90% of the calories for many millions of the poorer people in the world (Burton *et al.*, 1972). It has about 12% protein, 5% fat (high), 67% carbohydrates and energy value of 360 K cal $100g^{-1}$. The amino acid profile of pearl millet grain is better than that of normal sorghum or normal maize and is comparable to those of the small grains, wheat, barley and rice (Ejeta *et al.*, 1987) with a less disparate leucine/isoleucine ratio (Hoseney *et al.*, 1987; Rooney and McDonough, 1987). The lysine content in pearl millet grain ranges from 1.9 to 3.9 g 100 g^{-1} protein (Ejeta *et al.*, 1987). Pearl millet grain appears to be

generally free of some of the major anti-nutritional factors, such as the condensed tannins in sorghum grain having a pigmented testa, which reduces protein availability. The mineral profile especially of grain Fe and Zn content in pearl millet is relatively superior to other cereals (Chauhan *et al.*, 1986) and has wide genetic variability (Jambunathan and Subramanian, 1988). The special nutritional quality of its grain particularly for micronutrients such as Fe and Zn content facilitates its use in health food formulations and fits well in value addition (Kurien *et al.*, 1961).

The present review covers the importance of micronutrient malnutrition, biofortification strategies, magnitude of genetic variability, stability, physiology, genetics and bioavailability of grain Fe and Zn content in various crops, with an emphasis on pearl millet.

2.2. MICRONUTRIENT DEFICIENCY

At least 49 nutrients are required to meet the metabolic needs of human beings. Inadequate consumption of even one of these nutrient will result in adverse metabolic disturbances leading to sickness, poor health, impaired development in children, and large economic costs to society (Golden, 1991; Grantham-McGregor and Ani, 1999; Ramakrishnan *et al.*, 1999; Branca and Ferrari, 2002). Importantly, the primary source of all nutrients for people comes from agricultural products. If agricultural systems fail to provide enough products containing adequate quantities of all nutrients during all seasons, dysfunctional food systems result that cannot support healthy lives (McGuire, 1993; Schneeman, 2001; Graham *et al.*, 2001). Estimated over three billion people are afflicted with micronutrient malnutrition and the numbers are on rise (Mason and Garcia, 1993; World Bank, 1994; Welch *et al.*, 1997; WHO, 1999). Nearly two-thirds of all deaths of children are associated with nutritional deficiencies, many from micronutrient deficiencies (Caballero, 2002). The mineral elements most frequently lacking in human diets are Fe and Zn, although other elements, such as I, Ca, Mg, Cu and Se, can be

deficient in the diets of some populations (Welch and Graham, 2004). An estimated 3 billion people in the world suffer from the insidious effects of micronutrient deficiencies, especially of Fe and Zn (Welch and Graham, 2004). These deficiencies are caused by habitual diets that lack diversity (overly dependent on a single staple food), situations of food insecurity, where populations do not have enough to eat (FAO/WHO, 2002); and low intake of vegetables, fruits, and animal and fish products, which are rich sources of minerals.

2.2.1. Iron deficiency

Anaemia is defined as a reduction in the oxygen-carrying capacity of red blood cells, which occurs as a result either of decreased haemoglobin or of a reduction in the total number of red blood cells (i.e. a decline in red blood cell mass). Iron deficiency is the most common cause of anaemia. The level of haemoglobin in the blood is the most commonly used indicator to screen for iron deficiency anaemia (IDA). IDA is more prevalent in women than in men, and is also prevalent among children and the elderly. IDA during pregnancy can result in serious consequences for both mother and baby. Fe-deficient women have a higher mortality risk during childbirth and an increased incidence of low-birth-weight babies (WHO, 2002). Southeast Asia shows the highest prevalence of anaemia in women, with over 50 percent of pregnant women affected (Mason *et al.*, 2001). In addition to the effects of anaemia during pregnancy, much more is now known of the deleterious effects of anaemia on the cognitive performance, behaviour and physical growth of infants and children of pre-school and school age (WHO, 2001). IDA in adults diminishes their stamina and work capacity by as much as 10–15 percent, and it has been estimated that this deficiency results in losses in gross domestic product of up to 1.5 percent (FAO, 2002).

2.2.2. Zn deficiency

Zinc deficiency is a public health problem both in terms of its magnitude and its health consequences and affects a range of functions such as reproduction, growth,

immunity and brain development (WHO, 1996) Global attention to Zn deficiency has accelerated rapidly over the past 15 years However, there is still no information about the prevalence of this deficiency, although it is assumed to be widespread in areas lacking dietary diversity Furthermore, the evidence suggests that Zn deficiency affects the most vulnerable segments of a population, pregnant women and young children, especially in developing countries (WHO, 1996) Zinc is an essential component in over 300 enzymes needed by the body for metabolism (FAO/WHO, 2002) The wide distribution of Zn in the body tissues and fluids reflects its essential role in metabolic activity as a component of key cell enzymes Zn stabilizes molecular structure of cellular components and membranes and contributes in this way to the maintenance of cell and organ integrity Furthermore, Zn has an essential role in polynucleotide transcription and thus in the process of genetic expression Zinc plays a central role in the immune system, affecting a number of aspects of cellular and humoral immunity (Hambidge *et al*, 1986) The clearest indicator of Zn deficiency is stunted child growth (Brown and Wuehler, 2000)

2.3. MICRONUTRIENT MALNUTRITION MANAGEMENT

The main strategies for addressing micronutrient malnutrition are dietary diversification, food fortification, supplementation and biofortification Most micronutrient deficiencies can be addressed, to some extent, through dietary diversification Food fortification strategies are needed in areas where the traditional diet lacks a specific nutrient Food-based approaches to fulfilling micronutrient requirements have received strong support as a sustainable means of meeting the nutritional needs of population groups (WHO/FAO, 1996, FAO/WHO, 2002)

2.3.1. Supplementation

Supplementation is a technical approach in which nutrients are delivered directly by means of syrup or pills Supplementation is most appropriate for targeted populations with a high risk of deficiency or under special circumstances, such as during pregnancy or in an acute food shortage Under normal circumstances, supplementation programs are

used only as a short-term measure and are then replaced with long-term, sustainable food-based measures such as fortification and dietary modification, usually by increasing food diversity (UNICEF, 2002, Timmer, 2003)

2.3.2. Dietary diversification

In treating the problem of micronutrient deficiencies, food-based approaches that focus on improving overall dietary quality, rather than merely delivering a single nutrient, are particularly useful There are complex nutrient-nutrient interactions that increase bioavailability when nutrients are consumed simultaneously For example, Fe absorption is increased when it is combined with vitamin C (FAO, 1997)

2.3.3. Fortification

Fortification strategies utilize widely accessible, commonly consumed foods to deliver one or more micronutrients The most widespread effort to date has been fortification of salt with iodine However, many other foods may be used as vehicles for a variety of micronutrients Some of the more common combinations are wheat products (cereal, bread or pasta) with one or more nutrients that include calcium, iron, niacin, riboflavin, thiamine and zinc (Mannar and Gallego, 2002)

2.3.4. Biofortification

Biofortifying major staple crops (biofortification' is a word coined to increasing the bioavailable micronutrient content of food crops through genetic selection via plant breeding) can significantly improve the amount of essential micronutrients consumed by target populations (Welch and Graham, 1999) Consumption of a wide variety of foods, including those that contain an array of micronutrients, is still seen as the best long-term sustainable solution to eradicate hidden hunger Along the path to achieving this goal, biofortification may help to improve the health and welfare of many populations

Furthermore, it is a sustainable intervention, unlike traditional interventions that depend on supplementation and fortification programs that have not proved to be sustainable in many developing nations (Yip, 1997, Subbulakshmi and Naik, 1999) In

addition, increasing the micronutrients stored in the seeds and grains of staple food crops increases crop productivity when these seeds are sown in micronutrient poor-soils (Welch, 1999) Much of the developing world has significant areas of such soils (White and Zasoski, 1999) Increased micronutrient in the seeds will act as an incentive to farmers cultivating micronutrient-poor soils to adopt the micronutrient-enriched seeds for use on their farms (Graham *et al*, 2001)

2.4. GENETIC ENHANCEMENT OF MICRONUTRIENTS

Biofortification reduces malnutrition by breeding essential micronutrients into staple crops This approach bridges the fields of human nutrition, crop science and public health to develop a set of highly sustainable nutrition interventions in a cost-effective manner The HarvestPlus Challenge Program of the Consultative Group on International Agricultural Research (CGIAR) has recently embarked upon addressing this issue through the delivery of crop cultivars with elevated levels of these micronutrients

Exploiting the genetic variation in crop plants for micronutrient content is one of the most powerful tools to change the nutrient balance of a given diet on a large scale The genetic potential for increasing the concentrations of bioavailable Fe and Zn in edible portion of pearl millet and several staple food crops (including rice, wheat, maize and beans) has been reviewed in this section

2.4.1. Variability for grain Fe and Zn content

There is no systematic work done on the genetic variation of grain Fe and Zn content in pearl millet and the potential to improve it through plant breeding Hulse *et al* (1980) summarized the results of a few studies indicating as high as 38 mg kg^{-1} of Fe and 16 mg kg^{-1} of Zn in pearl millet grains Another preliminary study done with a limited number of 27 genotypes showed large variation for Fe (40 580 mg kg^{-1}) and Zn content (10 66 mg kg^{-1}) in pearl millet grains (Jambunathan and Subramanian, 1988) Some of

earlier reports on genetic variability for grain Fe and Zn content in pearl millet by various workers have been summarized and furnished in Table 1

Large variability for grain Fe and Zn has also been reported in other crops For instance, Monasterio (1998) found four-to-five-fold variation for grain Fe and Zn content in the several hundred accessions of wheat The highest concentrations were about twice those of popular modern cultivars However, Fe and Zn content in wild relatives were 50% higher than in the modern cultivated wheat

A diverse range of 132 wheat germplasm accessions was evaluated at International Centre for Wheat and Maize Improvement (CIMMYT), Mexico for Fe and Zn content in the whole grain The variability for Fe and Zn content were 28 8 56 5 mg kg^{-1} for Fe and 25 2 53 3 mg kg^{-1} for Zn (Monasterio and Graham, 2000) Imtiaz *et al* (2003) assessed the Zn content of prominent domestic wheat varieties and the varieties collected from different countries The grain Zn content ranged between 10 to 34 mg kg^{-1} and the highest Zn content (34 mg kg^{-1}) was observed in a variety Pirsabak and the lowest content was noticed in Turkish variety CBWF-96-151 (10 mg kg^{-1})

Core collection of over 1000 accessions of common bean (*Phaseolus vulgaris* L) was screened for grain Fe and Zn content at the International Centre for Tropical Agriculture (CIAT), Columbia The seed Fe content ranged between 34 to 89 mg kg^{-1} and Zn content ranged from 21 to 54 mg kg^{-1} Some bean accessions from Peru were found to contain high levels of Fe averaging over 100 mg kg^{-1} The range in seed-Zn content in the core collection was narrower than seed-Fe content (Beebe *et al*, 2000) Wild types tended to have lower Zn concentrations than common cultivated types House *et al* (2002) reported genetic variability of seed Zn content varying from 26 7 mg kg^{-1} to 62 4 mg kg^{-1} in different classes of dry bean cultivars

Table 1. Genetic variability for grain Fe and Zn content (mg kg^{-1}) in pearl millet genotypes.

No. of lines	Mean	Range	Reference
Fe content (mg kg^{-1})			
6	110	90–120	Rao and Swaminathan (1953)
163	60	20–120	Goswami *et al.* (1969a,b and 1970a,b)
5	70	50-90	Deosthale *et al* (1971)
12	380	250-460	Desai and Zender (1972)
8	20	10 20	Ganry and Bideau (1974)
12	80	40–120	Sawhney and Naik (1969)
12	130	110-150	Shukla and Bhatia (1971)
14	30	20–50	Uprety and Austin (1972)
27	–	40-580	Jambunathan and subramanian (1988)
	160	–	Alpana (1989)
–	180		Khetarpaul and Chauhan (1991)
–	120		Aggarwal (1992)
–	160	–	Kumar and Chauhan (1993)
-	30		Hadimani and Malleshi (1993)
–	–	89-97	Archana (1997)
–		89–94	Rekha (1997)
10	–	70-180	Abdalla *et al.* (1998)
–		83–99	Malik (1999)
–	82		Poonam (2002)
Zn content (mg kg^{-1})			
–	–	19–25	Kumar and Kapoor (1984)
–	–	38–55	Singh *et al.* (1987)
–	–	10-38	Hoseney *et al.* (1987)
27	–	10–66	Jambunathan and Subramanian (1988)
10	-	53–70	Abdalla *et al.* (1998)
–	–	28–32	Malik (1999)

Banziger and Long (2000) screened 1814 accessions of core collections, breeding lines and populations of maize at CIMMYT, Mexico for grain Fe and Zn content. The grain Fe content ranged between 9.6 to 63.2 mg kg^{-1} and grain Zn content varied between 12.9 to 57.6 mg kg^{-1}. The extent of genetic variation for grain Fe and Zn content in 109 maize inbred lines was evaluated by Maziya-Dixon *et al.* (2000). The grain Fe content varied from 15 to 159 mg kg^{-1} for mid-altitude and from 14 to 134 mg kg^{-1} for lowland maize inbred lines; Zn content varied from 12 to 96 mg kg^{-1} for mid-altitude inbreds and from 24 to 96 mg kg^{-1} for lowland inbred lines.

Gregorio *et al.* (2000) evaluated 1138 brown rice genotypes for grain Fe and Zn content at the International Rice Research Institute (IRRI), Philippines. The Fe content varied from 6.3 to 24.4 mg kg^{-1} and Zn content from 13.5 to 58.4 mg kg^{-1}. The highest grain Fe content (range of 18–22 mg kg^{-1}) and Zn content (24–35 mg kg^{-1}) were found in aromatic rice varieties.

Reddy *et al.* (2005) evaluated different classes of breeding lines and germplasm accessions (n = 84) for grain Fe and Zn content in sorghum at ICRISAT. The grain Fe and Zn varied from 20.1 to 37.0 mg kg^{-1} with an average of 28 mg kg^{-1}, and grain Zn content varied from 13.4 to 31.0 mg kg^{-1} with the mean of 19.0 mg kg^{-1}. The grain Fe and Zn contents in germplasm accessions were significantly higher than those in other classes of breeding materials.

2.5.2. Character association

Correlation between grain Fe and Zn has been studied in several crops, with the results, by a large, showing similar trends. For instance, highly significant and positive correlation ($r = 0.54$; $P<0.01$) between grain Fe and Zn content has been observed in maize populations (Arnold *et al.*, 1977). Similarly, a strong and positive relationship between Fe and Zn content has been observed for both mid-altitude ($r=0.88$; $P<0.01$) and

vland maize inbred lines (r=0 62, P<0 01), as well as in germplasm accessions (Maziya-Dixon *et al* 2000)

Positive and highly significant correlation between grain Fe and Zn content has been observed in milled rice genotypes evaluated for grain micronutrient content (Gregorio *et al* (2000) Beebe *et al* (2000) also observed significant positive correlation (r=0 66, P<0 01) between the Fe and Zn content in common bean

Similarly, positive significant correlation (r=0 70, P<0 01) was observed between grain Fe and Zn content in wheat (Monasterio and Graham, 2000) and sorghum (r=0 55, P<0 01) (Reddy *et al*, 2005) Thus, genetic factors for increasing Fe content are co-segregating with genetic factors for increasing Zn content

In order to realize the maximum impact of micronutrient dense cultivars, the micronutrient traits must be delivered in high-yielding cultivars with farmers-preferred traits such as bold grains and, in many cases early maturity Monasterio and Graham (2000) observed that there was no negative linkage between grain yield and Fe and Zn content in the wheat grain Gregorio *et al* (2000) observed that high grain Fe content highly correlated with good agronomic traits in rice Reddy *et al* (2005) found negative and weak correlation of agronomic traits such as days to 50% flower, plant height and seed size with grain Fe and Zn content in sorghum (r=-0 54, P<0 01 to 0 18)

2.5.3. Genetics of grain Fe and Zn content

An understanding of the inheritance is essential for systematic and efficient genetic enhancement of any trait or trait combinations Very limited information is available on the inheritance of grain Fe and Zn content in crops The first genetic study was conducted by Weiss (1943) on Fe efficiency in soybeans The Fe efficiency was controlled by a single, major dominant gene Epstein (1972) noted apparently simple genetic control of Fe efficiency in maize and tomato On the contrary, few genes have been found to be involved in Zn efficiency in rice (Ponnamperuma, 1976) Ripperger and Schreiber (1982) observed that Fe efficiency in tomato was controlled by a major gene

Another study in soybean indicated that several minor additive genes contributed to Fe efficiency (Fehr, 1982)

Arnold and Bauman (1976) studied the inheritance of grain elemental contents in maize with 6 × 6 partial diallel mating design The *gca* effects were highly significant for grain Fe and Zn content, indicating the preponderance of additive genetic effects Gorsline *et al* (1964) observed that grain Fe and Zn accumulation in maize was under additive gene control Long *et al* (2004) studied inheritance of grain Fe and Zn content with fourteen southern African-adapted white-grained maize inbred lines using half diallel model General combining ability (*gca*) effects for flour Fe and Zn concentration were significantly more important than specific combining ability (*sca*) effects in high yielding environments

Results from a diallel analysis in rice suggested that the genes controlling Zn efficiency have additive gene action and, to a lesser extent, the dominant gene action (Majumder *et al*, 1990) Hartwig *et al* (1991) suggested that only a few genes control the Zn efficiency in rice

One study in rice showed that genetic control of Fe content was relatively simple, with high Fe and Zn content linked to an aromatic trait making selection easy in early generations by smell (Graham *et al*, 1997) Gregorio *et al* (2000) carried out the genetic component analysis of grain Fe content using four traditional high Fe rice varieties The study revealed the presence of both additive and non-additive genetic variance for grain Fe content, with 43% narrow sense heritability

Zhang *et al* (2004) studied full diallel crosses of six black pericarp rice varieties and one white aromatic variety to analyze the genetic, maternal and cytoplasmic genetic effects on Fe and Zn contents in rice grains The genetic effects were found to be more influential than the maternal effects on Fe and Zn contents, and the additive effects constituted a major component of the genetic variation

Beebe *et al* (2000) studied genetics of mineral content in three different recombinant inbred line (RIL) populations developed for molecular marker analysis in common bean In all the three sets of populations, both Fe and Zn content in the RIL's had a continuous distribution indicating it a quantitative trait Cichy *et al* (2005) found a single dominant gene controlling the high seed Zn accumulation in Navy bean Another preliminary study in rye (*Secale cereale* L) addition lines showed several loci on as many different chromosomes involved in Zn efficiency in rye (Graham, 1984)

2.5.4. Genotype × environment interaction

Genotypes grown in multi-environment trials react differently to environmental changes The seasonal fluctuations and their interactions highly influence the performance of genotypes in relation to their grain quality traits The stability performance of genotypes for grain Fe and Zn and the G × F interaction with various factors are reviewed hereunder

2.5.4.1. Stability performance of grain Fe and Zn content: Graham *et al* (1999) evaluated ten rice lines/cultivars in different environments and observed that high Fe and Zn content were expressed in all environments tested, although there was some evidence of significant genotype × environment interactions that could ultimately affect grain Fe and Zn content in extreme environments Abilgos-Ramos *et al* (2004) evaluated ten Philippine rice cultivars for stability of grain Fe content in four test environments The level of Fe content across the genotypes tested remained stable, there was a significant environment effect, but no significant G × E effect Most of the cultivars had lower grain Fe content during the wet season

Monasterio and Graham (2000) observed significant genotype × environment interactions for grain Fe and Zn content in wheat Beebe *et al* (2000) observed that grain Fe and Zn contents in bean were stable across the environments, although there were a significant location and location × genotype effects demonstrating that environments can influence the concentration of Fe and Zn in bean seeds Reddy *et al* (2005) noticed the

bsence of genotype × environment interaction for grain Fe and Zn in a fertility level xperiment in sorghum

2.5.4.2. Effect of soil salinity on grain Fe and Zn content: It is well known that soil has a considerable influence on the nutrient content of grains Soil pH is the most decisive factor affecting availability of Zn to plant roots Increased soil pH stimulate Zn adsorption to surfaces of various soil constituents such as metal oxides and clay minerals, this resulting in substantial decreases in solubility and, hence, reduced availability of Zn to plants (Brummer *et al* , 1983, Barrow, 1987)

Crops grown on saline, sodic and calcareous soils can suffer from micronutrient deficiencies (Richard, 1954) Lins and Cox (1988) studied the effect of pH on soil Zn concentrations and found extractable Zn levels were not significantly affected by changes in pH, plant Zn content decreased as soil pH increased Sims (1986) reported that soil pH markedly alters the distribution and plant availability of Zn Soliman (1988) reported that increasing salinity progressively increased Zn and Fe contents of both shoots and roots of corn plants Rahman *et al* (1993) indicated that salinity increased the concentration of Zn, but it decreased the Fe concentration in the grains of maize

2.5.4.3. Effect of N on grain mineral content: Shukla and Bhatia (1971) conducted an experiment to find the effects of various nitrogen (N) levels on concentration of Fe in hybrid and local varieties of pearl millet The Fe concentration did not differ significantly with the various levels of N application Zebarath *et al* (1992) reported average increases in grain Zn concentrations by about 10 mg kg^{-1} and 4 mg kg^{-1} when winter wheat was fertilized with 160 kg N ha^{-1} and 40 kg N ha^{-1} as ammonium nitrate, respectively compared to the control with zero N ha^{-1}

Ahmadi *et al* (1993) studied the influence of N application on grain Fe and Zn concentration of six soft endosperm type corn hybrids Nitrogen fertilizer with two levels (34 and 200 kg N ha^{-1}) increased the grain Fe and Zn concentration in the soft endosperm

type corn Ten Triticale (*Tritico secale*) breeding lines, fertilized with different levels of NPK, showed a wider range of Zn (20 to 37 mg kg^{-1}) than Fe (30 to 35 mg kg^{-1}) with a clear negative relationship between grain yield and grain concentrations of grain Fe and Zn content (Feil and Fossati, 1995)

In brown rice, Fe content increased by an average of 15% with the addition of N levels between 0 and 135 kg ha^{-1} Zinc content increased by an average of 10% All combinations of N, P and K were tested and confirmed that the addition of N alone increases the iron concentration in the grain However, the addition of N and potassium maximizes the difference in Fe content between varieties The addition of N (in addition to phosphorus and potassium) increased both Fe and Zn content (Gregorio *et al* 2000) These experiments suggested that the N level is an important favourable factor determining grain mineral content, even under adverse soil conditions

Feil *et al* (2005) studied the influence of water stress and N supply on concentrations of grain minerals (Mg, Ca, Mn, Zn, and Cu) among tropical maize varieties The varieties were evaluated with two water regimes (drought vs irrigation throughout the vegetation cycle), three levels of N fertilization (0, 80, 160 kg N ha^{-1}, applied as ammonium sulfate) in the tropical lowlands of Thailand The water regime did not affect the mineral composition of the grains, however application of N fertilizer reduced the concentrations of Ca and Zn, and increased the concentration of Mn in the grains

2.5.4.4. Response to soil micronutrient fertilizers: Soil application of Fe and Zn increased the grain Fe and Zn concentrations in various cereal crops by a factor of two to three depending on species (Rashid and Fox, 1992) and crop genotype (Graham *et al* 1992) Soil type also influences the extent of increase in Zn concentration as a consequence of soil Zn fertilization In Paaloa soil (pH 6 3), the maximal Zn concentrations achieved in wheat was about 71 mg kg^{-1} even with high Zn fertilization of 27 mg Zn kg^{-1} soil (Rashid and Fox, 1992) In contrast, slightly acidic (pH 5 8), sandy

affer soil with low organic matter content and low CEC (Rengel and Graham, 1995) does not bind with Zn, leaving a relatively greater proportion of Zn in the plant available form, thus allowing for a considerable increase in Zn fertilization to 3 2 mg Zn kg^{-1} soil (up to 145 mg kg^{-1} grain)

In contrast to Zn, soil application of inorganic Fe fertilizers to Fe-deficient soils is usually ineffective because of quick conversion of Fe into plant unavailable Fe (III) forms In contrast, an application of synthetic Fe-chelates for correction of Fe deficiency is effective, but generally expensive (Godsey *et al* , 2003)

2.5.4.5. Interaction of Fe and Zn with other trace elements: Imtiaz *et al* (2003) studied antagonistic effect of Fe and Zn on other micronutrients in sand culture using Long Aston nutrient solution Zn application had adverse effect on Fe concentration and Fe uptake in plants Zinc deficient plants had significantly higher concentration of Fe, Zn also antagonized the uptake of Mn and Cu in the plants Hacisalihoglu *et al* (2001) observed Zn-efficient genotypes might be able to maintain the functioning of Zn-requiring enzymes under low Zn conditions, thus, biochemical Zn utilization may be an important component of Zn Efficiency

2.5.4.6. Influence of processing on grain mineral content: Borade *et al* (1984) reported that cooking and autoclaving does not influence the Fe and Zn content of legume seeds Roasting of soybean slightly changed Fe and Zn content (Ologhobo, 1989) Increase in Fe content after roasting of pearl millet grains was observed by Cisse *et al* (1998), in contrast negative effect of roasting on total Fe content was observed by Annapurani and Murthy (1985) Gregorio *et al* (2000) demonstrated a strong interaction between grain mineral content loss and milling or polishing in rice

2.6. BIOAVAILABILITY

Just increasing the concentration of micronutrients in the edible plant parts does not guarantee for the improved nutritional status of people with micronutrient

deficiencies. This is because micronutrients in plant parts are not fully bioavailable. Thus, determining the bioavailability of Fe and Zn in the genetically enhanced new lines is an important aspect of crop Biofortification program.

2.6.1. Fe bioavailability

Some of the dietary factors or ingredients that depress Fe absorption in human diet are interaction with mineral elements having similar physiochemical properties or shared absorptive pathways (Mills, 1985), protein from various plant or animal sources (Kim *et al.*, 1995), polyphenolic compounds such as tannins (Cook *et al.*, 1995) and phytates (inositol hexa-phosphate), and lesser phospharylated derivatives of inositol (Reddy *et al.*, 1996).

The mechanism by which phytate depresses the bioavailabiliy of Fe, as well as that of Zn is due to its ability to chelate divalent cations. Phytate forms insoluble complexes which co-precipitate as Fe–Ca phytate and there are not available for absorption. It should be noted that dietary phytate had little effect on the bioavailabiliy of Fe in some studies with humans (Beard *et al.*, 1996).

Several dietary factors increase the bioavailability of Fe; the absorption of heme and non-heme Fe is enhanced when meat is consumed (Bothwell *et al.*, 1989). The mechanism of 'meat factor' enhances Fe absorption through chelation of non-heme Fe by aminoacids, polypeptides, proteins or other factors and stimulation of increased secretion of gastric acid to solubilize the dietary Fe. Ascorbic acid (Vitamin A) markedly increases the absorption of non-heme Fe from various sources (Haltberg *et al.*, 1989). Ascorbate functions to improve the Fe absorption by converting Ferric Fe to Ferrous Fe, which is more soluble at the pH in the upper regions of the intestinal tract (Carpenter and Mahoney, 1992).

In addition to ascorbate, other dietary organic acids including citric acid, tartaric acid and lactic acid enhance Fe absorption (Bothwell *et al.*, 1989). Recent studies

indicated that supplemental vitamin A overcomes the depressing effect of phytic acid and polyphenols on Fe absorption (Layrisse *et al.*, 1997).

2.6.2. Zn bioavailability

Many factors affect the bioavailability of Zn in the human diet. Because of its capacity to bind with minerals, phytic acid has been considered to be an antinutrient. It was proposed that phytic acid depressed Zn bioavailability (Saha *et al.*, 1994). In addition to phytic acid, other dietary components have been assessed for their effect on Zn bioavailability to humans. Some of these factors include: interactions between Zn and other mineral elements (Davidsson *et al.*, 1995); protein quantity from various plant or animal sources (Davidsson *et al.*, 1996), and several other Zn binding such as picolinic acid, citric acid, arachidonic acid and amino acids (House *et al.*, 1996).

The nutrients of particular importance are phytic acid, a powerful inhibitor of Zn absorption. Addition of phytic acid to diets in amounts usually found in whole grain-based cereals reduced the absorption of Zn by one-half, from 34% to 17% (Turnlund *et al.*, 1984). The Zn absorption from cereal-based meals in humans shows a gradual decrease in Zn absorption as phytic acid concentrations increase (House *et al.*, 1996). At the concentrations of 400–500 mM phytic acid, Zn absorption was < 10% and it was reduced to <5% at the increased concentrations of 1000 mM (660 mg), indicating that substantial reductions in phytic acid would be necessary to significantly improve Zn absorption.

Donangelo *et al.* (2003) studied Zn absorption in young women fed on diets formulated from dry bean genotypes with different Zn levels. Although seed Zn content among the genotypes varied by two-fold, phytic acid levels were similar in all genotypes. The women who consumed a high Zn-bean diet compared with a low-Zn bean diet showed an increase in total Zn absorption by 40 per cent.

Unfortunately, it is impractical to test the bioavailability of selected micronutrients in numerous genotypes of staple plant foods that can be generated in a

plant breeding program (Graham and Welch, 1996). Therefore, one must use a bioavailability model to screen large numbers of promising lines of micronutrient-enriched genotypes identified in such breeding programs. Current breeding efforts to screen large numbers of promising micronutrient-dense lines of staple plant foods (rice, maize, wheat, beans, and cassava) at several CGIAR Centers for bioavailable Fe relies on an *in vitro* Caco-2 cell model. Previous bioavailability screens for Fe and Zn were based on a rat model (Welch *et al.*, 2000). Furthermore, plant foods contain substances (i.e. antinutrients and promoters (Phytic acid) that influence the bioavailability of these nutrients to humans. Thus, it is necessary to demonstrate the efficacy of micronutrient enrichment of plant foods towards improving the nutritional health of targeted populations. This requires that the bioavailability of Fe, Zn, and other micronutrients in selected micronutrient-enriched genotypes of staple plant foods be demonstrated, in order to assure a human health impact before advancing genotypes in breeding programs (Graham *et al.*, 2001).

2.6.3. Bioavailability reducers and promoters

Phytic acid is the primary storage form of phosphorus in most mature seeds and grains. Around 75% of total P in wheat grains is stored as phytic acid (Raboy *et al.*, 2001). Because phytic acid has high Zn binding and complexing ability, it hampers minerals biological availability in diets (Welch, 1993). However, phytic acid is required for early seed maturation, seedling growth, vigour, and viability (Graham and Welch, 1996). Phytic acid also plays an important role in determining the mineral nutrient reserves of seeds and as such contributes to the viability and vigour of the seedling. For these reasons, it has been argued that selecting genotypes with substantially lower phytic acid content could have unacceptable effects on agronomic performance, especially in regions of the world with low- phosphorus soils. However, initial results from research in low–phytic acid mutants suggested that these mutants perform well agronomically and there is no noticeable decline in yields (Raboy *et al.*, 1985).

There is large variability for phytic acid concentration in seed among wheat cultivars (Raboy, 1996). Similarly, a wide range of 590–1040 mg $100g^{-1}$ phytate content was reported among different varieties of pearl millet (Chauhan *et al.*, 1986) and, among ten pearl millet inbreds the range was 354–795 mg$100g^{-1}$ (Abdalla *et al.*, 1998). These results indicate that breeding genotypes for lower phytic acid is feasible and may be the solution for the phytate-induced nutritional problems in humans.

Another potentially complementary approach to increasing the bioavailability of minerals (Fe and Zn) in staple crops is to increase the concentration of sulfur- containing amino acids, methionine, lysine, and cysteine that are thought to promote their absorption. Results of a few studies in rats and in humans showed that both the amount of proteins and the concentration of cysteine, and histidine to a lesser extent, had positive effects on mineral absorption particularly Zn (Snedeker and Gregor, 1983). More recent work with marginally Zn-deficient rats showed that diets supplemented with amino acids increased the absorption of Zn from an initial 64% to 69% with lysine, 82% with methionine, and 86% with both amino acids (House *et al.*, 1996). Another experiment on testing the effect of supplemental cysteine and methionine in test meals also showed an increase in the absorption of Zn from 53% and 57% initially to 73% with either of the amino acid added.

MATERIALS AND METHODS

CHAPTER 3
MATERIALS AND METHODS

The present investigation was conducted during the period from January 2004 to July 2006 at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru (17° N; 78° E), Andhra Pradesh, India. The experiments were conducted in the Alfisol (red soil) fields at ICRISAT, Patancheru. This chapter describes details of the plant materials, experimental methods (both at the field and laboratory levels), and statistical methods used in this investigation.

3.1. EXPERIMENTAL MATERIALS

Magnitude of genetic variability in pearl millet for grain Fe and Zn content were studied in four different sets of experiments. First set (set I) comprised of 120 entries that included a diverse range of hybrid parents, improved populations, advanced population progenies and germplasm accessions. Second set (set II) consisted of 69 improved populations, mostly developed by ICRISAT pearl millet programs in Asia and African regions. Third set (set III) had initial and advanced recurrent selection cycle bulks of five composites. The fourth set (set IV) included 68 S_2 progenies of an open-pollinated variety (GB 8735) released in several countries of western and central Africa, and 64 S_3 progenies derived from another open-pollinated variety (AIMP 92901) released in India, to study the intra-population variability.

3.1.1. Variability experiment: set I trial

A diverse range of 120 pearl millet entries (Table 2) that included 30 each of improved populations and advanced population progenies, and 20 each of germplasm accessions, seed parents and pollinators were evaluated during the 2004 summer and rainy season. This trial was inclusive of the three controls, consisting of the first ICRISAT-bred and widely cultivated open-pollinated variety (WC-C75), and seed parent (81B) and pollen parent (ICMP 451) of the first ICRISAT-bred and widely cultivated

Table 2. Pedigree/origin of 120 entries evaluated for grain Fe and Zn content during the 2004 summer and rainy seasons (set I), ICRISAT- Patancheru.

Trt. No.	Entry	Pedigree/Origin
Seed parent		
1	81 B	Induced downy mildew resistant selection from Tift $23D_2B$
2	843 B	Selection from KSU line BKM 2068
3	863 B	Togo-13-4-1
4	ICMB 88004	Togo-11-5-2 selection
5	ICMB 89111	[843B × (GNS × SS-48-40-4)-1-9-8]-30-B-B-1
6	ICMB 91777	[843B × (J 1623 × 3/4 EB-96-1-10)]-5-2
7	ICMB 93333	(843B × ICMPS 900-9-3-8-2)-21-8-4
8	ICMB 94111	{(ICMB 89111×ICMB 88002) × [(81B×SRL53-1) ×843B]-3+× IP9402-2+}-31
9	ICMB 94555	{{[843B×(843B×700651)]+×1163B} × (ICMB 89111×ICMB 88004)}-5-3
10	ICMB 95222	{[843B×(GNS×SS-48-40-4)-29-7-4-B] × (843B×ICMPES-29)-23-2-3}-16
11	ICMB 97111	HTBC-48-B-1-1-1-1
12	ICMB 98222	ARD-288-1-10-1-2 (RM)-5
13	ICMB 00888	(843B×ICTP8202-161-5)-20-3-B-B-3
14	ICMB 91444	[843B × (Boudama - 481 × Ankautess-2)-4]-2
15	ICMB 01555	(BSECBPT/91-40×SPF3/S91-529)-12-1-1-3
16	ICMB 01888	{[(81B× SRL-53-1) × 843B]-3-5-3×[(843B×111B)-10-1-2-2]}-226-B-2-B-B-B
17	ICMB 00999	(ICMB 89111 × 863B)-65-8-B-B
18	ICMB 90111	Maintainers of EGP-261 cytoplasm selected from pollen parent ICMP 423
19	ICMB 98777	[(F4FC 1498-1-1-3 × J 104)-11-2-1-1]-7-3-1-B
20	ICMB 97333	(ICMB 89111 × ICMB 88004)-9-2-6-3-3-2-B
Advance population progenies		
21	AIMP 92901 S1-15-1-2-B	Aurangabad and ICRISAT
22	AIMP 92901 S1-183-2-2-B	Aurangabad and ICRISAT
23	AIMP 92901 S1-421-2-3-B	Aurangabad and ICRISAT
24	ICMR 312 S1-22-1-2-1-B	ICRISAT
25	ICMR 312 S1-25-1-1-1-3-B	ICRISAT
26	ICMR 312 S1-39-1-6-1-B	ICRISAT
27	ICMS 7704 S1-13-3-3-1-3-B	ICRISAT
28	ICMS 7704-S1-51-4-1-1-B	ICRISAT
29	ICMS 7704-S1-80-1-3-B	ICRISAT
30	ICMS 8274 S1-1-3-3-1-B	ICRISAT
31	ICMS 8282 S1-8-5 6-1-B	ICRISAT

Table 2. Contd.

Trt. No.	Entry	Pedigree/Origin
32	ICMS 8511 S1-14-3-1-B	ICRISAT
33	ICMS 8511 S1-17-2-1-1-B	ICRISAT
34	ICMV 91059 S1-11-3-2-2-B-B	ICRISAT
35	ICMV 91059 S1-14-2-4-2-2-B	ICRISAT
36	ICMV 91059 S1-58-2-2 2-B	ICRISAT
37	ICMV 93074 S1-9-1-1-1-B	ICRISAT
38	ICMV 93074 S1-9-2-1-1-1-B	ICRISAT
39	ICMV 93074 S1-9-2-2-1-B	ICRISAT
40	MC 94 C2-S1-3-1-1-2-B	ICRISAT
41	MC 94 C2-S1-46-1-1-B	ICRISAT
42	MC 94 C2-S1-47-1-1-B	ICRISAT
43	RCB-2 S1-33-1-3-3-2-B	ICRISAT
44	RCB-2-S1-51-3-2-1-B	ICRISAT
45	RCB-2-S1-121-1-2-1-B	ICRISAT
46	RCB-2-S1-138-2-2-1-B	ICRISAT
47	SDMV 90031-S1-84-1-1-2-B	ICRISAT
48	SDMV 90031-S1-93-3-1-1-B	ICRISAT
49	SDMV 93032-S1-5-2-1-1-B	ICRISAT
50	SDMV 93032-S1-93-3-2-2	ICRISAT
Pollen parent		
51	HTP 94/54 (HHB 146)	Hisar
52	G 73/107 (HHB 94)	Hisar
53	H 77/29-2 (HHB 117)	Hisar
54	H 77/833-2 (HHB 67)	Hisar
55	J 104 (Sel.)	Jamnagar
56	ICMP 451	LCSN 72-1-2-1-1
57	ICMR 356	(B 282 × J 104)-12-B-B-B
58	RIB 335/74 (RHB 30)	Rajasthan
59	RIB 3135-18 (RHB 121)	Rajasthan
60	CZI 98-11	Jodhpur
61	CZI 9621	Jodhpur
62	IPC 577 (Dual restorer)	[IP 2788 × (J9347 × 700544-7-2-1)]-1-4-1
63	IPC 804-2 (Dual restorer)	(S10LB-30 × LCSN 1225-6-3-1)-1-2-1-1-2
64	IPC 827-1 (Dual restorer)	(5054B × F4FC 1498-1-1-1)-3-1-1-1
65	IPC 873 (Dual restorer)	B Senegal -2-5 × EC 298 -2-2-3-1-1-3
66	IPC 1518 (Dual restorer)	ICRC -F4-146-3
67	IPC 94	EC-S3-211-1-2
68	IPC 802	(E 298 × LCSN 282-4-1-2)-12-2-1
69	IPC 843	[(J834 × 700516)]-1-4-4-2-4
70	MIR 97171	Mandor

Table 2. Contd.

Trt. No.	Entry	Pedigree/Origin
Open pollinated varieties		
71	NCD2 Bulk	NCD_2 C_0 bulk developed by random mating 41 dwarf BC_1F_4 progenies derived from introgression of the d_2 dwarfing gene from GAM 73 into Nigerian Composite (NC)
72	ICMR 312	BSEC TCP_2 C_1
73	Raj 171	Bred by random mating 8 S_1 progenies of Inter-varietal Composite selected at Patancheru
74	SRC II C4	Fourth cycle random mated full-sib bulk of Smut Resistant Composite II Developed at ICRISAT by random mating 71 selections from Inter-varietal Composite and 62 selections from Smut Resistant Composite in 1987
75	SOSAT C88	Developed by random mating 248 S_1 progenies from the composite Souna × Samo
76	ICMV 221	Bred by random mating 124 selected S_1 progenies of Bold-Seeded Early Composite (BSEC) drought trial
77	GB 8735	Developed by random mating four F_3 lines derived from crosses involving *Iniari* and Souna
78	MC 94	Developed by random mating 172 progenies visually selected at Patancheru from two varieties and three populations developed from *Iniari* landraces of Togo and Ghana origin, other African countries and India
79	ICMV 91059	Bred at ICRISAT by random mating 42 S_1 selections of ICMS 8359 at Patancheru in Kharif 1990
80	ICMS 7703	Bred from 7 inbreds selected at Patancheru in 1977
81	ICMS 7704	Synthetic bred from 6 inbred lines derived from Indian × African crosses selected at Tandojam in Pakistan in 1977
82	RCB 2	Developed from 20 inbreds of diverse genetic origin from Rajasthan
83	AIMP 92901	Bred by random mating 272 Bold Seeded Early Composites (BSEC) S_1 progenies selected at Aurangabad and BSEC S_1s bulk at Patancheru in Kharif 1991
84	ICMS 8511	Bred from 4 inbreds selected from Diallel Trial (DAT 1) in 1984
85	ICMV 93074	Reselected dwarf version of ICMV 82132 developed by backcrossing with D_2C_6
86	SDMV 90031	Recombination of ICMV 89003, ICMV 89004, ICMV 89005, ICMV 88908 and SDPM 2264
87	SDMV 93032	BC_3F_{1-7} SDGP 1514 × OK -1
88	Jakhrana pop	Landrace population from Jakharana, Rajasthan
89	Barmer pop	Bred at ICRISAT by random mating 6 germplasm accessions collected from Barmer, Rajasthan in 1992 (IC MP 94852)
90	EEBC	Extra early B-composite developed by random mating 286 S_2 progenies
91	WC-C 75	Bred from 7 full-sib progenies of World Composite selected at Coimbatore in 1975 Reselection at ICRISAT-Patancheru within a composite population originally developed in Nigeria
92	JBV 2	Bred at ICRISAT by random mating 140 S_1 progenies of GICV 92191
93	JBV 3	Bred jointly by Jawaharlal Nehru Krishi Vidyapeeth and ICRISAT by random-mating 15 Smut Resistant Composite II C3 full-sib progenies selected visually at Patancheru in 1995
94	CO 8	(732 A × Giant Bajra) (Composite), TNAU, Coimbatore
95	AFB 2	Anand Fodder Bajra variety
96	ICMV 88908	Mass-selected variety from the cross between BSEC-C_4 and 87901

Table 2. Contd.

Trt. No.	Entry	Pedigree/Origin
97	HHVDBC	High Head Volume Dwarf B-Composite developed by random mating of 35 S_1 progenies of HHVBC
98	E Raj pop	Early-Rajasthan Population Developed by four selected landraces collected from dry parts of Rajasthan
99	CZP 9802	Bred by random mating 14 early-maturing and high-tillering full-sib progenies of a population - Early Raj Population
100	MCNELC	Bred by random mating 23 selected F_1s of MC 94 and NELC II at ICRISAT
Germplasm accessions		
101	IP 3122	India
102	IP 3859	India
103	IP 5830	Senegal
104	IP 6244	Cameroon
105	IP 6271	Mali
106	IP 6764	Malawi
107	IP 9453	Ghana
108	IP 7946	ICRISAT
109	IP 8032	Ghana
110	IP 8964	Togo
111	IP 10781	Sudan
112	IP 12000	Nigeria
113	IP 12012	Nigeria
114	IP 12089	Nigeria
115	IP 12240	Nigeria
116	IP 12245	Nigeria
117	IP 12313	Nigeria
118	IP 12341	Nigeria
119	IP 12383	Nigeria
120	IP 12384	Nigeria

hybrid (ICMH 451). The trial was laid out in a randomized complete block design (RCBD) with two replications. Each entry was grown in 4 rows of 4 m length.

3.1.2. Variability experiment: set II trial

A diverse range of 69 improved populations mostly developed by ICRISAT in its regional programs in Asia, Western Central Africa (WCA) and South Eastern Africa (SEA) were evaluated during the 2004 rainy season and 2005 summer season. The experiment was conducted in a RCBD in two replications. Each population was planted in 2 rows of 4 m length. The parentage details of the improved populations used in this experiment are given in Table 3.

3.1.3. Variability experiment: set III trial

Five diverse composites had earlier been improved for 3-8 cycles for grain yield by various methods of recurrent selection at ICRISAT (Table 4). These advanced cycle bulks along with their respective base composite bulks were evaluated in RCBD in two replications during 2004 rainy season and 2005 summer season, to examine if the recurrent selection for grain yield led to any changes in grain Fe and Zn contents. Each composite was grown in a RCBD in two rows of 4 m length, replicated twice.

3.1.4. Intra-population variability experiment: set IV trial

A total of 64 (S_1) progenies from AIMP 92901 and 68 (S_2) progenies from GB 8735 that had been identified having high Fe and Zn in the 120-entries evaluation trials (set I), were field tested to determine the intra-population variability for grain Fe and Zn content. The trials were conducted in a RCBD design with two replications during the 2005 rainy and 2006 summer seasons. Each entry was grown in single row of 4 m length.

3.1.5. Stability trial

Based on the results of two seasons (2004 summer and rainy seasons) evaluation trials of 120 entries (set I), twenty-nine entries were selected to study the stability of performance for grain Fe and Zn content. The selected lines included 14 high, eight medium and six low both for Fe and Zn content with WC-C75 used as a control (Table 5).

Table 3. Pedigree details of improved populations evaluated for grain Fe and Zn content during 2004 rainy and 2005 summer seasons (set II), ICRISAT-Patancheru.

Trt. No	Population	Parentage	Origin
1	3 4 HK	Selected within BC_1F_2 from backcross between dwarf line (Hft) and recurrent parent (Niger landrace Haini kire)	Niger
2	Acid Tolerant Pop	Introduced from ICRISAT's later American program	ICRISAT
3	Ankoutess	Developed by random mating 16 S_1 progenies derived from Ankoutess	ICRISAT Niger
4	Bmr CIVT 133	Selected within BC_1F_1S from backcross between bmr line and recurrent parent CIVT	ICRISAT Niger
5	Bmr CIVT 153	Selected within BC_1F_1S from backcross between bmr line and recurrent parent CIVT	ICRISAT Niger
6	Bmr HKP 163	Selected within BC_1F_1S from backcross between bmr line and recurrent parent HKP	ICRISAT Niger
7	CGP	Selection from Togo germplasm (GGP bulk)	ICRISAT Niger
8	CIVT GMS	Developed by 4 generations of Gridded mass selection in CIVT	INRAN Niger
9	EDC	Developed by crossing early composites and dwarf composites constituted at ICRISAT	ICRISAT Patancheru
10	EXB	Selection from a landrace population from Bornu state in Nigeria	Nigeria
11	Faringuero	S_1 recurrent selection from cross of GRP1 and GB 8735	ICRISAT Niger
12	GGP Bulk (C)	Togo germplasm accessions collected in 1988 (Half sib recurrent selection)	ICRISAT Niger
13	GGT Bulk (C_0)	Togo germplasm accessions collected in 1988 (Half sib recurrent selection)	ICRISAT Niger
14	HKP GMS	Developed by 4 generations of Gridded mass selection in HKP	ICRISAT Niger
15	ICMV IS 85333	Developed by random mating 8 S_1 progenies derived from variety ITMV8001	ICRISAT Niger
16	ICMV IS 86330	Developed by random mating 6 S_1 progenies derived from Souna × Ankoutess	ICRISAT Niger
17	ICMV IS 88305	Developed by random mating 6 S_4 progenies derived from INMG1	ICRISAT Niger
18	ICMV IS 90311	Developed by S_1 recurrent selection from Haini kire × *iniari*	ICRISAT Niger
19	ICMV IS 92326	Developed by S_1 recurrent selection from ISC 851	ICRISAT Niger
20	ICMV IS 95303	Developed from F_4 normale plant derived from (bmr × CIVT)× CIVT	ICRISAT Niger
21	ICMV IS 99001	Developed from half sib recurrent selection in HKP	ICRISAT Niger
22	ICMV IS 99002	Developed from half sib recurrent selection in HKP	ICRISAT Niger
23	ICMV IS 99003	Developed from half sib recurrent selection in HKP	ICRISAT Niger
24	ICMV IS 99005	Developed by S_1 recurrent selection from IP10437	ICRISAT Niger
25	PVGGP 1	S_1 progeny selected from a Togo germplasm	ICRISAT Niger
26	PVGGP 6	S_1 progeny selected from a Togo germplasm	ICRISAT Niger
27	PVGGT 4	S_1 progeny selected from a Togo germplasm	ICRISAT Niger
28	PVGGT 5	S_1 progeny selected from a Togo germplasm	ICRISAT Niger
29	Sasank	Developed by S_1 recurrent selection from SOSAT × Ankoutess	ICRISAT Niger

Table 3. Contd.

rt. No	Population	Parentage	Origin
30	SDMV 89004	Developed by mass selection in SDMV 87014	ICRISAT-Zimbabwe
31	SDMV 92037	Developed by recombination of 8 S_1 progenies from SDGP2812	ICRISAT-Zimbabwe
32	SDMV 95022	[(IBMV8502 × ICMV 87901)-1-1-1]BC$_4F_2$	ICRISAT-Zimbabwe
33	SDMV 95045	Recombination of 6S_1S from OK - 1-S_1 trial (late)	ICRISAT-Zimbabwe
34	HKP GMS	Developed by 4 generations of Gridded mass selection in HKP	ICRISAT - Niger
35	IKMV Bulk	ICRISAT Kanboenze millet variety Recurrent selection from landrace	Burkina Faso
36	Guerianiari	Developed from a cross of GRP1 and GB 8735 The GRP1 is from Guerguera landrace from Maradi- Zinder area Niger	ICRISAT
37	HiGroP	High growth Population Developed by random mating 11 variety cross F_2's involving lines from West Africa, Pakistan and Western India	ICRISAT
38	HiTip 88	Developed by random mating 13 high-tillering progenies from ICRISAT pollinator collection and and open-pollinated variety (CZP 86) The CZP 86 was developed from crosses between ICRISAT-bred materials and germplasm from western Rajasthan	ICRISAT
39	HTBC	High-tillering B-composite developed by random mating 100 progenies derived from pedigree breeding	ICRISAT
40	ICMV 96752	Bred by random-mating best 15 Smut Resistant Composite II C_1 full-sib progenies selected visually at Patancheru in 1995	ICRISAT
41	ICMV 98109	Bred at ICRISAT by random mating 14 S_1 progenies of ICTHC6	ICRISAT
42	ICMV 99902	Bred at ICRISAT by random mating 15 S_1 progenies of HHVBC Tall C_0	ICRISAT
43	ICMV-IS 94206	ICMV-IS 94206 has a pedigree similar to ICMV-IS 92222, as it was bred at ISC-Sadore by reselection within a farmers' local (Haini-Kirei) collected at Say, Niger	SADC/ICRISAT
44	ICMV-IS 92222	Bred by reselection within a farmers' local (Haini-Kirei) collected at Say, Niger This improved version of Haini-Kirei was bred by S_1 selection against shibras and for downy mildew resistance, and earliness	SADC/ICRISAT
45	ICRC H	Developed at ICRISAT by random mating 21 restorer lines (17 from Patancheru, 3 from Indian National program and 1 from Kansas State University)	ICRISAT
46	ICTP 8203	Bred from 5 S_2 progenies selected from a landrace from Togo at Patancheru in 1982	ICRISAT
47	IAC-ISC-TCP-4	Bred by random mating two pollinators developed at ICRISAT-Patancheru, one inbred pollinator and two open-pollinated varieties from ICRISAT-Niger	ICRISAT
48	IAC-ISC-TCP-6	Bred by random mating two pollinators developed at ICRISAT-Patancheru, one inbred pollinator and one open-pollinated variety from ICRISAT-Niger	ICRISAT
49	Jakharana (ICMP 97880)	Bred at ICRISAT by random mating 48 S_1 progenies of Jakharana selected at Fatehpur in 1996 rainy season	ICRISAT
50	Lubasi	Developed at ICRISAT by random mating 32 selected varieties with the bulk of 210 open-pollinator selections of African introductions	ICRISAT
51	MC-C10	Tenth cycle random mated bulk of Medium Composite (MC) The MC was developed at ICRISAT by random mating197 geographically diverse lines from India and Africa that flowered in 45-55 days at Patancheru in 1973	ICRISAT
52	MRC	Mandor Restorer Composite, developed by random mating	ICRISAT

Table 3. Contd.

Trt. No	Population	Pedigree/Source population and selection method	Origin
53	NELC II	Developed at ICRISAT by random mating 7 improved varieties-populations in 1994.	ICRISAT
54	GAM 73	Dwarf synthetic introduced from Senegal	ICRISAT
55	SADC White grain	Developed by random mating 34 S_1 progenies selected for long panicles received from Zimbabwe.	ICRISAT
56	SenPop	Developed by back-crossing and selection involving a weedy selection from Senegal and the F_1 of the variety cross between ICMV 87901 (from the ICRISAT Bold-Seeded Early Composite) and ICMV 82132 (from the ICRISAT Smut Resistant Composite, released in Zambia as 'Kaufela')	ICRISAT
57	SRBC	Smut Resistant B-comp developed by random mating of 47 entries derived from pedigree breeding	ICRISAT
58	SRC III-C2	SRC III C2: Second cycle full-sib random mated bulk of Smut Resistant Composite III : Developed at ICRISAT by random mating 8 crosses involving IVC, ESRC II, Lubasi, SRC II, SenPop and ICMV 91059	ICRISAT
59	SSC-C7	Seventh cycle random mated bulk of Super Serere Composite (SSC) SSC was developed at ICRISAT in 1984 by random mating four composites [$SC_1(S_4)$, $SC_2(M)$, $SC_3(M)$ and $SC_4(M)$] originally developed at Serere Research Station, Uganda	ICRISAT
60	Ugandi	Developed at ICRISAT from Serere composite selection	ICRISAT
61	W Raj Pop	Developed at ICRISAT by random mating 29 Chadi type land race entries selected from Western Rajasthan in 1988	ICRISAT
62	EC 87 C_0	Early Composite 87 developed at ICRISAT by crossing between EC II, ICMV 87901, ICMV 87902 and ICMV 87119	ICRISAT
63	ELC C_0	Initial cycle bulk of Elite Composite II (ELC II). Developed at ICRISAT by random mating 292 elite lines from NELC, MC, SRC, IVC, Popular varieties and Synthetic varieties	ICRISAT
64	Afpop 90	Very tall, very long duration, feeder population developed by random mating inbred bulks selected at ICRISAT-Patancheru from breeding materials received from Zambia and Zimbabwe	ICRISAT
65	LaGrap	Large Grain Population developed at ICRISAT based on large-seeded germplasm accessions from Africa and Asia	ICRISAT
66	SSC C1 Brist	First cycle random mated bulk of Super Serere Composite (SSC). SSC was developed at ICRISAT by random mating four composites [$SC_1(S4)$, $SC_2(M)$, $SC_3(M)$ and $SC_4(M)$] originally developed at Serere Research Station, Uganda	ICRISAT
67	ICMS 7704	Bred from 6 inbred lines derived from Indian × African crosses selected at Tandojam in Pakistan in 1977	ICRISAT
68	RCB 2	Developed from 20 inbreds of diverse origin in Rajasthan	Rajasthan
69	WC-C 75	Bred from 7 full-sib progenies of World Composite selected at Coimbatore, India	ICRISAT

Table 4. Pedigree details of initial and advanced recurrent cycle bulks of populations evaluated for grain Fe and Zn content during 2004 rainy and 2005 summer seasons (set III), Patancheru.

Composite	Cycle bulks	Pedigree	Origin
Medium composite (MC)	C_0	Initial cycle bulk of Medium Composite Developed at ICRISAT from 197 geographically diverse lines from India and Africa	ICRISAT
	C_8	Eighth cycle random mated bulk of MC formed by random mating of 54 selected S_1 progenies from the C_7 cycle	ICRISAT
Super Serere Composite (SSC)	C_1	First cycle random mated bulk of Super Serere Composite Developed by merging four populations (SC_1(S4), SC_2(M), SC_3(M) and SC_{14}(M) received from Uganda in 1974	ICRISAT
	C_6	Sixth cycle random mated bulk of Super Serere Composite	ICRISAT
New Elite Composite (NELC)	C_0	Initial cycle random mated bulk of New Elite Composite Developed from 47 Elite progenies from different Composites in 1977	ICRISAT
	C_5	Fifth cycle random mated bulk of NELC formed by random mating 66 selected S_1 progenies from the C_4 cycle	ICRISAT
Serere Composite I (SC I)	C_1	First cycle random mated bulk of Serere Composite-I	ICRISAT
	C_4	Fourth cycle random mated bulk of Serere Composite-I	ICRISAT
Smut Resistant Composite (SRC)	C_0	Initial cycle random mated bulk of Smut Resistant Composite Developed in 1979 by random mating 37 smut resistant lines derived from West African germplasm	ICRISAT
	C_3	Third cycle random mated bulk of SRC	ICRISAT

The trial was repeated in a RCBD in two replications during the 2005 summer and rainy seasons. Each entry was grown in 2 rows of 4 m length.

3.1.6. Diallel crosses

Ten inbred lines were selected based on the results of two seasons (2004 summer and rainy seasons) trials of 120 entries (set 1) with four high and three each of medium and low Fe and Zn content lines for inheritance study. The details of the parental lines are given in Table 6. These were grown in a crossing block during the 2005 summer season. Full diallel crosses (including reciprocals) were made to produce 90 hybrids. Two staggered plantings were done at an interval of ten days in order to enable making all 90 crosses among parents which differed for flowering time. The 90 hybrids and 10 parental lines were evaluated during the 2005 rainy season and 2006 summer season for grain Fe and Zn content. The parents and their hybrids were laid out in a RCBD in three replications as two seperate experiments laid side by side (one for hybrids and one for the inbreds). Each entry was grown in 2 rows of 4 m length.

3. 2. AGRONOMIC PRACTICES AND OBSERVATIONS

All the five field experiments were conducted at ICRISAT-Patancheru. The experiments in all environments were machine-planted in plots of 4-m length with an inter-row distance of 75 cm and 15 cm spacing between the plants within the rows. The trials were conducted in Alfisols with applied fertilizer levels of 75 kg ha^{-1} N (50% basal and 50% top dressing) and 35 kg ha^{-1} P (basal dose) during all the seasons. The trials were irrigated to ensure no moisture stress and the plots were kept free from weeds. The standard cultural and agronomic practices were followed to raise good crop.

Observations were taken on two key characters (time to 50% flowering and seed mass) to determine if grain Fe and Zn could be influenced by these traits.

1). Days to 50% flowering: Days to 50% flowering was recorded as the number of days from sowing until 50% of the plants in each plot produced stigmas on their main panicles.

Table 5. Entries of stability trial with their mean levels of grain Fe and Zn content.

Class and range	No. of Entries	Entry
High **Fe** (51.2–75.7 mg kg^{-1}) and **Zn** (48.1–64 8 mg kg^{-1})	14	843B, 863B, AIMP 92901 S1-15-1-2-B, AIMP 92901 S1-183-2-2-B, EEBC, GB 8735, ICMB 00888, ICMB 00999, ICMB 88004, ICMB 94111, ICMB 98222, ICMS 7704-S1-51-4-1-1-B, IPC 843 and SDMV 90031-S1-84-1-1-2-B
Medium **Fe** (41.1–44.8 mg kg^{-1}) and **Zn** (40.7–45.6 mg kg^{-1})	8	CZI 96-21, CZI 98-11, ICMV 91059 S1-58-2-2-2-B, ICMV 93074 S1-9-1-1-1-B, Jakharana pop, MC 94 C2-S1-46-1-1-B, NCD_2 Bulk and SDMV 93032-S1-93-3-2-2
Low **Fe** (30.1–37.7 mg kg^{-1}) and **Zn** (24.5–33.7 mg kg^{-1})	6	81B, HTP 94/54, ICMB 90111, ICMS 8511 S1-17-2-1-1-B, SDMV 90031-S1-93-3-1-1-B and SOSAT C88
Check	1	WC-C75

Table 6. Inbred line parental diallel crosses with their mean levels of grain Fe and Zn content.

Class	Parental line
High Fe (60.4 75 7 mg kg^{-1}) and Zn (55.8–64.8 mg kg^{-1})	863B
	ICMB 94111
	ICMB 00888
	AIMP 92901 S1-15-1-2-B
Medium Fe (41.8–44.8 mg kg^{-1}) and Zn (40.9 47.1 mg kg^{-1})	ICMB 95222
	ICMV 93074 S1-9-1-1-1-B
	MC 94 C2-S1-46-1-1-B
Low Fe (30.1–36.3 mg kg^{-1}) and Zn (24.5–33.5 mg kg^{-1})	81B
	ICMS 8511 S1-17-2-1-1-B
	ICMV 91059 S1-14-2-4-2-2-B

2) 1000-grain mass Two hundred grains for each plot were counted and weighed to determine 1000-grain mass

3.3. GRAIN PRODUCTION AND SAMPLING FOR IRON AND ZINC ANALYSIS

Sib-mated grain samples were produced for laboratory analysis of grain Fe and Zn content in all the experiments except for progenies in the intra-population variability study, where grain samples were produced by selfing From the 120 entries trial (set 1), three sources of grain samples (sib-mated, selfed and open-pollinated) were produced for comparison of different sources of grains for grain Fe and Zn content

Sib-mated grain samples were produced by hand pollination with bulk pollen collected from 50–60 plants crossed on 20 plants of same entry to produce representative sample of each entry unaffected by foreign pollen effect (if any) and dust Placing a parchment paper bag over a panicle prior to stigma emergence produced selfed seed and the open-pollinated (OP) grains were collected from open panicles The sib-mated, selfed and OP panicles were hand harvested after physiological maturity (85 90 days after planting) and dried in the sun to <12% post-harvest grain moisture content The dried panicles were threshed with well-cleaned thresher (Wintersteiger ID780S I4-Single head thresher) The threshed grains were manually cleaned free from glumes, panicle chaff and debris Fifty-gram grain samples were taken from the grain lot of each plot and transferred to labeled new, clean, metal fold envelops for grain Fe and Zn analysis Care was taken at each step to avoid any contamination of the grains with dust particles and any other extraneous matter

3.4. LABORATORY ANALYSIS OF MICRONUTRIENTS

3.4.1. Soil micronutrient analysis

3.4.1.1. Soil sampling

To assess the soil micronutrient level of the experimental fields, six soil samples were collected by using hand auger at each of the two soil depths viz , 0 15 and 16 30 cm by adopting random sampling procedure The soil samples were air-dried, crushed with a wood mallet and sieved through a 6 mm nylon screen Precautions were taken to

avoid contamination during sampling, drying, crushing and storage. For the laboratory analyses, a representative sub-sample of each soil sample was further pulverized with a wooden rolling pin and screened through a 1mm stainless sieve.

3.4.1.2. Laboratory analysis (DTPA extractable method)

The soil Fe and Zn content was analyzed at Central Analytical Services Laboratory, ICRISAT, Patancheru The available soil Fe and Zn contents were analyzed using DTPA extractable method. The laboratory protocol and DTPA extracting solution for soil Fe and Zn analysis is followed using the procedure described by Sahrawat *et al.* (2002). Ten grams of air-dried soil was placed in a 125 ml conical flask and 20 ml of the DTPA extracting solution was added. Each flask was covered with stretchable and secured upright on a horizontal shaker with a stroke of 8.0 cm with a speed of 120 cycles min^{-1}. After 2 hours shaking, the suspensions were filtered by gravity through Whatman no. 42 filter paper. The filtrates were analyzed for Fe and Zn using atomic absorption spectrophotometry (AAS) with appropriate standards included in the analysis.

3.4.2. Grain micronutrient analysis

3.4.2.1. National Institute of Nutrition, Hyderabad

All the experiments except intra-population variability (set IV) experiment were analyzed at the National Institute of Nutrition, Hyderabad using dry ashing and Atomic Absorption Spectrophotometry (AAS) method. Dry ashing and mineral solution preparation was carried out according to the method described by Jorhem (1993).

Pearl millet samples were powdered to pass through 10 mm-mesh using cyclone sample mill (Udy Corporation, USA). Aliquots of the powdered sample were taken in duplicate and ashed. About 10 -20 g of grain sample weighed in crucible and dried in drying oven or hot plate at 100°C. Samples were placed in programmable furnace at initial temperature <100°C and increased stepwise to 450°C at <50°C h^{-1}. The samples were kept overnight in furnace until they were completely combusted i.e., ash turning into white/grey colour. Crucibles were removed from furnace and cooled down. Five ml of

6M HCl were added to crucible, ensuring that all ash comes into contact with the acid. Acid was evaporated on water bath or hot plate. Residue dissolved in a volume (10-30 ml) of 0.1 M HNO_3. Crucibles were swirled with care so that all ash comes into contact with acid and the sample was allowed for digestion (1 2 hours). Then the solution in the crucible was thoroughly stirred with stirring rod and transferred to plastic bottle, with blanks also treated similarly.

All samples were analyzed in duplicate using Atomic Absorption Spectrophotometer (Thermo Electron Corporation) fitted with GFS97 auto sampler. Fe concentration was determined at 248.3 nanometer (nm) with the Nitrous oxide/acetylene gas mixture, whereas Zn content was determined at 213.9 nm with Air/acetylene gas mixture.

The analytical method used was validated using NIST standard certified reference material (1584A). With every day-to-day run, in-house quality control sample was ashed and quantified together with blank to expose any systematic error. Discrepancies between in-house quality control sample and concentrations quantified were below 5% and the coefficient of variation for single measurements was below 10 per cent

3.4.2.2. Central Analytical Services Laboratory, ICRISAT

Selfed grain samples of population progenies produced during the 2005 rainy season and 2006 summer seasons, and sib-mated grain samples of 12 entries from set I trial (2004 summer and rainy seasons), and sib-mated, selfed and open-pollinated grain samples of 30 entries (2004 summer season) from set I trial were analyzed

Pearl millet grain Fe and Zn content was determined by triacid mixture method. The grain samples were finely ground (<60 mesh for grain samples) using cyclone mill then oven dried at 60°C for 48 h before analysis.

Ground and dried grain samples of 0.5 g were transferred to 125 ml conical flasks. Twelve ml of tri-acid mixture of nitric acid, sulfuric acid and perchloric acid (9:2:1(v/v)) were added to the flasks. The flour samples were digested in a room temperature for 3 h

followed by digestion for 2–3 h on a hot plate, until the digest was clear or colorless. The flasks were allowed to cool and contents were diluted to an appropriate volume The digests were used for Fe and Zn determination using Atomic Absorption Spectrophotometry (AAS).

3.4.2.3. Waite Analytical Laboratory, Adelaide University, Australia

The same 12 entries from the set 1 trial, that were analyzed at ICRISAT, were also analyzed at Waite Analytical laboratory, Adelaide University, Australia, following the method described by Zarcinas *et al.* (1987). The samples were digested with di-acid (nitric/perchloric acid) mixture and the digests were used for Fe and Zn determination using Spectro CIROS Axial Inductively Coupled Plasma Optical Emission Spectrometer (ICPOES).

Grain samples were oven-dried overnight at 85° C prior to digestion and the samples was grounded to pass a 1 mm stainless steel sieve using Christie and Norris hammer mill and stored in screw-top polycarbonate vials without further drying prior to analysis.

Ten ml of nitric acid and 1ml of perchloric acid was added into a 1 0 g flour sample and allowed to stand overnight at room temperature. The samples were heated for 1 h at 120° C and then increased the temperature to 175° C (if the digests turn black, add nitric acid drop wise until the digest clears). Further, increased the temperature to 225° C and digested at this temperature for approximately 10 min to ensure complete dissolution. After cooling, the digests were diluted with 20 ml of 1% nitric acid

Amorphous silica was separated from the digests solution by settling overnight then decanting the supernatant into an auto-sampler test tube before aspirating directly into the plasma for the determination of Fe and Zn. An increase in the background emission occurs if silica is aspirated into the plasma, resulting in false high analyses.

The digested solution was introduced into the plasma using a modified Babington Pneumatic nebulizer. A Gilson Minipuls 2 peristaltic pump with a Tygon red-

red (1.14 mm) pump tube was used for solution delivery to the nebulizer. A stabilization time of 30 seconds was followed by three 20 seconds integrations. The Fe content was read at 259.94 nm and Zn content at 213.86 nm in the ICPOES. Reagent bottles, volumetric ware (plastic and glass) and digestion tubes were cleaned after usage by soaking overnight in 2 N HCl, rinsed with water and oven dried at 60° C. Double distilled water was used for all analytical purposes.

3.5. COST EFFECTIVE APPROACHES FOR IRON AND ZINC ANALYSES

3.5.1. Perl's Prussian blue staining method

The cost of Fe and Zn analysis using the above laboratory methods when applied to a large number of grain samples would be fairly high. Further, laboratory analysis is also time consuming. Thus, a simple, rapid and cheaper method of Perl's Prussian blue staining that has been followed effective in earlier studies in rice (Prom-U-Thai, 2003) was tested for its usefulness as the first-stage selection of lines with high grain Fe content.

Initially, 12 pearl millet lines (6 B-lines and 6 advanced population progenies) with wide range of grain Fe content (30.8 74.7 mg kg^{-1}) identified through Atomic Absorption Spectrophotometry (Jorhem, 1993) at the National Institute of Nutrition, Hyderabad, India were used to standardize this procedure for distinguishing lines with high (51.7–74.7 mg kg^{-1}), medium (40.3 40.8 mg kg^{-1}) and low (30.8 35.7 mg kg^{-1}) Fe content. Subsequently, the procedure was validated using 20 B-lines (counterparts of the designated A-lines) developed at ICRISAT, Patancheru. The grain samples of the genotypes, obtained by sib-mating during 2004 summer season from set I trial were used for this test.

The selected 12 lines with high, medium and low Fe content lines were used for the standardization of the protocol by altering the concentration of potassium ferrocyanide (1%, 2%, 3% and 5%) and hydrochloric acid (1%, 2%, 3% and 5%); and mixing the two in various combinations. Following are the chemicals used in the final protocol for 2% concentration.

1. 2% aqueous potassium ferrocyanide: Mix well 10.0 g potassium ferrocyanide with 500 ml distilled water, pour into an acid-cleaned brown bottle. The solution will be stable for 6 months.
2. 2% Aqueous hydrochloric acid: Mix well 10.0 ml concentrated hydrochloric acid (HCl) with 490 ml distilled water, pour into brown bottle. The dilute HCl will be stable for 6 months.
3. Prussian blue solution: Mix equal volumes of 2% HCl and 2% ferrocyanide solution. This solution needs to be made fresh every time during the experimentation and the remaining solution need to discarded.

The ferric Fe is released from any attachments in protein by treatment with dilute hydrochloric acid and then reacts with a dilute solution of potassium ferrocyanide to produce an insoluble compound, ferric ferro cyanide (Prussian blue) with blue colour. The intensity of blue color shows the concentration of Fe in the grain.

Dry pearl millet grains were made into flour by Pestle and Mortar and 0.5 g of the flour of each sample was transferred into a quarter (of the 4) of the Petri dish. Pestle and Mortar was cleaned adequately after grinding each sample to avoid contamination of the flour from the previous sample. The flour samples were submerged in freshly prepared Prussian blue solution (10 ml) for 10 minutes. The development of the blue colour in flour was recorded and compared with the actual Fe content of the genotypes. The colour intensity was visually scored using 1 4 scales, as no colour (1), less intense blue color (2), medium blue color (3) and more intense blue color (4). Rank correlation between colour-score and actual Fe content ranks was worked out following Gomez and Gomez (1984).

3.5.2. Sib-mated, selfed and open-pollinated grain sources

Production of selfed grains for micronutrient analysis is more cost effective than producing sib-mated grains. Production of open-pollinated grains is the cheapest. In this regard, sib-mated, selfed and open-pollinated grain samples of 30 entries (15 inbred lines and 15 populations) produced from the 120-entries trial (set I) were selected and analyzed

for grain Fe and Zn content at the ICRISAT laboratory to examine the correlation between different grain sources

3.6. STATISTICAL ANALYSIS

All statistical analyses were performed using Genstat version 8 from Rothamsted, UK and Microsoft spreadsheet (Genstat 8th edition, 2002)

3.6.1. Analysis of variance (ANOVA)

Analysis of variance for all characters under the study was carried out for individual trial/environment separately, as follows

Source of variation	Degree of freedom	Mean square	Expected mean square (EMS)	F ratio
Replication	(r-1)	MSr	$\sigma^2_e + t\,\sigma^2_r$	MSr/MSe
Treatment	(t-1)	MSt	$\sigma^2_e + r\,\sigma^2_g$	MSt/MSe
Error	(r-1) (t-1)	MSe	σ^2_e	
Total	(rt-1)			

where,

r – number of replications

t – number of treatments or genotypes

σ^2_g = genotypic variance of character X

σ^2_r = replication variance of character X

σ^2_e = error variance of character X and

MSr, MSt and MSe stand for mean squares due to replications, treatments and error, respectively

3.6.2. STATISTICS

3.6.2.1. Mean

Mean value ($\overline{X}$) of each character in each trial environment was worked out by dividing the sum of the observed values by the corresponding number of observations

$$\overline{X} = \sum X_{ij} / N$$

where,

X_{ij} = observation in the i_{th} treatment and j_{th} replication, and

N = total number of observations

3.6.2.2. Standard error

Standard errors of means were calculated for each character for each trial from the corresponding mean square error values from the analysis of variance tables as

$$S.E.(m) = \frac{\sqrt{\sigma^2_e}}{r}$$

Where,

σ^2_e = estimated error variance

$S.E._{(m)}$ = the standard error of the mean, and

r = number of replications

3.6.2.3. Least significant difference (LSD)

Least significant difference at values greater than which all the differences are significant and the LSD values were calculated as suggested by Fukey (1953)

$$LSD = \sigma^2_d \times t_{(5\%)}$$

t = 0.05 at error df

Where,

$\sigma^2_d = 2 \times MSe/n$

n = Number of observations (n= number of replications)

3.6.2.4. Genotypic and phenotypic variances

The Genotypic and phenotypic variances for each trial were calculated as follows

Genotypic variance of character X = σ^2_g = (MSt MSe) / r

Phenotypic variance of character X = σ^2_p $\sigma^2_g + \sigma^2_e$

3.6.2.5. Heritability (in broad sense):

Heritability in broad sense was calculated according to the formula suggested by Lush (1940) for each character as given below

$$H = \frac{\sigma^2_g \times 100}{\sigma^2_p}$$

3.7. CORRELATION ANALYSIS

The correlation co-efficients between grain Fe and Zn content as well as among the agronomic traits were worked out for genotypes in individual and pooled environments Phenotypic correlation co-efficients were estimated using the formula as suggested by Al-Jibouri *et al* (1958),

$$r_p = \frac{Cov_p(X, Y)}{\sqrt{Var_p(X)\, Var_p(Y)}}$$

where,

r_p = phenotypic correlation coefficient

$Cov_p(X, Y)$ = phenotypic covariance between characters X, Y

Var_p (X) = phenotypic variance in character X

Var_p (Y) = phenotypic variance in character Y

The observed correlation co-efficient was compared with the tabulated value for (n-2) degrees of freedom for test of significance

3.8. STABILITY ANALYSIS

Stability of the selected 29 entries tested in the four different environments for grain Fe and Zn content was assessed through Eberhart and Russell (1966) and AMMI (Zobel *et al*, 1988) models

The linear model for pooled analysis of variance is

$$Y_{ijk} = \mu + e_k + (r_{jk} \cdot g_i) + (ge)_{ik} + e_{ijk}$$

where

μ = denotes the general mean

e_k = denotes the effect of environment k

r_{jk} = denotes the effect of replication j within environment k

g_i = denotes the effect of genotype i

$(ge)_{ik}$ = denotes effect of interaction of genotype i within environment k, and

e_{ijk} = denotes residual effect

3.8.1. Pooled analysis of variance

Pooled analysis of variance (Macintosh, 1983) was carried out using Genstat version 8 (2002) for both Fe and Zn content across the environments as follows

Source of variation	Degree of freedom	Mean square	Expected mean square	F ratio
Environment	(e-1)	MSe		MSe/MSE
Genotype	(g-1)	MSg	$\sigma^2_e + r\sigma^2_{ge} + re\sigma^2_g$	MSg/MSE
G × E	(e-1) (g-1)	MSge	$\sigma^2_e + r\sigma^2_{ge}$	MSge/MSE
Pooled error	r(e-1)(g-1)	MSE	σ^2_e	

where,

r = number of replications

g = number of treatments or genotypes

σ^2_g = genotypic variance of character X

σ^2_{ge} = variance of character x due to g × e

σ^2_e = error variance of character X, and

MSr, MSg, MSge and MSE stand for mean sums of squares due to replications, genotypes, genotype × environment interactions and error, respectively

3.8.2. Eberhart and Russell (1966) model

Eberhart and Russell (1966) method was followed to estimate the three parameters of stability viz., mean, regression coefficient (b_i) and mean squared deviation ($\bar{S}^2_d$) for each genotype

The linear model proposed by Eberhart and Russell (1966) is

$$Y_{ij} = \mu_i + b_i I_j + \delta_{ij}$$

Where,

Y_{ij} = Mean performance of i^{th} genotype in j^{th} environment

μ_i = Average performance of i^{th} genotypes over all environments

b_i = Regression coefficient that measures the response of the i^{th} genotype to varying environments

δ_{ij} = Deviation from regression of the i^{th} genotype at j^{th} environment

I_j = Environmental index as the deviation of the mean of all genotypes in j^{th} environment from grand mean

The analysis of variance as proposed by Eberhart and Russell (1966) is given below

Source	d.f.	Expected M.S.	
Total	(ge-1)	$\Sigma_i \Sigma_j Y_{ij}^2 - CF$	
Genotype	(g-1)	$1/e\Sigma_i Y_i^2 - CF$	MS_1
Environment + (G × E)	g(e-1)	$\Sigma_i \Sigma_j Y_{ij}^2 - Y^2/e$	
Environment (linear)	1	$1/g(\Sigma_j Y_{ij} I_j)^2 / \Sigma_j I_j^2$	
Genotype × Environment (linear)	(g-1)	$\Sigma_i [(\Sigma_j Y_{ij} I_j)^2 / \Sigma I_j^2] - [1/g(\Sigma_j Y_{ij} I_j)^2 / \Sigma_j I_j^2]$	MS_2
Pooled deviation	g(e-2)	$\Sigma_i \Sigma_j \sigma_{ij}^2$	MS_3
Deviation due to genotypes -1	(e-2)	$[\Sigma_i Y_{ij}^2 - (Y_i)^2/e] - [(\Sigma_j Y_{ij} I_j)^2 / \Sigma_j I_j^2] - \Sigma_i \sigma_{ij}^2$	MS_{3-1}
Genotypes-g	(e-2)	$[\Sigma_j Y_{gj}^2 - (Y_g^2)^2/e] - [(\Sigma_j Y_{gj} I_j)^2 / \Sigma_j I_j^2] - \Sigma$	MS_{3-g}
Pooled error	e(r-1)(g-1)		$\sigma^2 e$

Where, g= genotype, r= replication, e– environment

The regression coefficient (b_i) and mean square deviation from the linear regression $\overline{S}_d^2$ were estimated as follows

The regression co-efficient is the regression of performance of each genotype under different environment on the environmental means of over all genotypes This was estimated as follows

$$b_i = \frac{\Sigma_j Y_{ij} I_j}{\Sigma_j I_j^2}$$

Where,

$\Sigma_j Y_{ij} I_j$ = the sum of products of environmental index (I_j) with corresponding mean of that genotypes at each environment (Y_{ij}),

$\Sigma_j I_j^2$ = the sum of squares of the environmental index (I_j)

(a) For each value of regression coefficient I_j^2 is common and equal to

$$\Sigma_j I_j^2 = I_1^2 + I_2^2 + I_{2g}^2$$

(b) On the other hand $\Sigma_j Y_{ij} I$ for each genotype is the sum of products of environmental index (I_j) with the corresponding mean ($\bar{X}$) of the genotype in each environment

These values may be obtained in the following manner

$$[\bar{X}] \times [I_j] = [\Sigma_j Y_{ij}] = [S]$$

Where,

$[\bar{X}]$ = Matrix of means

$[I_j]$ = Vector for environmental index

[S] = Vector for sum of products (i e) Σ Y I

b_i values for each genotype was calculated by dividing $\Sigma_j Y_{ij} I_j$ for each genotype by $\Sigma_j I_j^2$

Where,

I_j = environmental index of j^{th} environment which can be calculated as follows

3.8.2.1. Computation of environmental index (I_j)

$$I_j = \frac{\Sigma_j Y_{ij}}{g} - \frac{\Sigma_i \Sigma_j Y_{ij}}{g_e} \text{ with } \Sigma_j I_j = 0$$

Total of all the genotypes at j^{th} location / Grand total

Number of genotypes / Total number of observations

3.8.2.2. Computation of mean square deviation ($\overline{S}_d^2$) from linear regression

$$(S_d^2) = \frac{\Sigma_j \delta_{ij}^2}{e-2} - \frac{S_e^2}{r}$$

Where,

$$\Sigma_j \delta_{ij}^2 = [\Sigma_j Y^2_{ij} - Y_i^2 / e] - \frac{(\Sigma_j Y_{ij} I_j)}{\Sigma_j I_j^2}$$

Where,

$\Sigma_j \delta_{ij}^2$ = variance due to deviation from the regression for i^{th} genotype,

$\Sigma_j Y^2_{ij} - Y_i^2 / g$ = variance due to dependent variable,

$$\frac{(\Sigma_j Y_{ij} I_j)^2}{\Sigma_j I_j^2} - \frac{(\Sigma_j Y_{ij} I_j)(\Sigma_j Y_{ij} I_j)}{\Sigma_j I_j^2} = b_i \Sigma Y_{ij} I_j$$

From $\Sigma_j \delta_{ij}^2$ values the stability parameters ($\overline{S}_d^2$) for each genotypes is computed as follows

$$(\overline{S}_d^2) = \frac{\Sigma_j \delta_{ij}^2}{e-2} - \frac{S_e^2}{r}$$

Mean squared deviation =

Deviation from regression / Pooled error

Degrees of freedom for each environment / Number of replications

S_e^2 = estimate of pooled error

e = number of environments

g = number of genotypes

r = number of replications

Test of significance

The following tests of significance were carried out,

(1) To test the significance of the differences among genotypic means namely

$M_0 = M1 = M2 = M3 =$ M 29

The F' test used was

The 'F' used was

$$F = \frac{\text{Mean squares due to genotypes}}{\text{Mean squares due to pooled deviation}} \quad \frac{MS_1}{MS_3}$$

(2) To test that the genotypes did not differ due to regression on environmental index

M0 = b1 = b2 = b29

The 'F' used was

$$F = \frac{\text{Mean squares due to genotypes} \times \text{environment (linear)}}{\text{Mean squares due to pooled deviation}} \quad \frac{MS_2}{MS_3}$$

(3) Individual deviation from linear regression was tested as follows

$$F = \frac{\Sigma_i \delta_{ij}^2}{e-2} \Big/ \text{(Pooled error)}$$

P = 0 05 at g-2 df

(4) The hypothesis that any regression coefficient does not differ from unity or from zero was tested by the appropriate 't' test (i e) for (1-b) 1-b/S E (b) t, $P = < 0\ 05$ for (g-e) df

$$S\ E\ (b) = \frac{\sqrt{\Sigma_i \delta i_i^2 / e-2}}{\Sigma_i I_i^2}$$

Stable genotype

A genotype with unit regression coefficient (b_i=1) and the deviation not significantly differing from zero ($\overline{S}_d^2$ =0) is said to be stable genotype

Mean and standard error of 'b'

$$\text{Mean of b} = \frac{\Sigma_i b_i}{}$$

$$\text{S E (b)} = \frac{\sqrt{MS \text{ due to pooled deviation}}}{\Sigma 1}$$

Population mean (μ) and standard error was calculated as

$$\text{Population mean} = \frac{\text{Grand mean}}{\text{Number of observations}}$$

$$\text{S E (mean)} = \frac{\sqrt{MS \text{ due to pooled deviation}}}{\text{Number of environments}}$$

3.8.3. Additive main effects and multiplicative interaction (AMMI) model

The AMMI statistical model is a hybrid model involving both additive and multiplicative components of two-way data structure It makes use of standard ANOVA procedures to separate the additive variance from the multiplicative variance (genotype × environment interaction) and then uses a multiplicative procedure (PCA Principle Component Analysis) to extract the pattern from the G × E portion of the ANOVA analysis The result is the least square analysis, which with further graphical representation of the numerical results (Biplot analysis), often allows a straight forward interpretation of the underlying causes of G × E the mathematical statement of the hybrid model is,

$$Yge = \mu + \alpha_g + \beta_e + \sum_{n=1}^{N} \lambda_n \xi_{gn} \eta_{en} + \theta_{ge} + \varepsilon_{ge}$$

where,

Yge	=	Value of genotype 'g' in environment 'e'
μ	=	Grand mean
α_g	=	Genotype mean deviation

= Environment mean deviation

N = Number of PCA axes (Interaction Principal Component Axis) retained in the model

λ_n = Eigen value for PCA axis 'n

ξ_{gn} = Genotypes PCA scores for PCA axis 'n

η_{en} = Environment PCA scores for PCA axis 'n'

θ_{ge} = Residuals

ε_{ger} = Error term, resulting from deviation of Y_{ge} from the observation in r^{th} replication

The AMMI biplot was developed by placing both genotype and environment values on the X axis, and the respective PCA axis eigen-vectors on the Y-axis

3.9. DIALLEL ANALYSIS

The diallel analysis of all possible crosses among ten inbreds was performed by using Hayman's (1954) model for the estimation of genetic components of variations The graphical analysis of regression of Wr (parent offspring covariance of the r^{th} array) on Vr (variance of the r^{th} array) to complement the former via graphical representation

The data from 10 × 10 diallel study for grain Fe and Zn content were subjected to analysis of variance and combining ability estimates appropriate for diallel mating design (Model I and Method I) as suggested by Griffing's (1956)

3.9.1. Hayman's approach

Genetic analyses were conducted according to the diallel model of Hayman (1954) The adequacy of additive-dominance genetic model was tested by using uniformity test (t^2) and the consistency of differences between Wr and Vr across the parental arrays The graphical analysis of regression line and the limiting parabola constructed by calculating its points ($Wr^2 - Vr \times Vol o$) and plotting the Vr, ($Wr \times VoLo$)$^{1/2}$ points as determined through Smith's formula adopted by Aksel and Johnson (1963) The Vr-Wr arrays were supplemented by Wr-Vr graphs adopting the procedure of

Mather and Jinks (1974). Standardized deviation graphs of parental measurements (Yr) and parental order of dominance (Wr+Vr) were produced, where the deviations of the Yr's and Wr+Vr's from their respective means were standardized by dividing them with their standard deviations.

Under an additive-dominance genetic model, the components of genetic variance (D: additive; H_1 and H_2: dominance; and F: average covariance of additive and dominance effects over all the parental arrays) were estimated following Jinks and Hayman (1953). The average degree of dominance was estimated as $\sqrt{H_1/D}$. The ratio of dominant and recessive genes in the parents was estimated as the ratio of $[\sqrt{(4DH_1)}+F)/[\sqrt{(4DH_1)}-F)$. The relative distribution of increasing (positive) and decreasing (negative) genes among common parents of arrays was assessed using the ratio $H_2/(4H_1)$. The narrow-sense heritability was estimated using Hayman (1954) approach. The proportion of phenotypic variance that can be attributed to additive genetic variance is narrow sense heritability, this was estimated as,

$$h^2_n = \frac{1/2\,D + 1/2\,H_1 - 1/2\,H_2 - 1/2\,F}{1/2\,D + 1/2\,H_1 - 1/4\,H_2 - 1/2\,F + E}$$

3.9.2. Griffing's combining ability analysis (method I)

The linear mathematical model for combining ability is,

$$Yij = \mu + g_i + g_j + r_{ij} + s_{ij} + \frac{1}{bc}\sum_{k}\sum_{l} e_{ijkl}$$

where,

μ = Population mean

g_i and g_j = General combining ability (*gca*) effects of i^{th} and j^{th} inbred lines

r_{ij} = Reciprocal effects

s_{ij} = Specific combining ability (*sca*) effect of ij th cross

e_{ijkl} = Environmental effects associated with ijklth individual observation

The analysis of variance and mean square expectations as suggested by Griffing's (1956) are as follows.

Source of variation	Degree of freedom	SS	MS	Expected mean square
Replication	(r-1)	S_g	M_b	
General combining ability effects	(p-1)	S_s	M_s	
Specific combining ability effects	$\frac{p(p-1)}{2}$	S_r	M_r	
Reciprocal effects	$\frac{p(p-1)}{2}$		M_e	
Error				σ^2

The sum of squares are obtained by

$$S_g = \frac{1}{2p}\sum (\quad + \quad) \quad \frac{2}{p}Y$$

$$S_s = \frac{1}{2}\sum\sum \quad (\quad + \quad) \quad \frac{1}{2p}\sum (\quad + \quad) + \frac{2}{p}Y$$

$$S_r = \frac{1}{2}\sum\sum (\quad + \quad)$$

Variance components

The estimates of *gca* variance (σ^2_g) equivalent to half of the additive variance component ($\sigma^2_g = 1/2V_A$), that of *sca* variance (σ^2_s) equivalent to dominance (non-additive) variance component ($\sigma^2_s = V_D$) can be obtained from expectations of mean squares of combining ability estimates

$$\sigma^2_g = \frac{1}{2P}\left[MS_g - MS_e\right]$$

$$\sigma^2_S = \left[MS_s - MS_e\right]$$

σ^2_e = Mse

Hence, the total genetic variance among single-cross progeny is equal to twice the general combing ability component of the variance ($\sigma^2_g \times 2$) plus the specific combining ability component of the variance (σ^2_s). Based on this relationship, it would seem that the

relative importance of general and specific combining ability in determining progeny **performance** should be assessed by estimating the components of variance and expressing **them** in the ratio, $2\sigma^2_g/(2\sigma^2_g + \sigma^2_s)$ (Baker, 1978).

Estimates of combining ability effects

i. ***gca* effect of parents**

The estimate of general combining ability effect (*gca*) of i[th] parent derived as:

$$g_i = \frac{1}{2p}(Y_{i.} + Y_{.i}) - \frac{1}{p^2}Y_{..}$$

ii. ***sca* effects of hybrids**

Estimate of specific combining ability of ij[th] cross are calculated as:

$$S_{ij} = \frac{1}{2}(y_{ij} + y_{ji}) - \frac{1}{2p}(Y_{i.} + Y_{.i} + Y_{j.} + Y_{.j}) + \frac{1}{p_2}Y_{..}$$

iii. Reciprocal effects of hybrids

The estimate of reciprocal effect for the ij[th] are calculated as:

$$r_{ij} = \frac{1}{2}(y_{ij}-y_{ji})$$

Test of significance of combining ability effects

The standard errors (S.E) are calculated as:

S. E. of *gca* effects

i. S. E. $g_i = \sqrt{\frac{p-1}{2p^2}\sigma^2}$

ii. S. E. $(g_i-g_j) = \sqrt{\frac{1}{p}\sigma^2}$

S. E. of *sca* effects of hybrids

iii. S. E. $S_{ij} = \sqrt{\frac{1}{2p^2}(p^2-2p+2)\sigma^2}$

iv. S. E. $(S_{ij}-S_{ik}) = \sqrt{\frac{(p-1)}{p}\sigma^2}$

v. S. E. $(S_{ij}-S_{kl}) = \sqrt{\frac{(p-2)}{p}\sigma^2}$

S. E. of *sca* of reciprocal effects

i. S. E. rij = $\sqrt{\frac{1}{2}\sigma^2}$

ii. S. E. $(r_{ij}-r_{kl}) = \sqrt{\sigma^2}$

Testing of significance

't' test for general combining ability

$$t = \frac{g_i - 0}{S.E.g_i}$$

't' test for specific combining ability

$$t = \frac{g_i - g_j}{S.E.g_i - g_j}$$

t' test for reciprocal effects

$$\frac{r_{ij} - 0}{S.E\, r_{ij}}$$

The calculated 't' value was compared with table 't' value at error degrees of freedom to test the significance

3.9.3. Estimation of heterosis

The heterosis was worked out by utilizing the mean values of each trait The mean values used to estimate heterosis per cent under three categories (Fonseca and Patterson, 1968).

I. Mid parent heterosis

Deviation of hybrid from mid-parent is derived as,

$$d_i = \frac{F_1 - MP}{MP} \times 100$$

Where,

F1 = mean value of hybrid

MP = mid parental value

Better parent heterosis

Deviation of hybrid from better parent is calculated as,

$$d_b = \frac{F_1 - BP}{BP} \times 100$$

where,

BP = mean value of better parent

Test of significance of heterosis

Significance of estimates of heterosis was tested at error degrees of freedom as suggested by Turner (1953)

't' for mid parent heterosis $\frac{F_1 - MP}{\sqrt{\frac{Me}{r} \times \frac{3}{2}}}$

't' for better parent heterosis $\frac{F_1 - BP}{\sqrt{Me} \times 2}$

where,

Me = error variance

r = number of replications

RESULTS

CHAPTER 4

RESULTS

The objective of this study was to develop an understanding of the possibility of enhancing the grain Fe and Zn content in pearl millet. For this, it is necessary to generate basic genetic information to formulate effective breeding strategies. Hence, the present study was carried out to determine the magnitude of genetic variability available among a diverse range of breeding lines, improved populations and germplasm accessions, explore the inter and intra-population variability, assess the degree of stability of grain Fe and Zn content over different environments, examine the relationships between grain Fe and Zn content and with two key agronomic traits (time to 50% flower and 1000-grain mass), which may have some association with the grain Fe and Zn content; and the nature of gene action for grain Fe and Zn content. The results have been presented for each of these objectives of the study below.

4.1. VARIABILITY IN SOIL IRON AND ZINC CONTENT

The initial soil Fe and Zn contents were determined at two different depths (0–15 cm and 16–30 cm) in all the experimental fields used for the study at ICRISAT-Patancheru, during summer and rainy seasons of 2004 and 2005, and 2006 summer season. Three samples at two depths were analyzed in the fields used during the 2004 and six samples were analyzed in the fields used during the 2005 and 2006. All the experimental sites had excessive available soil Fe and Zn content (DTPA extractable), when compared to critical limits (2.0 mg kg^{-1} Fe and 0.75 mg kg^{-1} Zn) (Appendix I). The soil Fe and Zn contents of the fields used in all the seasons varied widely. The soil Fe content of summer season fields varied from 4.9 to 11.5 mg kg^{-1} and of the rainy season fields from 10.0 to 18.5 mg kg^{-1}. Similarly, the soil Zn ranged from 0.8 to 3.1 mg kg^{-1} in the summer season fields and from 1.1 to 5.1 mg kg^{-1} in the rainy season fields. The soil Fe content of 2004 (15.8 ± 3.11 mg kg^{-1}) and 2005 (13.4 ± 2.44 mg kg^{-1}) rainy season fields were 155% and 43% higher compared to the 2004 (6.2 ± 0.45 mg kg^{-1}) and 2005 (9.4 ± 2.77 mg kg^{-1}) summer fields; and 131 to 172% higher than the 2006 summer

season fields (5 8 ± 0 76 mg kg^{-1}) Similarly, the soil Zn content of 2004 (2 2 ± 0 63 mg kg^{-1}) and 2005 (3 6 ± 1 21 mg kg^{-1}) rainy season fields were 38% and 64% higher compared to the 2004 (1 6 ± 0 52 mg kg^{-1}) and 2005 (2 2 ± 0 86 mg kg^{-1}) summer fields and 47 to 140% higher compared to 2006 summer season fields (1 5 ± 0 78) The surface soil (0 15 cm) had 2 41% higher Fe and 8 142% higher Zn content than the sub-surface soil (16 30 cm)

4.2. GENETIC VARIABILITY

Magnitude of genetic variability for grain Fe and Zn content were studied in four different sets of experiments First set (set I) comprised of 120 entries that included a diverse range of hybrid parents, improved populations advanced population progenies and germplasm accessions Second set (set II) consisted of 69 improved populations to examine the extent of variability in mostly the improved populations Third set (set III) had initial and advanced recurrent cycle bulks of five composites to examine if the recurrent selection for grain yield led to any changes in grain Fe and Zn contents The fourth set (set IV) included 68 S_1 progenies of an open-pollinated variety (GB 8735) released in several countries of western and central Africa, and 64 S_1 progenies derived from another open-pollinated variety (AIMP 92901) released in India, to study the intra population variability

4.2.1. Variability in hybrid parents, advanced population progenies, improved populations and germplasm accessions

The analysis of variance showed highly significant differences among entries for grain Fe and Zn content, days to 50% flower and 1000 grain mass (Table 7) Considering the analysis across two environments, the mean differences among genotypes, environments and G × E interaction effects were highly significant for all the four traits The magnitude of genotypic variance was more than the G × E interaction variance for grain Fe and Zn content and days to 50% flower whereas for 1000 grain mass G × E interaction variance was higher than genotypic variance (Table 8) Though the G × E interaction was significant, the correlation co-efficient between the two seasons was

Table 7. Analysis of variance for grain Fe and Zn content, days to 50% flower and 1000-grain mass in different classes of breeding materials and germplasm accessions (set 1 trial), 2004 summer and rainy seasons, ICRISAT-Patancheru.

Source	df	Mean square							
		Fe content (mg kg^{-1})		Zn content (mg kg^{-1})		Days to 50% flower		1000-grain mass (g)	
		Summer 04	Rainy 04	Summer 04	Rainy 04	Summer 04	Rainy 04	Summer 04	Rainy 04
Genotype	119	99 08**	291 87**	61 31**	181 23**	86 70**	78 16**	6 29**	6 88**
Block	1	66 26	70 20	18 21	3 78	12 85	11 20	4 10	2 45
Error	119	28 00	72 20	10 30	31 40	2 40	2 30	0 20	0 22
σ^2_g		35 5	109 8	25 5	74 9	42 2	38 0	3 1	3 4
σ^2_p		63 5	182 1	35 8	106 3	44 6	40 2	3 2	3 5
h^2(%)		56	60	72	71	95	95	94	95

* Significant at 5% and 1% probability levels respectively

Table 8. Pooled analysis of variance over two environments for grain Fe and Zn content, days to 50% flower and 1000-grain mass in variability trials, ICRISAT-Patancheru.

Trial set	Source	df	Mean square			
			Fe content (mg kg^{-1})	Zn content (mg kg^{-1})	Days to 50% flower	1000-grain mass (g)
I	Environment	1	7627 29**	6817 67**	9514 10**	8 18**
	Replication	1	76 96	20 75	16 50	4 40
	Genotype	119	308 17**	194 17**	144 71**	12 93**
	Genotype × Environment	119	82 77**	48 37**	20 14**	28 86**
	Error	239	49 95	21 00	2 30	0 18
	σ^2_g		129 11	86 59	71 21	6 38
	σ^2_{ge}		16 41	13 69	8 92	14 31
II	Environment	1	363 80**	633 85**	524 25**	6 44**
	Replication	1	96 30	6 53	12 50	3 80
	Genotype	68	260 20**	85 98**	69 80**	9 57**
	Genotype × Environment	68	94 90	42 99	35 80	15 47**
	Error	137	103 40	31 78	11 50	0 30
	σ^2_g		78 40	21 50	29 15	4 64
	σ^2_{ge}		4 25	5 61	12 15	7 59
III	Environment	1	63 76	32 76	28 21	3 25
	Replication	1	0 09	10 20	8 51	1 50
	Genotype	9	46 92	33 87	15 47	5 80
	Genotype × Environment	9	27 12	17 82	15 45	6 87
	Error	19	32 03	18 94	8 87	0 05
	σ^2_g		7 45	7 47	3 30	2 88
	σ^2_{ge}		2 46	0 56	3 29	3 41
AIMP 92901 progeny trial (IV)	Environment	1	3726 15**	6825 55**	4752 44**	10 54**
	Replication	1	649 21	198 73	200 24	4 80
	Genotype	63	1021 38**	325 31**	487 50**	13 25**
	Genotype × Environment	63	172 56**	77 32**	65 87**	22 35**
	Error	127	46 60	32 48	32 54	0 31
	σ^2_g		487 39	146 42	227 48	6 47
	σ^2_{ge}		62 98	22 42	16 67	11 02
GB 8735 progeny trial (IV)	Environment	1	8038 80**	16023 29**	6012 45**	9 45**
	Replication	1	99 18	131 03	100 21	3 90
	Genotype	67	600 04**	253 50**	300 14**	14 25**
	Genotype × Environment	67	166 47**	60 00**	45 21**	31 00**
	Error	135	95 38	60 66	23 15	0 40
	σ^2_g		252 33	96 42	138 50	6 93
	σ^2_{ge}		35 55	0 33	11 03	15 30

**Significant at 5% and 1% probability levels, respectively

positive and highly significant for grain Fe (r = 0.66, $P < 0.01$) and Zn content (r = 0.69, $P < 0.01$) (Table 9, Fig 1 and 2)

Based on pooled data from two environments, the grain Fe content had larger variability (30.1–75.7 mg kg^{-1}) and higher mean (45.5 mg kg^{-1}) than grain Zn content (24.5–64.8 mg kg^{-1} with the mean of 43.9 mg kg^{-1}) (Table 10). The average grain Fe and Zn content in the rainy season (49.5 mg kg^{-1} Fe and 47.7 mg kg^{-1} Zn) were 17.20% higher than those in the summer season (41.5 mg kg^{-1} Fe and 40.1 mg kg^{-1} Zn). This might have largely to do with the Fe and Zn levels in the soil, which had 155% more Fe and 38% more Zn in the field used during the rainy season than the ones used during the summer season (Appendix I). The variability for grain Fe content in the summer season was smaller (29.3–74.7 mg kg^{-1}) than in rainy season (29.4–102.0 mg kg^{-1}). Similarly, a large variability of grain Zn content was observed during rainy season (24.0–80.8 mg kg^{-1}) compared to summer (25.0–61.6 mg kg^{-1}). The range of grain Fe and Zn varied among the different classes of breeding materials and germplasm accessions. Larger variability for grain Fe and Zn content were observed in advanced population progenies (30.1–75.7 mg kg^{-1} Fe and 24.5–64.8 mg kg^{-1} Zn) followed by seed parents (34.4–72.7 mg kg^{-1} Fe and 27.3–59.6 mg kg^{-1} Zn), open pollinated varieties (32.4–62.7 mg kg^{-1} Fe and 33.7–53.1 mg kg^{-1} Zn), pollinators (34.9–55.7 mg kg^{-1} Fe and 31.7–55.2 mg kg^{-1} Zn) and germplasm accessions (34.5–54.4 mg kg^{-1} Fe and 35.4–49.3 mg kg^{-1} Zn) (Table 10, Fig. 3 and 4). Although wider range of values for grain Fe and Zn content were observed in advanced population progenies, the mean Fe (50.3 mg kg^{-1}) and Zn content (47.3 mg kg^{-1}) were higher in seed parents compared to other class of breeding materials and germplasm accessions. In all classes of breeding materials the mean grain Fe content was slightly higher than the Zn content and it was almost equal in germplasm accessions.

4.2.1.1. Fe content

Among the seed parents, the grain Fe content of nine lines during summer (43.7–69.0 mg kg^{-1}) and 12 lines during rainy season (53.9–76.5 mg kg^{-1}) was significantly higher than the check 81B (32.4 mg kg^{-1} in summer and 36.4 mg kg^{-1} in

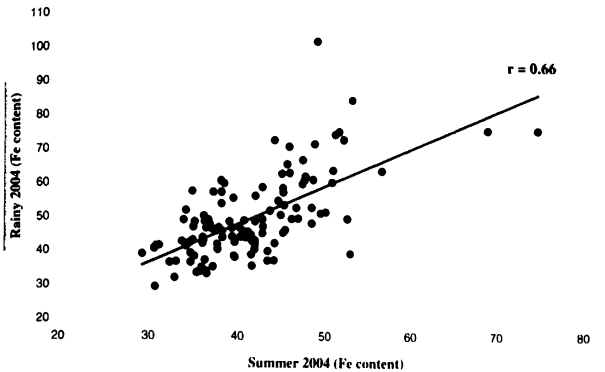
Table 9. Correlation co-efficient between seasons (summer and rainy) for grain Fe and Zn content, and days to 50% flower and 1000-grain mass in variability trials, ICRISAT-Patancheru.

Character	Set I	Set II	Set III	AIMP 92901 progeny trial (Set IV)	GB 8735 progeny trial (Set IV)
Fe content (mg kg^{-1})	0.66**	0.57**	0.70**	0.75**	0.59**
Zn content (mg kg^{-1})	0.69**	0.44**	0.51**	0.80**	0.64**
Days to 50% flower	0.98**	0.89**	0.85**	0.91**	0.87**
1000-grain mass (g)	0.93**	0.94**	0.99**	0.88**	0.91**

** Significant at 5% and 1% probability levels, respectively.

Table 10. Range and mean of grain Fe and Zn content in different classes of breeding material and germplasm accessions in set I trial, 2004 summer and rainy seasons, ICRISAT - Patancheru.

Class of material	No. of lines	Summer 2004		Rainy 2004		Pooled mean	
		Range	Mean	Range	Mean	Range	Mean
		Fe content (mg kg^{-1})					
Inbred lines	20	32.4 – 69.0	43.4	35.0 – 76.5	57.3	34.4 – 72.7	50.3
Potential pollinators	20	33.1 – 52.7	39.1	33.2 – 60.4	44.5	34.9 – 55.7	41.8
Partial inbreds	30	29.3 – 74.7	42.2	29.4 – 102.0	52.3	30.1 – 75.7	47.2
Open-pollinated varieties	30	32.9 – 52.3	41.8	32.0 – 73.1	48.7	32.4 – 62.7	45.2
Germplasm	20	34.7 – 50.2	40.7	33.5 – 61.1	43.7	34.5 – 54.4	42.2
Total	120	29.3 – 74.7	41.5	29.4 – 102.0	49.5	30.1 – 75.7	45.5
		Zn content (mg kg^{-1})					
Inbred lines	20	26.5 – 54.0	40.8	28.0 – 71.9	53.8	27.3 – 59.6	47.3
Potential pollinators	20	31.1 – 52.8	38.8	32.3 – 57.5	44.2	31.7 – 55.2	41.5
Partial inbreds	30	25.0 – 61.6	40.8	24.0 – 80.8	49.1	24.5 – 64.8	44.9
Open-pollinated varieties	30	32.8 – 45.5	39.6	34.6 – 61.0	47.1	33.7 – 53.1	43.4
Germplasm	20	34.1 – 46.3	40.5	34.2 – 54.7	43.5	35.4 – 49.3	42.1
Total	120	25.0 – 61.6	40.1	24.0 – 80.8	47.7	24.5 – 64.8	43.9



Relationship between 2004 summer and rainy seasons for grain Fe content (mg kg^{-1}) in set I trial, ICRISAT- Patancheru.

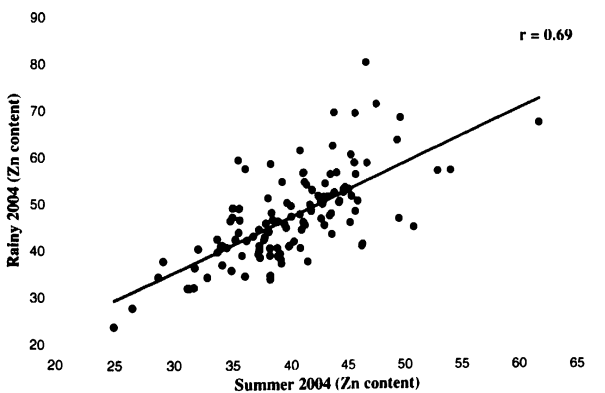


Fig 2. Relationship between 2004 summer and rainy seasons for grain Zn content (mg kg^{-1}) in set I trial, ICRISAT-Patancheru.

rainy season) (Table 11, Appendix II). Based on pooled data, highest level of grain Fe was observed in 863B (72.7 mg kg^{-1}) followed by ICMB 94111 (63.6 mg kg^{-1}), ICMB 98222 (62.9 mg kg^{-1}), ICMB 88004 (60.4 mg kg^{-1}) and ICMB 00888 (60.4 mg kg^{-1}). In case of pollinators, the grain Fe content of IPC 843 (52.7 mg kg^{-1}) and MIR 97171 (50.9 mg kg^{-1}) was significantly higher during summer, and MIR 97171 (60.4 mg kg^{-1}) was significantly higher during rainy season when compared to ICMP 451 check (39.6 mg kg^{-1} during summer and 38.4 mg kg^{-1} in rainy season). Based on the pooled data, the grain Fe content of IPC 843 was 31% higher and that of MIR 97171 was 43% higher than the grain Fe content of check ICMP 451 (39.0 mg kg^{-1}).

The grain Fe content of nine advanced population progenies in rainy season (57.4–102.0 mg kg^{-1}) and three in summer season (53.0–74.7 mg kg^{-1}), with two common across seasons, was significantly higher than the check ICMP 451 (39.6 mg kg^{-1} in summer season and 38.4 mg kg^{-1} in rainy season). Based on the pooled data, the grain Fe content of two S_4 progenies derived from the open-pollinated variety AIMP 92901 [AIMP 92901 S1-15-1-2-B (75.7 mg kg^{-1}) and AIMP 92901 S1-183-2-2-B (75.6 mg kg^{-1})] was highest, followed by the S_4 progeny derived from another variety SDMV 90031 (SDMV 90031-S1-84-1-1-2-B: 69.0 mg kg^{-1}). The Fe levels of the two AIMP 92901 progenies mentioned above exceeded the grain Fe content of original population AIMP 92901 (50.8 mg kg^{-1}) by 49% and SDMV 90031 progeny had 44% higher grain Fe content than the original population SDMV 90031 (47.8 mg kg^{-1}). A large variability was observed for grain Fe among three AIMP 92901 progenies that were tested (54.6–75.7 mg kg^{-1}).

Among the open-pollinated varieties, the grain Fe content of GB 8735 (73.1 mg kg^{-1}), ICMV 221 (67.0 mg kg^{-1}), EEBC (64.1 mg kg^{-1}), HHVDBC (61.2 mg kg^{-1}), MC 94 (59.8 mg kg^{-1}) and AIMP 92901 (58.8 mg kg^{-1}) was significantly higher during rainy season than the check WC-C75 (41.9 mg kg^{-1}), whereas during summer, the grain Fe content of GB 8735 (52.3 mg kg^{-1}) and EEBC (51.0 mg kg^{-1}) was ~20% higher than the check WC-C75 (42.0 mg kg^{-1}). Based on the pooled data, the grain Fe content of six populations (GB 8735, EEBC, HHVDBC, ICMV 88908, AIMP 92901 and ICMR 312)

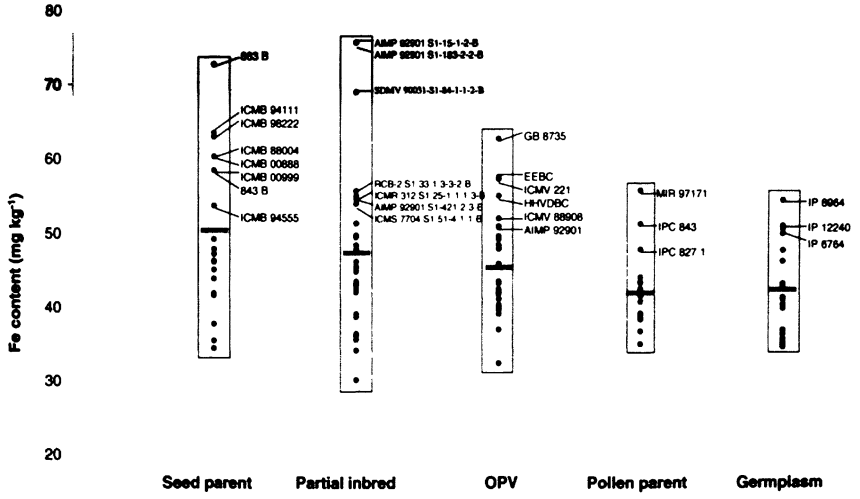


Fig 3. Range and mean (═) of grain Fe content (mg kg^{-1}) in various classes of breeding material and germplasm accessions (mean of two seasons), ICRISAT-Patancheru.

was 21 to 50% higher than the WC-C75 (41.9 mg kg^{-1}). There was no germplasm accession that significantly differed from the check WC-C75 during both the seasons for grain Fe content. Based on pooled data, the grain Fe content of four germplasm accessions was more than 20% higher when compared to WC-C75 (41.9 mg kg^{-1}), among which a germplasm accession IP 8964 had the highest grain Fe content (54.4 mg kg^{-1}).

4.2.1.2. Zn content

Based on two seasons pooled mean, Zn content varied from 24.5 to 64.8 mg kg^{-1} with the mean of 43.9 mg kg^{-1} (Table 11, Appendix II). The grain Zn content of 15 entries was 25–62% higher than that of the popular variety WC-C75 (39.5 mg kg^{-1}).

Among the seed parents, the grain Zn content of 12 lines during summer (38.1–54.0 mg kg^{-1}) and 16 lines during rainy season (44.4–71.9 mg kg^{-1}) was significantly higher than the 81B check (31.3 mg kg^{-1} in summer and 32.2 mg kg^{-1} in rainy season). Based on pooled data, highest level of grain Zn content was observed in 843B (59.6 mg kg^{-1}) followed by ICMB 00999 (59.2 mg kg^{-1}), ICMB 94111 (56.8 mg kg^{-1}) and ICMB 00888 (56.7 mg kg^{-1}) and 863B (55.8 mg kg^{-1}). In case of pollinators, the grain Zn content of IPC 843, J 104 (sel.), IPC 827-1 (dual-restorer) and MIR 97171 was significantly higher than the control ICMP 451 during both summer (36.0 mg kg^{-1}) and rainy season (34.9 mg kg^{-1}). Based on the pooled data, the same four pollinators had 32 to 56% higher grain Zn over the check ICMP 451 (35.4 mg kg^{-1}).

Ten advanced population progenies in both summer and rainy season and additional 9 in rainy seasons had significantly higher Zn content when compared to check ICMP 451 (36.0 mg kg^{-1} in summer season and 34.9 mg kg^{-1} in rainy season). Based on the pooled data across two seasons, the grain Zn content of 13 advanced population progenies was 29–83% higher than the check ICMP 451, among which two S_4 progenies [AIMP 92901 S1-15-1-2-B (64.8 mg kg^{-1}) and AIMP 92901 S1-183-2-2-B: 63.7 mg kg^{-1}] derived from AIMP 92901 had the highest grain Zn content, followed by a S_5 progeny derived from another improved population ICMS 7704 (57.7 mg kg^{-1}). The AIMP 92901 progenies had more than 50% excess grain Zn content over that of original population

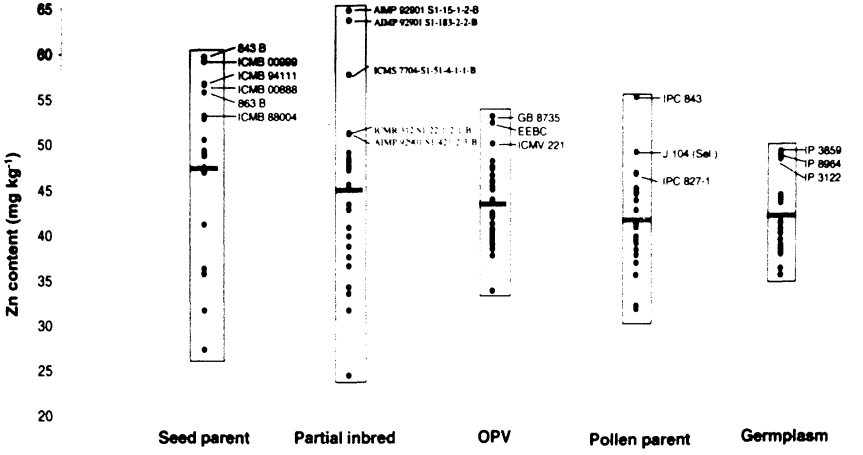


Fig 4. Range and mean (▬) of grain Zn content (mg kg^{-1}) in various classes of breeding material and germplasm accessions (mean of two seasons), ICRISAT-Patancheru.

AIMP 92901 (42.0 mg kg^{-1}). Similarly, a ICMS 7704 progeny had 26% higher grain Zn content than the original population ICMS 7704 (45.8 mg kg^{-1}). The progenies derived from an open-pollinated variety AIMP 92901 had large within-population variability for grain Zn content (51.2–64.8 mg kg^{-1}).

Among the open-pollinated varieties, the grain Zn content of EHBC (45.5 mg kg^{-1}), GB 8735 (45.2 mg kg^{-1}) and ICMS 7704 (45.2 mg kg^{-1}) was significantly higher during summer season than that of WC-C75 (38.2 mg kg^{-1}), whereas during rainy season, the grain Zn content of GB 8735 (61.0 mg kg^{-1}), EHBC (59.2 mg kg^{-1}), ICMV 221 (56.7 mg kg^{-1}), Raj 171 (55.0 mg kg^{-1}) and ICMS 7703 (52.1 mg kg^{-1}) was significantly higher than that of WC-C75 (40.9 mg kg^{-1}). Based on pooled data across the seasons, the grain Zn content of three populations (GB 8735, EHBC and ICMV 221) was 27–34% higher than the check WC-C75 (39.5 mg kg^{-1}). None of the germplasm accessions significantly differed from the check WC-C75 in both the seasons. Based on pooled data across seasons, the grain Zn content of germplasm accessions, IP 3859 (49.3 mg kg^{-1}), IP 8964 (48.8 mg kg^{-1}) and IP 3122 (48.7 mg kg^{-1}) was ~20% higher than that of WC-C75 (39.5 mg kg^{-1}).

4.2.1.3. Days to 50% flower

The time to 50% flower ranged around 37 to 70 days with a mean of 48 days during the summer and rainy seasons and also based on pooled data from two seasons (Table 11; Appendix II). Among the 21 short-listed entries for high grain Fe and Zn content, flowering ranged from 38 to 57 days with 15 entries flowering one to nine days earlier than the check WC-C75, which flowered in 47 days (10 days earlier than the other check entry 81B and similar to ICMP 451). Three entries flowered in 47 days and two-flowered one day late. Among these, the population EHBC (38 days) was the earliest to flower, followed by a seed parent 843B (42 days) and a pollinator, MIR 97171 and two populations, GB 8735 and ICMV 221 (all 43 days). A germplasm accessions IP 8964 (61 days) was late flowering. The early to mid-early flowering entries (35–50 days) had large variability for grain Fe (32.4–75.7 mg kg^{-1}) and Zn content (27.3–64.8 mg kg^{-1}) with

in set 1 trial, 2004 summer and rainy seasons, ICRISAT- Patancheru.

Sl. No.	Entry	Fe content (mg kg^{-1})			Zn content (mg kg^{-1})			Days to 50% flower			1000 grain mass (g)		
		Summer	Rainy	Mean	Summer	Rainy	Mean	Summer	Rainy	Mean	Summer	Rainy	Mean
	Seed parent (n=20)												
1	863 B	69.0	76.5	72.7	54.0	57.7	55.8	46	48	47	11.8	12.6	12.2
2	ICMB 94111	51.7	75.4	63.6	43.7	69.9	56.8	45	47	46	9.1	10.0	9.6
3	ICMB 98222	51.3	74.6	62.9	46.6	59.1	52.9	45	47	46	10.9	11.2	11.1
4	ICMB 00888	56.7	64.1	60.4	49.3	64.1	56.7	44	44	44	10.9	10.7	10.8
5	ICMB 88004	48.9	71.8	60.4	43.6	62.8	53.2	45	47	46	8.2	8.8	8.5
6	ICMB 00999	44.3	72.7	58.5	49.5	68.9	59.2	46	46	46	7.4	7.8	7.6
7	843 B	46.0	70.9	58.4	47.4	71.9	59.6	43	42	42	7.8	8.2	8.0
8	ICMB 94555	47.5	59.9	53.7	44.0	57.1	50.5	44	45	45	7.0	7.5	7.3
9	MIR 97171	50.9	60.4	55.7	42.8	50.7	46.7	42	44	43	9.0	9.2	9.1
	Population progenies (n=30)												
10	AIMP 92901 S1-15-1-2-B	74.7	76.6	75.7	61.6	68.0	64.8	46	45	46	12.8	11.5	12.2
11	AIMP 92901 S1-183-2-2-B	49.2	102.0	75.6	46.5	80.8	63.7	45	45	45	10.8	10.0	10.4
12	SDMV 90031-S1-84-1-1-2-B	53.2	84.8	69.0	50.8	45.4	48.1	43	47	45	9.9	10.8	10.4
13	RCB-2 S1-33-1-3-3-2-B	45.7	65.7	55.7	41.1	57.1	49.1	49	47	48	11.0	9.8	10.4
14	ICMR 312 S1-25-1-1-1-3-B	47.8	62.1	55.0	43.8	52.8	48.3	47	48	47	10.0	9.6	9.8
15	AIMP 92901 S1-421-2-3-B	46.0	63.1	54.6	45.6	56.8	51.2	46	45	46	10.2	10.0	10.1
16	ICMS 7704-S1-51-4-1-1-B	45.1	62.9	54.0	45.6	69.8	57.7	47	47	47	10.9	10.6	10.8
	Improved population (n=30)												
17	GR 8735	52.3	73.1	62.7	45.2	61.0	53.1	42	44	43	12.7	12.2	12.5
18	FEBC	51.0	64.1	57.5	45.5	59.2	52.4	39	38	38	9.6	10.0	9.8
19	ICMV 221	47.5	67.0	57.3	43.4	56.7	50.1	43	43	43	11.9	11.5	11.3
20	HHVDBC	48.7	61.2	55.0	43.2	51.9	47.6	49	48	48	9.2	9.6	9.4
	Germplasm accession (n=20)												
21	IP 8964	47.7	61.1	54.4	43.0	54.7	48.8	60	62	61	9.2	8.6	8.9
	Control												
	S1 B	32.4	36.4	34.4	31.3	32.2	31.7	55	58	57	7.2	7.5	7.4
	ICMP 451	39.6	38.4	39.0	36.0	34.9	35.4	48	47	47	10.0	10.5	10.3
	WC-C 75	42.0	41.9	41.9	38.2	40.9	39.5	47	48	47	9.0	8.7	8.9
	Trial mean	41.5	49.5	45.5	40.1	47.7	43.9	47	48	47	9.0	9.0	9.0
	Minimum	29.3	29.4	30.1	25.0	24.0	24.5	37	38	38	6.5	6.1	6.5
	Maximum	74.7	102.0	75.7	61.6	80.8	64.8	68	70	69	12.8	12.6	12.5
	LSD ($P = 0.05$)	10.5	16.8	13.9	6.4	11.1	9.0	3.1	3.0	4.2	0.8	0.8	1.2
	CV (%)	12.8	17.2	15.5	8.0	11.8	10.4	5.4	6.5	7.2	4.8	4.7	6.8

higher mean (46.6 mg kg^{-1} Fe and 44.9 mg kg^{-1} Zn), whereas the late flowering entries (56–70 days) had lesser variability for grain Fe (34.4–50.4 mg kg^{-1}) and Zn content (31.7–48.8 mg kg^{-1}) with lower mean (45.7 mg kg^{-1} Fe and 44.5 mg kg^{-1} Zn) (Appendix III).

4.2.1.4. 1000-grain mass

The variability for 1000-grain mass was almost similar in both summer (6.5–12.8 g) and rainy season (6.1–12.6 g), and also based on pooled mean across two seasons (Table 11). Among the 21 entries selected for high Fe and Zn content, 16 had higher grain mass (9.1–12.5 g), compared to the check WC-C75 (8.9 g). The open-pollinated variety, GB 8735 had highest 1000-grain mass (12.5 g), followed by a seed parent 863B (12.2 g) and an advanced population progeny AIMP 92901 S1-15-1-2-B (12.2 g). The entries with higher grain mass (11.1–12.5 g) had larger variability for Fe (32.4–78.7 mg kg^{-1}) and Zn content (31.7–64.8 mg kg^{-1}) with higher mean (57.7 mg kg^{-1} Fe and 51.3 mg kg^{-1} Zn) (Appendix III). The entries with lower grain mass (6.5–9.5 g) had lesser variability for Fe (30.1–60.4 mg kg^{-1}) and Zn content (24.5–59.6 mg kg^{-1}) with a lower mean (44.1 mg kg^{-1} Fe and 43.9 mg kg^{-1} Zn).

4.2.2. Variability in improved populations from diverse origin

Highly significant differences were observed among populations for grain Fe and Zn content, days to 50% flower and 1000-grain mass (Table 12). The pooled analysis over two environments showed that the mean differences among genotypes and environments were highly significant for all the four traits, but there was non-significant G × E interaction. Also, the magnitude of genotypic variance was more than the G × E interaction variance for grain Fe and Zn content and days to 50% flower, whereas for 1000-grain mass G × E interaction variance was higher than genotypic variance (Table 8). Consistency of relative rankings of the populations for grain Fe and Zn content was again confirmed by positive and highly significant correlation co-efficient between the two seasons for grain Fe ($r = 0.57$, $P < 0.01$) and Zn content ($r = 0.44$, $P < 0.01$) (Table 9).

Table 12. Analysis of variance for grain Fe and Zn content, days to 50% flower and 1000-grain mass in 69 improved populations (set II trial), 2004 rainy and 2005 summer seasons, ICRISAT- Patancheru.

Source	df	Mean square							
		Fe content (mg kg^{-1})		Zn content (mg kg^{-1})		Days to 50% flower		1000-grain mass (g)	
		Rainy'04	Summer'05	Rainy'04	Summer'05	Rainy'04	Summer'05	Rainy'04	Summer'05
Genotype	68	221.29**	85.03**	219.77**	62.70**	31.50**	30.25**	0.21**	0.22*
Block	1	2148.17**	704.04	0.09	15.27	10.57	8.97	1.10	0.78
Error	68	22.95	27.21	38.93	24.41	2.02	2.14	0.07	0.08
σ^2_g		99.2	90.4	28.9	19.2	14.7	14.1	0.07	0.07
σ^2_p		122.2	129.3	56.1	43.6	16.8	16.2	0.14	0.15
h^2(%)		81	52	70	44	88	87	50	47

* Significant at 5% and 1% probability levels, respectively

4.2.2.1. Fe content

The grain Fe content among 69 improved populations ranged from 41.1 to 85.3 mg kg^{-1} in the rainy season, whereas it ranged from 37.6 to 82.2 mg kg^{-1} in summer season (Table 13, Appendix IV). The average grain Fe content was 30% higher in rainy season (57.6 mg kg^{-1}) than the summer season (52.1 mg kg^{-1}). The mean Fe content across the seasons varied from 42.0 to 79.9 mg kg^{-1} with the overall mean of 54.8 mg kg^{-1}. In all the populations grain Fe content was 20-80% higher than Zn content. The grain Fe content of eight populations during rainy season (74.7 to 85.3 mg kg^{-1}) and three populations in the summer season (71.7-82.2 mg kg^{-1}) was significantly higher than the check WC-C75 (56.3 mg kg^{-1} in summer and 58.9 mg kg^{-1} in rainy season). Based on pooled data across two seasons, 12 populations were identified with high Fe content (63.2-79.9 mg kg^{-1}), which had 15 to 40% higher Fe over the popular variety WC-C75 (57.6 mg kg^{-1}). Among them ICTP 8203 had highest grain Fe (79.9 mg kg^{-1}) followed by GGP bulk (78.5 mg kg^{-1}) and CGP (73.8 mg kg^{-1}).

4.2.2.2. Zn content

The grain Zn content during rainy season ranged from 24.2 to 51.7 mg kg^{-1}, whereas it ranged between 27.9 and 57.6 mg kg^{-1} in the summer season (Table 13, Appendix IV). The mean grain Zn content (40.2 mg kg^{-1}) was 13% higher in summer season compared to rainy season (35.7 mg kg^{-1}). Based on pooled mean, grain Zn content varied from 27.2 to 50.2 mg kg^{-1} with the mean of 38.0 mg kg^{-1}. The grain Zn content of six populations during rainy season (45.4-51.7 mg kg^{-1}) and a population, Uganda (57.6 mg kg^{-1}) during summer season was significantly higher than the check WC-C75 (34.4 mg kg^{-1} in summer and 43.2 mg kg^{-1} in rainy season). Based on pooled mean, eight populations had 45.6-50.2 mg kg^{-1} Zn content, which was 18 to 29% higher than the check WC-C75 (38.8 mg kg^{-1}). Among these GGP bulk had highest grain Zn (50.2 mg kg^{-1}) followed by Uganda (49.8 mg kg^{-1}), PVGGI-5 (47.7 mg kg^{-1}) and ICTP 8203 (47.1 mg kg^{-1}).

Table 13. Selected populations with high grain Fe and Zn content in set II trial, 2004 rainy and 2005 summer seasons, ICRISAT- Patancheru.

Sl. No.	Population	Fe content (mg kg^{-1})			Zn content (mg kg^{-1})			Days to 50% flower			1000-grain mass (g)		
		Rainy 2004	Summer 2005	Mean	Rainy 2004	Summer 2005	Mean	Rainy 2004	Summer 2005	Mean	Rainy 2004	Summer 2005	Mean
1	ICTP 8203	77.6	82.2	79.9	47.7	46.5	47.1	43	42	42	13.0	13.5	13.3
2	GGP Bulk (C3)	85.3	71.7	78.5	51.7	48.7	50.2	48	46	47	12.6	12.1	12.4
3	CGP	83.7	63.8	73.8	41.1	50.1	45.6	45	46	46	11.9	11.0	11.5
4	IAC-ISC-TCP-4	74.7	71.1	72.9	47.1	47.4	47.2	48	51	50	10.6	10.9	10.8
5	PVGGT-4	84.2	59.1	71.6	41.3	42.3	41.8	46	48	47	10.2	9.8	10.0
6	PVGGT-5	76.2	66.6	71.4	45.4	49.9	47.7	46	47	46	9.5	9.7	9.6
7	Ugandi	74.7	65.5	70.1	42.1	57.6	49.8	52	55	54	10.2	10.0	10.1
8	Higrop	64.4	69.5	66.9	40.6	48.0	44.3	49	52	51	9.6	9.8	9.7
9	MRC	54.2	79.1	66.7	29.3	44.8	37.0	41	41	41	9.5	9.8	9.7
10	Lubasi	61.9	71.3	66.6	42.2	47.3	44.7	52	50	51	11.8	11.0	11.4
11	LaGrap	70.2	60.3	65.2	45.9	45.7	45.8	48	52	50	10.2	11.0	10.6
12	PVGGP-6	79.3	47.1	63.2	51.4	40.1	45.7	45	43	44	12.1	10.9	11.5
Check													
	ICMS 7704	54.3	51.6	52.9	25.4	36.6	31.0	46	47	46	9.8	10.2	10.0
	RCB 2	57.7	42.8	50.2	40.0	36.3	38.1	43	43	43	10.2	9.2	9.7
	WC-C 75	56.3	58.9	57.6	34.4	43.2	38.8	46	45	46	9.2	9.5	9.4
	Trial mean	57.6	52.1	54.8	35.7	40.2	38.0	46	46	46	9.8	9.7	9.7
	Minimum	41.1	37.6	42.0	24.2	27.9	27.2	41	40	41	7.2	7.0	7.1
	Maximum	85.3	82.2	79.9	51.7	57.6	50.2	57	58	57	14.0	13.5	13.6
	LSD ($P = 0.05$)	14.6	12.8	10.2	10.3	10.1	11.1	5.8	6.7	7.5	2.8	3.2	4.2
	CV (%)	12.7	12.3	17.9	14.5	12.6	15.1	13.4	10.5	12.0	3.1	3.0	6.2

4.2.2.3. Days to 50% flower

Time to 50% flower varied from 40 to 58 days in both rainy season and summer season, with the overall mean of 46 days (Table 13, Appendix IV). The mean flowering data based on two seasons showed, five populations out of 12 having high Fe and Zn content, as early flowering (41–46 days) compared to the popular check WC-C75 (46 days). In total, 35 populations were flowered early (41–46 days) when compared to WC-C75 (46 days). The early flowering populations (41–45 days) had larger variability for grain Fe (49.0–79.9 mg kg^{-1}) and Zn content (34.2–50.2 mg kg^{-1}) with higher mean (58.4 mg kg^{-1} Fe and 40.3 mg kg^{-1} Zn). The populations with late flowering (51–60 days) had lesser variability for grain Fe (44.4–70.1 mg kg^{-1}) and Zn content (30.4–49.8 mg kg^{-1}) with lower mean (55.7 mg kg^{-1} Fe and 38.8 mg kg^{-1} Zn) (Appendix III).

4.2.2.4. 1000-grain mass

The 1000-grain mass ranged from 7.0 to 14.0 g during both rainy and summer seasons with the mean of 9.8 g (Table 13, Appendix IV). Based on the data across two seasons, all the selected 12 high Fe and Zn content populations had larger seed size (9.6–13.3 g) when compared to popular check WC-C75 (9.4 g). Among the populations identified for high grain Fe and Zn content, ICTP 8203 (13.3 g) had highest 1000-grain mass followed by GGP bulk (12.4 g). The populations with higher grain weight (11.1–14.0 g) had more variability for grain Fe (44.1–79.9 mg kg^{-1}) and Zn content (35.8–50.2 mg kg^{-1}) with higher mean (72.1 mg kg^{-1} Fe and 45.6 mg kg^{-1} Zn) (Appendix III). Whereas the populations with lower grain weight (6.5–9.5 g) had less variability for grain Fe (43.5–59.7 mg kg^{-1}) and Zn content (28.6–43.2 mg kg^{-1}) with lower mean (51.3 mg kg^{-1} Fe and 36.6 mg kg^{-1} Zn).

4.2.3. Variability in initial and advanced recurrent cycle composites trial

Highly significant genetic variability was observed among the composites for grain Fe content, whereas non-significant differences existed among the composites for grain Zn content, days to 50% flower and 1000-grain mass (Table 14). The difference between the initial vs. advanced generation bulks of composites was highly significant for

Table 14. Analysis of variance for grain Fe and Zn content, days to 50% flower and 1000-grain mass in initial and advanced recurrent cycle bulks of different composites (set III trial), 2004 rainy and 2005 summer seasons, ICRISAT-Patancheru.

Source	df	Mean square							
		Fe content (mg kg^{-1})		Zn content (mg kg^{-1})		Days to 50% flower		1000-grain mass (g)	
		Rainy 2004	Summer 2005	Rainy 2004	Summer 2005	Rainy 2004	Summer 2005	Rainy 2004	Summer 2005
R	1	27.85	23.55	12.96	0.84	25.23	18.27	1.25	0.80
Genotype	9	73.88**	100.16**	22.42	29.26	18.25	22.47	0.06	0.08
Composite	4	109.11**	121.89**	9.75	17.02	8.75	12.41	0.12	0.22
Initial *Vs* Advanced bulk	1	78.41**	14.96	22.26	30.5	32.12	28.96	1.20	0.95
Bulks/Groups	4	37.51	99.73**	35.14	41.19	37.45	42.12	0.80	0.70
E	9	14.67	47.24	10.75	8.1	8.2	6.8	0.02	0.03
		29.6	26.5	5.8	10.6	5.0	11.5	0.02	0.02
		44.3	73.7	16.6	18.7	13.2	18.3	0.04	0.05
G		67	36	35	57	38	63	50	40

*, ** Significant at 5% and 1% probability levels, respectively.

Fe content during 2004 rainy season, whereas non-significant for the remaining traits. Pooled analysis over two environments exhibited the mean differences among the composites, environments and G × E interaction effects were non-significant. Also, the variances due to composites were higher than the variance due to G × E interaction component (Table 8). The correlation co-efficient between seasons was highly significant and positive for both Fe ($r = 0.70$; $P < 0.01$) and Zn content ($r = 0.51$; $P < 0.01$) (Table 9).

4.2.3.1. Fe content

Fe content of the composites ranged from 37.9 to 60.7 mg kg^{-1} during rainy and 43.8 to 68.0 mg kg^{-1} during summer seasons. Based on pooled mean, Fe content varied from 43.3 to 64.3 mg kg^{-1} with the average of 50.6 mg kg^{-1} (Table 9 and 15). The advance bulk (C_4) of Serere Composite 1 (52.1 mg kg^{-1}) had significantly lower Fe than the initial bulk (64.3 mg kg^{-1}), whereas the advanced generation bulk (C_3) of Smut Resistant Composites (C_0) showed slight improvement of grain Fe content (47.7 mg kg^{-1}) over its original bulk (43.3 mg kg^{-1}).

4.2.3.2. Zn content

The grain Zn content among the composites ranged from 31.4 to 42.0 mg kg^{-1} during the rainy season and 32.7 to 43.5 mg kg^{-1} during summer seasons (Table 9 and 15). In the pooled data across seasons, Zn content varied from 32.7 to 42.6 mg kg^{-1} with an overall mean of 38.3 mg kg^{-1}. The Zn content in advanced generation bulks of SC1 (35.6 mg kg^{-1}) and SRC (32.7 mg kg^{-1}) was lesser compared to their original bulks (SC1: 42.3 mg kg^{-1} and SRC: 39.7 mg kg^{-1}). However, Zn content in advanced generation bulk of Super Serere Composite (42.6 mg kg^{-1}) was higher than that of original bulk (37.9 mg kg^{-1}).

4.2.3.3. Days to 50% flower

The time to flower varied from about 42 to 46 days with the mean of 43 days in two seasons (Table 9 and 15). The pooled mean also had similar range. There was no significant difference of time to flowering of the initial/original bulks and advanced bulks

Table 15. Grain Fe and Zn content in original/initial and advanced recurrent cycle bulks of different composites, 2004 rainy and 2005 summer seasons, ICRISAT- Patancheru.

Composite	Cycle bulk	Fe content (mg kg^{-1})			Zn content (mg kg^{-1})			Days to 50% flower			1000 grain mass (g)		
		Rainy 2004	Summer 2005	Mean	Rainy 2004	Summer 2005	Mean	Rainy 2004	Summer 2005	Mean	Rainy 2004	Summer 2005	Mean
Medium composite (MC)	C_0	53.0	54.7	53.8	37.6	41.1	39.3	44	44	44	8.8	9.2	9.0
	C_8	48.9	47.5	48.2	41.9	32.7	37.3	43	44	44	8.4	8.5	8.5
Super Serere Composite (SSC)	C_1	50.8	51.5	51.1	39.3	36.6	37.9	42	43	42	9.8	10.5	10.1
	C_9	49.2	58.0	53.6	42.0	43.1	42.6	43	42	42	9.0	9.2	9.1
New Elite Composite (NELC)	C_0	49.2	45.6	47.4	41.3	35.5	38.4	43	43	43	8.0	8.1	8.1
	C_5	37.9	50.3	44.1	38.8	36.1	37.4	46	46	46	8.6	8.9	8.7
Serere Composite I (SC1)	C_1	60.7	68.0	64.3	41.0	43.5	42.3	43	42	42	10.2	10.8	10.5
	C_4	53.0	51.3	52.1	36.5	34.8	35.6	42	42	42	8.9	9.1	9.0
Smut Resistant Composite (SRC)	C_0	42.9	43.8	43.3	41.9	36.4	39.1	44	43	43	8.2	8.2	8.2
	C_3	47.7	47.8	47.7	31.4	34.0	32.7	46	46	46	9.2	9.6	9.4
Mean		49.3	51.9	50.6	39.2	37.4	38.3	43.6	43.5	43.4	8.9	9.2	9.1
Minimum		37.9	43.8	43.3	31.4	32.7	32.7	42.0	42.0	42.0	8.0	8.1	8.1
Maximum		60.7	68.0	64.3	42.0	43.5	42.6	46.0	46.0	46.0	10.2	10.8	10.5
LSD ($P = 0.05$)		7.2	12.4	8.8	8.5	7.8	8.5	2.1	2.0	2.8	1.0	0.8	1.1
CV (%)		8.0	9.2	7.3	8.7	9.5	10.2	8.0	6.0	6.2	7.5	8.8	6.3

of the composites [MC (44 days), SSC (42 days) and SC1 (42 days)], whereas three days flowering difference was observed for the composites NELC and SRC (43-46 days).

4.2.3.4. 1000-grain mass

The 1000-grain mass ranged from about 8.0 to 10.5 g with the average of 9.0 g during summer and rainy seasons and also based on pooled data from two seasons (Table 9 and 15). The SSC and SC1 composites had larger seed size (9.0 10.5 g). The seed size in the initial/original bulk of MC (9.0 g), SSC (10.1 g) and SC1 (10.5 g) was higher than their advanced generation bulks (8.5 g for MC, 9.1 g for SSC and 9.0 g for SC1). On the contrary, the seed size of original bulks of NELC (8.1 g) and SRC (8.2 g) was lower than the advanced generation bulks (8.7 g for NELC and 9.4 g for SRC).

4.2.4. Intra-population variability for grain Fe and Zn content

A total of 64 S_3 progenies from a released variety AIMP 92901 and 68 S_2 progenies from another released variety GB 8735, that had been identified for high Fe and Zn in the previous trials (set I), were field tested during rainy season 2005 and summer season 2006 to determine the intra-population variability. Highly significant genetic differences were observed among the progenies derived from both varieties (Table 16). Considering the analysis across two environments, highly significant mean differences among genotypes, environments and G × E interaction effects was noticed for all the four traits. The magnitude of genotypic variance was more than the G × E interaction variance for grain Fe and Zn content and days to 50% flower, whereas for 1000-grain mass G × E interaction variance was higher than genotypic variance (Table 8). Though the G × E interaction was significant, the correlation co-efficient between the two seasons for progenies derived from both the populations were highly significant for grain Fe ($r = 0.75$; $P < 0.01$ for AIMP 92901 and $r = 0.59$; $P < 0.01$ for GB 8735) and Zn content ($r = 0.80$; $P < 0.01$ for AIMP 92901 and $r = 0.64$; $P < 0.01$ for GB 8735) (Table 9).

Table 16. Analysis of variance for grain Fe and Zn content, days to 50% flower and 1000-grain mass in population progeny trials (set IV trial), 2005 rainy and 2006 summer seasons, ICRISAT- Patancheru.

Source	df	Mean square							
		Fe content (mg kg^{-1})		Zn content (mg kg^{-1})		Days to 50% flower		1000-grain mass (g)	
		Rainy 2005	Summer 2006	Rainy 2005	Summer 2006	Rainy 2005	Summer 2006	Rainy 2005	Summer 2006
Genotype	63	488.49**	705.45**	141.59**	268.86**	71.25**	85 33**	8.97**	12.58**
Block	1	73.50	754.05	73.52	129.11	38.12	18.25	7.25	12.25
Error	63	34.54	56.57	16.07	47.45	12.51	11.41	2.15	3.00
σ^2_g		227.0	324.4	62.8	110.7	29 4	37.0	3.4	4.8
σ^2_p		261.5	381.0	78.9	158.2	41.9	48.4	5.6	7.8
h^2(%)		87	85	80	70	70	76	61	62

*, ** Significant at 5% and 1% probability levels, respectively.

i) AIMP 92901 progenies trial

Source	df	Mean square							
		Fe content (mg kg^{-1})		Zn content (mg kg^{-1})		Days to 50% flower		1000-grain mass (g)	
		Rainy 2005	Summer 2006	Rainy 2005	Summer 2006	Rainy 2005	Summer 2006	Rainy 2005	Summer 2006
Genotype	67	328.92**	458.80**	117.93**	195.56**	86.25**	100 21**	10.25**	8.78**
Block	1	27.36	373.10	165.44	843.95	17.50	55.42	7 58	9.98
Error	67	52.06	131.60	17.11	92.00	19.20	18.52	1.98	2.58
σ^2_g		138.4	163.6	50 4	51.8	33 5	40.9	4.1	3.1
σ^2_p		190.5	295.2	67.5	143.8	52.7	59 4	6 1	5.7
h^2(%)		73	55	75	36	64	69	67	54

* Significant at 5% and 1% probability levels, respectively.

ii) GB 8735 progenies trial

4.2.4.1. AIMP 92901 progenies

AIMP 92901 (S_3) progenies showed approximately three-fold variation for grain Fe in 2005 rainy (34.5–104.0 mg kg^{-1}) and 2006 summer season (44.0–134.3 mg kg^{-1}) and more than two-fold variation for grain Zn content in both rainy (28.5–68.0 mg kg^{-1}) and summer season (34.7–95.4 mg kg^{-1}) (Appendix V; Fig. 5). The mean grain Fe and Zn contents were higher in summer season (70.4 mg kg^{-1} Fe and 56.8 mg kg^{-1} Zn) than in rainy season 2005 (63.0 mg kg^{-1} Fe and 46.6 mg kg^{-1} Zn). Based on pooled data, grain Fe ranged from 40.9 to 118.9 mg kg^{-1} with the mean of 66.8 mg kg^{-1} and grain Zn varied from 31.8 to 82.7 mg kg^{-1} with the mean of 51.9 mg kg^{-1}. Twenty-three progenies had >70 mg kg^{-1} Fe content and nine progenies had >60 mg kg^{-1} Zn content, of which a progeny had >100 mg kg^{-1} Fe content and >80 mg kg^{-1} Zn content. In total, 13 progenies had higher grain Fe content and eight progenies had higher Zn content than the trial mean (81.0–118.9 mg kg^{-1} Fe and 63.5–82.7 mg kg^{-1} Zn). The grain Fe and Zn content of progenies derived from the S1's 383, 406 and 480 was significantly higher.

The time to 50% flower ranged around 41–53 days in 2005 rainy and 2006 summer season. Based on pooled data, 53 progenies were significantly earlier in flowering than the mean (46 days). The early-flowering progenies (41–50 days) had larger variability for grain Fe (40.9–118.9 mg kg^{-1}) and Zn content (31.8–82.7 mg kg^{-1}) than the medium-flowering progenies (51–55 days) that had 51.9 to 93.7 mg kg^{-1} Fe and 38.8 to 68.2 mg kg^{-1} Zn content. The average grain Fe and Zn content were higher in medium flowering (69.9 mg kg^{-1} Fe and 52.2 mg kg^{-1} Zn) than the early-flowering progenies (66.5 mg kg^{-1} Fe and 51.9 mg kg^{-1} Zn) (Appendix III).

The 1000-grain mass varied around 8.8 to 14.0 g during 2005 rainy and 2006 summer season with the average of 11.0 g. In total, 28 progenies had higher 1000-grain mass (11.0–13.8 g) compared to mean (Appendix V). The progenies with higher 1000-grain mass had larger variability for grain Fe (44.2–118.9 mg kg^{-1}) and Zn content (36.1–80.3 mg kg^{-1}) with higher mean for grain Fe (68.2 mg kg^{-1}) and Zn content (55.3 mg kg^{-1}), whereas the progenies with lower grain mass had lesser variability for

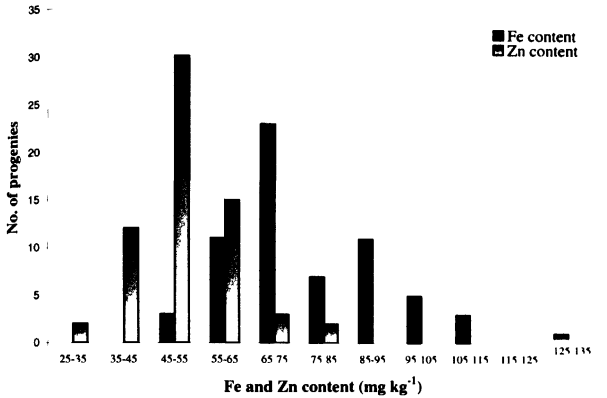


Fig 5. Frequency distribution of AIMP 92901 (S_3) progenies for grain Fe and Zn content, 2005 rainy and 2006 summer seasons, ICRISAT- Patancheru

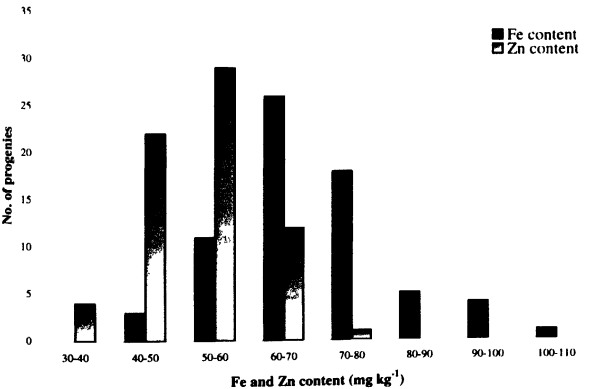


Fig 6. Frequency distribution of GB 8735 (S_2) progenies for grain Fe and Zn content, 2005 rainy and 2006 summer seasons, ICRISAT- Patancheru.

grain Fe (40.9–93.7 mg kg^{-1}) and Zn content (31.8–68.2 mg kg^{-1}) with lesser mean for Fe (67.5 mg kg^{-1}) and Zn (53.1 mg kg^{-1}) (Appendix III).

4.2.4.2. GB 8735 progenies

GB 8735 (S_2) progenies exhibited approximately three-fold variation for grain Fe in both 2005 rainy (39.5–104.5 mg kg^{-1}) and 2006 summer season (48.9–112.0 mg kg^{-1}) and two-fold variation for grain Zn in 2005 rainy (28.5–59.5 mg kg^{-1}) and 2006 summer season (39.2–83.0 mg kg^{-1}) (Appendix VI; Fig. 6). The mean grain Fe and Zn content was higher in summer season (75.1 mg kg^{-1} Fe and 60.4 mg kg^{-1} Zn) compared to rainy season (64.7 mg kg^{-1} Fe and 45.0 mg kg^{-1} Zn). Based on pooled data, Fe content ranged from 45.5 to 108.3 mg kg^{-1} with the mean of 69.8 mg kg^{-1} and Zn content ranged between 33.8 to 70.5 mg kg^{-1} with the mean of 52.7 mg kg^{-1}. The grain Fe content of six progenies and Zn content of one progeny was significantly higher than the mean. In total, 10 progenies had >80 mg kg^{-1} grain Fe and 13 progenies had >60 mg kg^{-1} grain Zn content.

Flowering time of the progenies varied around 38 to 52 days with the trial mean of 44 days during both rainy and summer seasons. Of these, 24 progenies were early-flowering (39 to 43 days). The early-flowering progenies (35–50 days) had larger variability for grain Fe (45.9–108.3 mg kg^{-1}) and Zn content (38.1–70.5 mg kg^{-1}) with higher mean (79.3 mg kg^{-1} Fe and 57.7 mg kg^{-1} Zn). The medium flowering progenies (51–55 days) had lower mean for grain Fe (61.6 mg kg^{-1}) and Zn content (50.1 mg kg^{-1}) (Appendix III).

The 1000-grain mass varied from 8.0 to 14.0 g with the mean of 11.0 g during both the seasons and also based on pooled data. In total, 33 progenies had larger seed size (11.1–13.6 g) when compared to mean (Appendix VI). The large-seeded progenies had larger variability for grain Fe (45.9–108.3 mg kg^{-1}) and Zn content (39.5–70.5 mg kg^{-1}) with higher mean Fe (73.8 mg kg^{-1}) and Zn content (53.6 mg kg^{-1}), whereas the small seeded progenies had relatively lesser variability for grain Fe (45.5–84.4 mg kg^{-1}) and Zn

content (33.8–67.2 mg kg^{-1}) with lesser mean Fe (70.9 mg kg^{-1}) and Zn (53.0 mg kg^{-1}) (Appendix III).

4.3. CORRELATION STUDIES

Correlation co-efficients between grain Fe and Zn content and their association with 1000-grain mass and days to 50% flower were estimated based on individual environments as well as over two environments (Table 17).

4.3.1. Correlation between grain Fe and Zn content

Highly significant positive correlation was observed between grain Fe and Zn content in all the experiments (r=0.68 to 0.91; P <0.01), except in the set III trial (r=0.39) during 2004 rainy season. Highly significant positive correlation was observed between grain Fe and Zn content among the 120 entries (set I) in both the summer (r=0.78; P <0.01) and rainy season (0.82; P <0.01) and also based on pooled mean from two environments (r =0.84; P <0.01) (Fig. 7).

Among the improved populations (n=69), positive significant correlation existed between grain Fe and Zn content in both the seasons (r= 0.76; P <0.01 in 2004 rainy and r=0.81; P <0.01 in 2005 summer season) and also based on combined mean from two seasons (r=0.84; P <0.01) (Fig. 8). In set III trial, positive significant correlation existed between grain Fe and Zn content in 2005 summer season alone (r=0.91; P <0.01) and also based on pooled mean (r=0.76; P <0.01).

Significant and fairly high positive correlation was also observed between grain Fe and Zn content in population progenies derived from AIMP 92901 (r=0.68; P <0.01 in 2005 rainy and r=0.76; P <0.01 in 2006 summer) and GB 8735 (r=0.76; P <0.01 in 2005 rainy and r=0.73; P <0.01 in 2006 summer season). Also, based on pooled data highly significant positive correlation was observed for AIMP 92901 progenies (r=0.75; P <0.01) and GB 8735 progenies (r=0.77; P <0.01). Similarly, highly significant positive correlation observed between grain Fe and Zn content among the hybrids used in the diallel crosses (r=0.84; P <0.01).

Table 17. Correlation co-efficient matrix of grain Fe and Zn content (mg kg^{-1}) with days to 50% flower and 1000-grain mass in different trials, ICRISAT – Patancheru.

Trials	Season	Correlation co-efficient	Zn content (mg kg^{-1})	Days to 50 % flower	1000-grain mass (g)
Set I trial (n=120)	Summer 2004	Fe	0 78**	-0 17	0 31**
		Zn		-0 07	0 24*
	Rainy 2004	Fe	0 82**	-0 31**	0 26**
		Zn		-0 32**	0 20*
	Pooled mean	Fe	0 84**	-0 18	0 29**
		Zn		-0 14	0 21*
Set II trial (n = 69)	Rainy 2004	Fe	0 76**	-0 53**	0 68**
		Zn		-0 53**	0 41**
	Summer 2005	Fe	0 81**	-0 15	0 55**
		Zn		-0 08	0 47**
	Pooled mean	Fe	0 84**	-0 04	0 43**
		Zn		-0 12	0 33**
Set III trial (n = 14)	Rainy 2004	Fe	0 39	-0 47	0 79**
		Zn		-0 38	0 22
	Summer 2005	Fe	0 91**	-0 44	0 81**
		Zn		-0 25	0 38
	Pooled mean	Fe	0 76**	-0 48	0 78**
		Zn		-0 45	0 32
AIMP 92901 progenies trial (n = 64)	Rainy 2005	Fe	0 68**	-0 14	0 26*
		Zn		0 17	0 12
	Summer 2006	Fe	0 76**	-0 08	0 24*
		Zn		-0 11	0 18
	Pooled mean	Fe	0 75**	-0 14	0 22
		Zn		-0 07	0 20
GB 8735 progenies trial (n = 68)	Rainy 2005	Fe	0 76**	-0 08	0 19
		Zn		-0 11	0 09
	Summer 2006	Fe	0 73**	0 19	0 19
		Zn		-0 21	0 12
	Pooled mean	Fe	0 77**	-0 18	0 18
		Zn		-0 09	0 17
Diallel inheritance trial (n = 45)	Rainy 2005	Fe	0 84**	-0 60**	0 46**
		Zn		-0 53*	0 42**

*, ** Significant at 5% and 1% probability levels, respectively

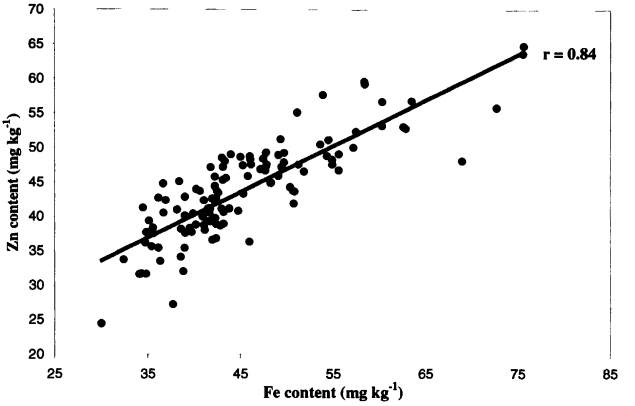


Fig 7. Relationship between grain Fe and Zn content in different classes of breeding materials and germplasm accessions (set I trial), ICRISAT-Patancheru.

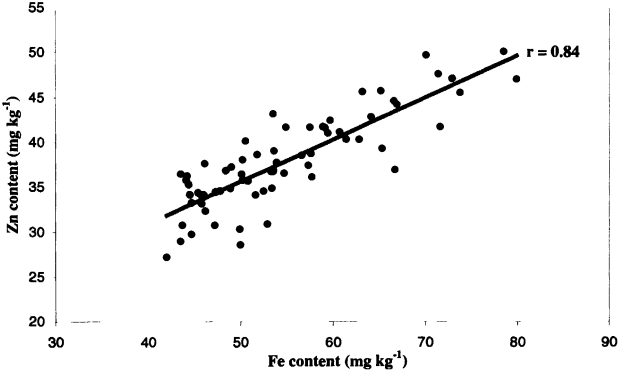


Fig 8. Relationship between grain Fe and Zn content in improved populations (set II trial), ICRISAT- Patancheru.

4.3.2. Correlation of days to 50% flower with grain Fe and Zn content

Although the correlations of days to 50% flower with grain Fe and Zn was non-significant in most of the trials, it was negative and significant in 2004 rainy season trials of set I (r=-0.31; P <0.01 for Fe and r=0.32; P <0.01 for Zn) and set II (r=-0.53; P <0.01 for Fe and Zn) and in diallel crosses (r= -0.60; P <0.01 for Fe; r= -0.53; P <0.05 for Zn) (Table 17).

4.3.3. Correlation of 1000-grain mass with grain Fe and Zn content

There was positive and significant correlation between 1000-grain mass and Fe content in set I trial (r =0.26 to 0.31; P <0.01) as well as in set II trial (r =0.55 to 0.68; P<0.01) in both seasons (Table 17). Based on the pooled data for the two seasons, this correlation was again positive and significant for both Fe (r=0.29; P <0.05 in set I trial and r= 0.43; P <0.01 in set II trial) and Zn content (r=0.21; P<0.05 in set I trial and r = 0.33; P<0.01 in set II trial). Similarly, highly significant positive correlation was observed in hybrids used in diallel crosses for both grain Fe (r=0.46; P <0.01) and Zn content (r=0.42; P <0.01).

There was positive and significant correlation between 1000-grain mass and Fe content in set III trial and AIMP 92901 progenies trial in both seasons (r=0.79 to 0.81; P<0.01 in set III trial and r=0.24 to 0.26; P <0.05 in AIMP 92901 progenies trial), whereas no correlation observed between 1000-grain mass and Zn content in these two trials during both the seasons. There was no correlation between 1000-grain mass and Fe, as well as with Zn content in GB 8735 progenies trial.

4.3.4. Inter-laboratory correlation for grain Fe and Zn content

Randomly selected 12 genotypes with a wide range of grain Fe and Zn levels as revealed from the laboratory analysis at NIN, Hyderabad were also analyzed at ICRISAT-Patancheru, India and Waite Analytical laboratory, Adelaide University, Australia. The inter-laboratory correlations were positive and highly significant for both grain Fe (r=0.77 to 0.93; P <0.01) and grain Zn (r=0.89 to 0.98; P <0.01) (Table 18). The correlation

Table 18. Correlation co-efficient of grain Fe and Zn content in randomly selected genotypes across different laboratories, 2004 summer and rainy seasons, ICRISAT- Patancheru.

Laboratory	NIN	ICRISAT	Waite
NIN	--	0.86**	0.77**
ICRISAT	0.89**	--	0.93**
Waite	0.93**	0.98**	--

Above diagonal Fe; below diagonal Zn

*, ** Significant at 5% and 1% probability levels, respectively.

Table 19. Correlation co-efficient between different grain sources for grain Fe and Zn content, 2004 summer season, ICRISAT - Patancheru.

Grain source	Sibbed	Selfed	Open-pollinated
Sibbed	--	0.68**	0.43**
Selfed	0.78**	--	0.44**
Open-pollinated	0.51**	0.53**	--

Above diagonal Fe; below diagonal Zn

*, ** Significant at 5% and 1% probability levels, respectively

between Waite laboratory and ICRISAT laboratory was higher for both grain Fe (r=0.93; P <0.01) and Zn (r=0.98; P <0.01), compared to NIN and ICRISAT (r=0.86; P <0.01 for Fe and r=0.89; P <0.01 for Zn) and NIN and Waite laboratory (r=0.77; P <0.05 for Fe and r=0.93; P <0.01 for Zn).

4.3.5. Correlation of Fe and Zn content among different grain sources

Production of selfed or open-pollinated (OP) grain for micronutrient analysis is much more cost-effective than producing sib-mated grain. Hence, relationship between sib-mated, selfed and open-pollinated (OP) grain samples of 30 entries produced from the set I trial during the 2004 summer season were analyzed for grain Fe and Zn content. Results showed that highly significant positive correlation observed between selfed and sib-mated grain for both grain Fe (r=0.68; P <0.01) and Zn content (r=0.78; P <0.01), also positive and highly significant correlation of OP grain with sib-mated grain was noticed for both grain Fe (r=0.43; P <0.01) and Zn content (r=0.51; P <0.01) but in lower magnitude (Table 19). Similarly, significant positive correlation observed between OP and selfed grain for both grain Fe (r=0.44; P <0.01) and Zn content (r=0.53; P <0.01).

4.4. ASSESSMENT OF GRAIN IRON CONTENT BY PERLS' PRUSSIAN BLUE STAINING METHOD

Twelve pearl millet inbreds with four high, three medium and five low Fe content were used to standardize the Prussian blue staining protocol. The Fe content measured from Atomic Absorption Spectrophotometer (AAS) of set I trial (n =120) was used for reference. The flour of pearl millet grains, when treated with 2% Prussian blue solution in the Petri dishes produced varying intensity of blue colour in genotypes having medium to high Fe content (Plate 1). In the genotypes having high Fe content (51.7–74.7 mg kg^{-1}), the blue colour was more intense than those having medium Fe content (40.3–40.8 mg kg^{-1}) (Table 20). No colour developed in the genotypes with low Fe content (31.2–40.6 mg kg^{-1}). The rank correlation of measured grain Fe content and the colour score was highly significant and positive (r=0.92; P <0.01).

Table 20. Prussian blue staining pattern and grain Fe content in different genotypes, 2004 summer and rainy seasons, ICRISAT- Patancheru.

Inbred/partial inbred	Class	Fe content (mg kg^{-1})*	Colour expression	Colour score
863B	High	69.0	More intense blue colour	4
ICMB 94111	High	51.7	More intense blue colour	4
ICMB 00888	High	56.7	More intense blue colour	4
AIMP 92901 S1-15-1-2-B	High	74.7	More intense blue colour	4
ICMV 91059 S1-58-2-2-2-B	Medium	40.6	Less intense blue colour	2
ICMV 93074 S1-9-1-1-1-B	Medium	40.3	Medium blue colour	3
MC 94 C2-S1-46-1-1-B	Medium	40.8	Medium blue colour	3
ICMB 95222	Low	35.2	No colour	1
81B	Low	32.4	No colour	1
ICMB 90111	Low	34.2	No colour	1
ICMS 8511 S1-17-2-1-1-B	Low	30.8	No colour	1
ICMV 91059 S1-14-2-4-2-2-B	Low	31.2	No colour	1

* Analysed through Atomic Absorption Spectrophotometry.

Table 21. Differential Prussian blue staining reaction and grain Fe content in 20 pearl millet inbred lines, 2004 summer season, ICRISAT- Patancheru.

Inbred	Fe content (mg kg^{-1})*	Colour expression	Colour score
81B	32.4	No colour	1
ICMB 90111	34.2	No colour	1
ICMB 97333	34.9	No colour	1
ICMB 95222	35.2	No colour	1
ICMB 98777	35.9	No colour	1
ICMB 89111	36.5	No colour	1
ICMB 93333	37.3	Less intense blue colour	2
ICMB 97111	38.2	No colour	1
ICMB 01888	39.1	No colour	1
ICMB 01555	42.1	Less intense blue colour	2
ICMB 91444	42.9	Medium blue colour	3
ICMB 91777	43.7	Medium blue colour	3
ICMB 00999	44.3	Medium blue colour	3
843B	46.0	Medium blue colour	3
ICMB 94555	47.5	Less intense blue colour	2
ICMB 88004	48.9	More intense blue colour	4
ICMB 98222	51.3	More intense blue colour	4
ICMB 94111	51.7	More intense blue colour	4
ICMB 00888	56.7	More intense blue colour	4
863B	69.0	More intense blue colour	4
Mean	43.4		
LSD (P=0.05)	11.1		
CV (%)	12.3		

* Analysed through Atomic Absorption Spectrophotometry.

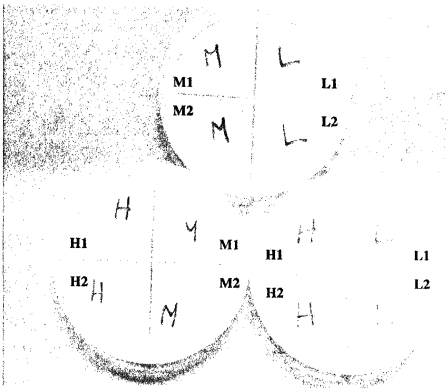


Plate 1. Differential Prussian blue staining of pearl millet grain flour with varying levels (H- high, M- medium and L-low) of grain Fe content. (H_1- 863B, H_2 - AIMP 92901 S1-15-1-2-B; M_1 - MC 94 C2-S1-46-1-1-B, M_2 - ICMV 93074 S1-9-1-1-1-B; L_1 - 81B, L_2 - ICMB 90111).

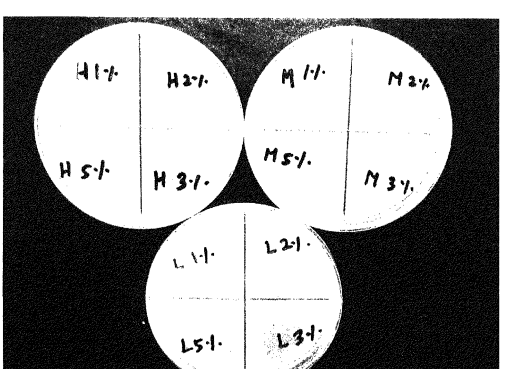


Plate 2. The intensity of blue colour in high (H), medium (M) and low (L) genotypes with varying concentrations of Prussian blue staining solution.

The protocol was fine-tuned by varying the concentration of Prussian blue solution (1%, 2%, 3% and 5%). But, the intensity of blue colour did not differ with varying concentrations in high, medium and low genotypes (Plate 2). However, the colour development was slow when treated with 1% solution.

This standardized protocol using 2% Prussian blue solution was validated using 20 diverse B-lines with a wide range of Fe content (32.4–69.0 mg kg^{-1}) (Table 21). The colour development was more intense in the B-lines with Fe content ranging from 48.9 to 69.0 mg kg^{-1}. On the contrary, no colour developed in the B-lines having Fe content from 32.4 to 39.1 mg kg^{-1} with the exception of ICMB 93333, which in spite of having low Fe content (37.3 mg kg^{-1}) showed but less intense blue colour as against the expected no colour. The medium colour intensity was noticed in the B-lines having Fe content 42.9–46.0 mg kg^{-1}. The seed parent ICMB 94555, in spite of having moderate levels of Fe (47.5 mg kg^{-1}) showed less intense blue staining, which could be considered as the exception. All the B-lines having Fe content above average of 43.4 mg kg^{-1} showed either medium or high intensity blue colour staining. There was highly significant and positive correlation ($r=0.91$; $P<0.01$) between the measured Fe content and colour score.

4.5. STABILITY ANALYSIS

Twenty-nine entries (out of 120 entries of set I trial) were selected for stability analysis that included 14 high, 8 medium and 6 low Fe and Zn content lines with WC-C75 as a control. The data from four test environments viz., summer 2004 (E_1), rainy 2004 (E_2), summer 2005 (E_3) and rainy season 2005 (E_4) were analyzed by using Eberhart and Russell (1966) and Additive Main effects and Multiplicative Interaction (AMMI) models (Zobel *et al.*, 1988) for grain Fe and Zn content, 1000-grain mass and days to 50% flower.

4.5.1. Analysis of variance (Eberhart and Russell model)

The analysis of variance indicated that the mean squares due to entries, environments and entries × environment interaction were significant for all the traits except 1000-grain mass (Table 22). The linear component of environment was significant

Table 22. Analysis of variance of grain Fe and Zn content, days to 50% flower and 1000-grain mass for stability analysis (Eberhart and Russell model), summer and rainy seasons of 2004 and 2005, ICRISAT-Patancheru.

Source	df	Mean squares			
		Fe content (mg kg^{-1})	Zn content (mg kg^{-1})	Days to 50 % flower	1000-grain mass (g)
Entry (G)	28	1472.86**	730.89**	225.00**	20.49**
Environment (E)	3	2270.78**	1804.44**	1304.33**	1.92*
G × E	84	126.32**	64.91**	8.14**	0.58
E + (G × E)	87	2397.10**	1869.35**	1312.47**	2.50
Environment (Linear)	1	3396.93**	2713.90**	1956.50**	2.77*
G × E (linear)	28	59.03	64.84**	9.63**	0.39
Pooled deviation (non-linear)	58	63.07*	15.63	1.25	0.23
Pooled error	116	72.68	24.91	1.65	0.62

* Significant at 5% and 1% probability levels, respectively.

Table 23. Environmental indices for grain Fe and Zn content, days to 50% flower and 1000-grain mass in stability trials, summer and rainy seasons of 2004 and 2005, ICRISAT-Patancheru.

Sl. No.	Character	Environment			
		Summer 2004 (E_1)	Rainy 2004 (E_2)	Summer 2005 (E_3)	Rainy 2005 (E_4)
1	Fe content	-8.99	5.23	0.89	2.88
2	Zn content	-1.55	8.09	-1.78	-4.75
3	Days to 50% flower	-5.05	-3.02	3.66	4.41
4	1000-grain mass (g)	-0.07	0.18	-0.22	0.10

for all the traits. The mean squares due to entries × environment (linear) were significant for grain Zn content and days to 50% flower when tested against mean squares due to pooled deviation (non-linear), whereas non-significant for grain Fe content and 1000-grain mass.

4.5.1.1. Environmental indices

The environmental indices for grain Fe content varied from -8.99 (E_1) to 5.23 (E_2), while the grain Zn content indices varied from -4.75 (E_4) to 8.09 (E_2) (Table 23). All the environments were positive for grain Fe (0.89–5.23) except for E_1 (-8.99) and all environments were negative for Zn content (-4.75 to -1.55) except E_2 (8.09). The minimum environmental index of -5.05 (E_1) and maximum of 4.41 (E_4) was observed for days to 50% flower, and E_1 and E_2 were negative environments. For 1000-grain mass, environmental indices ranged from -0.22 (E_3) to 0.18 (E_2), and E_2 and E_4 had positive environmental indices. The environmental indices for all the characters were highest and positive in E_2 (0.18–8.09) except for days to 50% flower (-3.02), followed by E_4 that had positive environmental index for all the characters (0.10 to 2.88) except Zn content (-4.75). The environmental indices were lowest and negative in E_1 (-8.99 to -0.07) for all the traits.

4.5.1.2. Stability parameters

The estimates of stability parameters viz., mean, regression coefficient (b_i) and deviation from regression ($\overline{S}_d^2$) for grain Fe and Zn content and days to 50% flower were calculated according to Eberhart and Russell (1966) model are given in Table 24.

The mean grain Fe content across four environments ranged from 31.2 to 84.5 mg kg^{-1} with the mean of 53.1 mg kg^{-1} and Zn content varied from 22.7 to 62.6 mg kg^{-1} with average of 43.1 mg kg^{-1} (Table 24; Appendix VII). Larger range of grain Fe was observed in E_2 (29.4–102.0 mg kg^{-1}), followed by E_3 (31.8–94.5 mg kg^{-1}) and E_4 (32.8–92.1 mg kg^{-1}), whereas smaller range was observed in E_1 (29.3–74.7 mg kg^{-1}). Similarly, for Zn content the wider range values were observed in E_2 (24.0–80.8 mg kg^{-1}) followed by E_4

(21.7–64.3 mg kg^{-1}) and E_1 (25.0–61.6 mg kg^{-1}), whereas smaller range was observed for E_3 (20.4–56.6 mg kg^{-1}). In general, the entries with high grain Fe and Zn remained high (84.5–54.2 mg kg^{-1} Fe and 62.6–44.2 mg kg^{-1} Zn), medium remained medium (47.6–42.5 mg kg^{-1} Fe and 41.9–37.9 mg kg^{-1} Zn) and low remained low (39.9–31.2 mg kg^{-1} Fe and 31.5–22.7 mg kg^{-1} Zn) across four environments.

Among the 14 entries with high grain Fe and Zn content, significant mean squared deviation was observed only in 3 entries, AIMP 92901 S1 183-2-2-B, SDMV 90031 S1-84-1-1-2-B and ICMB 00888 for both Fe (259.0–948.1; P <0.01) and Zn content (80.5–182.1; P<0.01) and, in an advance population progeny AIMP 92901 S1-15-1-2-B (292.6; P<0.01) for Fe content, and in a seed parent 843B (110.5; P<0.05) for Zn content. Thus, there were 9 entries, which were stable for both Fe and Zn, and one additional entry that was stable for only Fe and another additional entry that was only stable for Zn. Of these 10 stable entries, seven entries had unit regression for Fe (b_i = 0.68 to 2.52) and Zn (b_i = 0.69 to 1.79). Three entries for Fe (ICMB 98222, EEBC and GB 8735) had positive regression slope significantly deviating from unity (b_i= 1.76 to 2.15; P <0.05) and three entries for Zn (EEBC, AIMP 92901 S1-183-2-2-B and ICMS 7704 S1-51-4-1-1-B) had positive regression slope significantly deviating from unity (b_i= 2.07 to 3.38; P <0.05).

Among the top entries for grain Fe and Zn content, a seed parent 863B (75.1 mg kg^{-1} Fe and 53.9 mg kg^{-1} Zn) had regression coefficient around unity (0.68 to 0.69) and non-significant deviation from regression (b_i = 9.9 to 14.9). For Zn content, an advance population progeny AIMP 92901 S1-15-1-2-B (62.6 mg kg^{-1}) had regression coefficient around unity (b_i = 0.82) with non-significant mean squared deviation (5.0). Among the open-pollinated varieties, GB 8735 having higher mean values for both Fe (67.2 mg kg^{-1}) and Zn (49.8 mg kg^{-1}), had regression slope around unity for Zn (b_i = 1.42), and non-significant mean squared deviation from the regression for both Fe (0.6) and Zn content (16.5).

Table 24. Stability parameters for grain Fe and Zn content, days to 50% flower and 1000-grain mass in stability trials (Eberhart and Russell model), summer and rainy seasons of 2004 and 2005, ICRISAT- Patancheru.

Sl. No.	Class	Entry	Fe content (mg kg^{-1})			Zn content (mg kg^{-1})			Days to 50% flower			1000-grain mass (g)
			Mean	b_i	S^2_d	Mean	b_i	S^2_d	Mean	b_i	S^2_d	Mean
1	High	AIMP 92901 S1-15-1-2-B	84.5	0.93	292.6*	62.6	0.82	5.0	46	1.05*	0.03	12.5
2	High	863B	75.1	0.68	14.9	53.9	0.69	9.9	47	1.09	3.01	12.1
3	High	AIMP 92901 S1-183-2-2-B	70.1	2.36	817.1**	51.4	3.38*	80.5*	48	0.61**	0.14	9.7
4	High	ICMB 98222	68.1	2.15**	5.8	49.3	1.17	2.0	48	0.83	44.85**	11.2
5	High	GB 8735	67.2	1.77**	0.6	49.8	1.42	16.5	45	1.09	1.57	12.2
6	High	ICMB 94111	66.2	1.96	132.6	49.9	0.98	30.4	53	1.11	0.51	10.7
7	High	EEBC	66.2	1.76*	9.7	51.1	2.26*	20.0	38	1.15	1.81	11.2
8	High	ICMB 00999	63.7	2.52	117.8	55.1	1.79	33.3	51	1.41*	0.75	8.9
9	High	ICMB 88004	62.2	1.39	60.8	49.0	1.59	29.0	50	1.45*	2.60	9.8
10	High	SDMV 90031-S1-84-1-1-2-B	60.1	0.46	948.1**	44.2	0.39	88.7*	59	0.81	0.14	10.2
11	High	IPC 843	59.5	0.84	216.5	53.8	0.64	10.4	54	0.98	0.94	9.1
12	High	843B	59.4	1.49	73.3	55.3	1.55	110.5*	45	0.56**	0.07	8.8
13	High	ICMS 7704-S1-51-4-1-1-B	56.0	1.17	35.8	52.1	2.07*	15.8	48	1.80**	0.12	9.5
14	High	ICMB 00888	54.2	-0.48	259.0**	45.5	2.60	182.1**	48	0.82	1.09	10.5
15	Medium	ICMV 91059 S1-58-2-2-2-B	47.6	0.90	87.9	39.8	0.84	30.0	57	1.10	0.34	9.5
16	Medium	ICMV 93074 S1-9-1-1-1-B	47.4	0.86	21.4	41.9	0.75	10.5	57	0.88	0.61	9.9
17	Medium	Jakharana pop	47.3	0.99	79.2	41.6	0.36	66.6	44	1.02	0.04	10.1
18	Medium	MC 94 C2-S1-46-1-1-B	46.5	0.78	1.5	37.9	0.21*	4.3	50	1.52*	1.54	8.8
19	Medium	SDMV 93032-S1-93-3-2-2	44.9	0.45	55.9	37.1	0.62	19.7	50	0.88	0.28	9.2
20	Medium	CZI-98-11	44.8	0.65	54.7	38.7	1.28	42.6	54	0.94	0.84	10.6
21	Medium	CZI 96-21	44.3	1.00	32.2	39.5	1.08	33.9	46	0.73	2.12	7.9
22	Medium	NCD2 Bulk	43.9	1.00	11.9	38.7	1.00	5.6	45	1.06	0.30	8.6
23	Low	ICMB 90111	42.5	0.53	13.3	39.6	0.85	13.7	51	0.94	2.16	9.1
24	Low	SDMV 90031-S1-93-3-1-1-B	39.9	0.73	3.3	26.4	0.20**	1.0	54	1.58*	1.78	7.8
25	Low	HTP 94/54 (HHB 146)	38.4	1.28	44.0	30.0	0.32*	10.1	55	0.71	2.53	10.2
26	Low	81B	36.6	-0.01	100.3	31.4	0.02**	1.3	55	1.57**	0.26	9.7
27	Low	SOSAT C88	36.5	0.52	10.2	30.2	0.20**	1.1	56	0.21**	0.15	7.0
28	Low	ICMS 8511 S1-17-2-1-1-B	34.1	0.17	27.4	31.5	0.27*	9.1	55	0.15**	0.72	11.1
	Check	WCC 75	31.2	0.19	130.3	22.7	-0.32*	22.9	58	0.94	1.07	7.2
		Mean	53.1	-	-	43.1	-	-	51	-	-	9.7
		Minimum	31.2	-0.48	0.6	22.7	0.02	1.0	38	0.15	0.03	7.0
		Maximum	84.5	2.15	948.1	62.6	3.38	182.1	59	1.8	44.85	12.5
		LSD ($P = 0.05$)	7	-	-	4.1	-	-	9.8	8.8	-	2.1
		CV (%)	9.8	-	-	9.5	-	-	12.5	11	-	4.3

*, ** Significant at 5% and 1% probability levels, respectively.

The time to flower ranged from 38 to 59 days with the grand mean of 51 days (Table 24). All the 14 high Fe and Zn content entries were early-flowering (34-54 days) except SDMV 90031 S1-84-1-1-2-B (59 days), with all of them having non-significant mean squared deviation (-0.81 to 0.68) except an seed parent ICMB 98222, which had highly significant mean squared deviation (44.85; P <0.01). Of these 13 stable entries for days to flower with high Fe and Zn, 7 entries had unit regression slope (b_i = 0.81 to 1.15) and six entries had positive regression slope significantly deviating from unity (0.56 to 1.80; P <0.01).

Among the high Fe and Zn content entries, 863B, ICMB 00888 and ICMB 88004 were early to flower (47–50 days) than the mean days to flower (51 days) and regression coefficient approaching unity (0.82–1.45) with non-significant deviation from regression (1.09 to 3.01). Similarly, among the medium grain Fe and Zn content entries, CZI 96–21, CZI–98–11, WC-C75 and Jakharana pop were earlier to flower (44–50 days), with unit regression (0.88 to 1.02) and non-significant deviation from regression (0.04 to 2.16).

The mean 1000-grain mass across the four environments ranged from 7.0 to 12.5g with the overall mean of 9.7 g (Table 24). Since the G × E interaction when tested against pooled error was not significant so the need for estimation of the b_i and $\overline{S}_d^2$ values did not arise.

4.5.2. AMMI analysis of variance

The analysis of variance in AMMI model split the total variances into additive main effects (entries, environments and entry × environments) and multiplicative interactions (PCA I and PCA II). Analysis of variance showed highly significant differences among main effects such as entries and environments for grain Fe and Zn content, 1000-grain mass and days to 50% flower. Further, the entries × environments interaction was significant for grain Fe and Zn content and days to 50% flower and was not significant for 1000-grain mass (Table 25). In all the traits, where entries × environment interaction was significant, the first interaction principal component axis

Table 25. AMMI analysis of variance for grain Fe and Zn content, days to 50% flower and 1000-grain mass, summer and rainy seasons of 2004 and 2005, ICRISAT - Patancheru.

Source	Fe content (mg kg^{-1})				Zn content (mg kg^{-1})			Days to 50% flower			1000 grain mass (g)		
	df	SS	MS	Explained SS (%)	SS	MS	Explained SS (%)	SS	MS	Explained SS (%)	SS	MS	Explained SS (%)
Entries (G)	28	41240	1472.9**	61.47	20465	730.9**	59.80	6300	225.0**	56.82	633.3	20.6**	83.15
Environments (E)	3	6812	2270.8**	10.15	5413	1804.4**	15.82	3913	1304.3**	35.29	5.6	1.9*	0.74
G × E	84	10611	126.3**	15.82	5452	64.9**	15.93	684	8.1**	6.17	49.5	0.6	6.50
PCA I	30	5985	199.5**	56.40	3740	124.7**	68.60	547	18.2**	79.97	27.6	0.9	55.76
PCA II	28	3313	118.3	31.22	1040	37.1	19.10	116	4.1**	17.00	16.4	0.6	33.13
Residual	26	1312	50.5	12 36	672	25.8	9.10	21	0.8	2.41	5.6	0 2	10.31
Pooled error	112	8316	72.3		2729	24.4		185	1.7		70.4	0 6	

*, ** Significant at 5% and 1% probability levels, respectively.

(PCA I) captured maximum interaction variation followed by the PCA II, which were interpretable interaction parameters and discarded noise affecting predictive accuracy. As PCA I was significant for all the traits except 1000-grain mass, it was used as a rough approximation of the true interaction in our experiments.

For grain Fe content, a large portion of total variation was attributed to effects of entries (61.47%) followed by effects due to entries × environment interaction (15.82%) and environmental effects (10.15%). The first interaction principal component axis (PCA I) of the entries × environments interaction variation was significant and captured 56.40% of the G × E sum of squares, while PCA II capturing 31.22% of the G × E sum of squares was non-significant. Similarly, major portion of variation for grain Zn content was due to by entries (59.80%), followed by G × E effects (15.93%) and environmental effects (15.82%). Among the entries × environment interaction components, the PCA I was significant, capturing 68.60% of the G × E interaction sum of squares. PCA II, accounting for 19.10% of the G × E interaction sum of squares was non-significant.

For days to 50% flower, 56.82% of the total sum of squares was attributable to effects of entries, 35.29% to environmental effects, and only 6.17% to entries × environment interaction effects. The interaction components PCA I and PCA II were highly significant, but PCA I explained 80% of the interaction sum of squares. For 1000-grain mass, the largest proportion of total variation was contributed by effects due to entries (83.15%). The G × E interaction was non-significant, and so were the PCA I and PCA II.

4.5.2.1. AMMI stability parameters

Interpretation of G × E interaction in the AMMI analysis was done using biplot graph, where the abscissa represents entry or environment means and ordinate represents entry or environment interaction PCA scores. The biplot shows not only average performance of the entries but also how it is achieved. The biplot graph is divided into four quadrants from lower micronutrient content environments III and IV to high

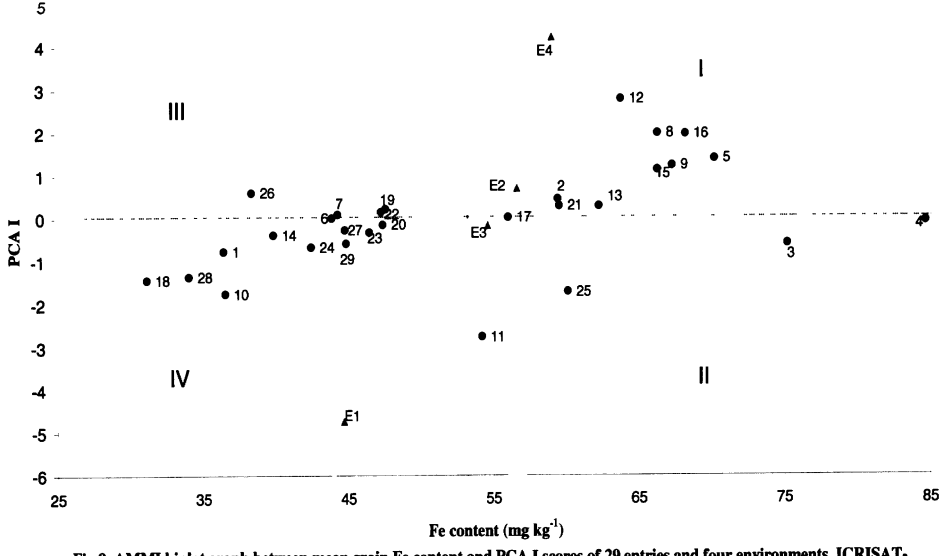


Fig 9. AMMI biplot graph between mean grain Fe content and PCA I scores of 29 entries and four environments, ICRISAT-Patancheru. (E1: summer 2004; E2: rainy 2004; E3: summer 2005; E4: rainy 2005 and the list of 29 entries given in Table 26)

micronutrient content environments in quadrants I and II. When an entry and an environment have the same sign on the PCA axis (ordinate), their interaction is positive; if different their interaction is negative. If an entry or an environment has a PCA score of nearly zero, it has small interaction effects. For any entry-environment combination, the additive part (main effects) of the AMMI model equals the entry mean plus the environment mean minus grand mean, and the interaction (multiplicative part) is the entry score times the environment score (Zobel *et al.*, 1988). For example, ICMB 00999 (No. 12) grown in environment E_1 has an additive effect 63.7+ 44.7-53.1=55.3 mg kg^{-1}, and interaction estimated is 2.78 × (-4.74)= - 13.2 mg kg^{-1}. Therefore the AMMI model gives grain Fe estimation for ICMB 00999 grown in summer 2004 (E_1) of 55.3-13.2 = 42.1 mg kg^{-1}. This prediction is as accurate as the raw data, where grain Fe content was 44. 3 mg kg^{-1} (Appendix VII).

Of the 14 entries with high grain Fe content occupying position in quadrant I and II, six had small interaction effects, as their PCA scores were nearly zero (-0.59 to 0.46) (Table 26; Fig. 9). The high grain Fe in the inbred line AIMP 92901 S1-15-1-2-B (No. 4) (84.5 mg kg^{-1}) and a seed parent 863B (No. 3) (75.1 mg kg^{-1}) was due to larger main effects and smaller interaction effects as these two had PCA scores nearly zero (-0.07 to -0.59). Similar were the case with other high Fe content lines, ICMB 88004 (No. 13) (62.2 mg kg^{-1}), IPC 843 (No. 21) (59.5 mg kg^{-1}), 843B (No. 2) (59.4 mg kg^{-1}) and ICMS 7704-S1-51-4-1-1-B (No. 17) (56.0 mg kg^{-1}). Six more entries (of the 14) with high positive PCA scores, that clustered in quadrant I, had higher grain Fe content (54.2–70.1 mg kg^{-1}), but had larger interaction effects (-2.76 to 2.78) suggesting that they perform well in environments such as E_4 (4.2) and E_2 (0.71). The same six entries (having positive PCA scores) have opposite performance especially in E_1 (-4.7) as that had negative PCA score. On the contrary, two entries, SDMV 90031 S1 84-1-1-2-B (60.1 mg kg^{-1}) and ICMB 00888 (54.2 mg kg^{-1}) had negative PCA scores (-1.70 to -2.76). The lines ICMB 00999 (63.7 mg kg^{-1}) and SDMV 90031 S1 84-1-1-2-B (60.1 mg kg^{-1}), had almost same main additive effects but had contrasting interaction effects as indicated by their positive

Table 26. Mean and first principle component axis scores (PCA I) for grain Fe and Zn content and days to 50% flower (AMMI model) in stability trials, summer and rainy seasons of 2004 and 2005, ICRISAT-Patancheru.

Trt. No.	Class	Entry	Fe content (mg kg^{-1})		Zn content (mg kg^{-1})		Days to 50% flower	
			Mean	PCA 1	Mean	PCA 1	Mean	PCA 1
1	L	81B	36.5	-0.76	30.2	1.18	56	1.57
2	H	843B	59.4	0.46	55.3	-0.98	45	0.88
3	H	863B	75.1	-0.59	53.9	0.49	47	-0.22
4	H	AIMP 92901 S1-15-1-2-B	84.5	-0.07	62.6	0.32	46	-1.16
5	H	AIMP 92901 S1-183-2-2-B	70.1	1.40	51.4	-0.69	48	0.37
6	M	CZI 96-21	43.9	0.02	38.7	0.04	45	-0.04
7	M	CZI-98-11	44.3	0.10	39.5	0.06	46	0.07
8	H	EEBC	66.2	1.99	51.1	0.10	38	1.70
9	H	GB 8735	67.2	1.24	49.8	-0.51	45	0.63
10	L	HTP 94/54 (HHB 146)	36.6	-1.75	31.4	1.39	55	0.26
11	H	ICMB 00888	54.2	-2.76	45.5	-2.47	48	-0.11
12	H	ICMB 00999	63.7	2.78	55.1	-1.00	51	-0.22
13	H	ICMB 88004	62.2	0.30	49.0	-0.98	50	-0.11
14	L	ICMB 90111	39.9	-0.38	26.4	1.13	54	-0.21
15	H	ICMB 94111	66.2	1.14	49.9	-1.90	53	0.76
16	H	ICMB 98222	68.1	1.97	49.3	-0.27	48	0.51
17	H	ICMS 7704-S1-51-4-1-1-B	56.0	0.03	52.1	-1.59	48	-0.80
18	L	ICMS 8511 S1-17-2-1-1-B	31.2	-1.42	22.7	1.79	58	-0.95
19	M	ICMV 91059 S1-58-2-2-2-B	47.6	0.23	39.8	0.37	57	-1.20
20	M	ICMV 93074 S1-9-1-1-1-B	47.4	-0.14	41.9	0.34	57	-0.33
21	H	IPC 843	59.5	0.30	53.8	0.61	54	-1.08
22	M	Jakharana pop	47.3	0.16	41.6	0.83	44	0.14
23	M	MC 94 C2-S1-46-1-1-B	46.5	-0.32	37.9	1.14	50	0.53
24	M	NCD2 Bulk	42.5	-0.66	39.6	0.31	51	0.10
25	H	SDMV 90031-S1-84-1-1-2-B	60.1	-1.70	44.2	0.91	59	-1.60
26	L	SDMV 90031-S1-93-3-1-1-B	38.4	0.61	30.0	1.00	54	0.10
27	M	SDMV 93032-S1-93-3-2-2	44.8	-0.26	38.7	-0.26	54	-0.22
28	L	SOSAT C88	34.1	-1.35	31.5	0.98	55	0.39
29	Check	WCC 75	44.9	-0.58	37.1	0.65	50	0.26

H; high, M; medium and L; low for grain Fe and Zn content.

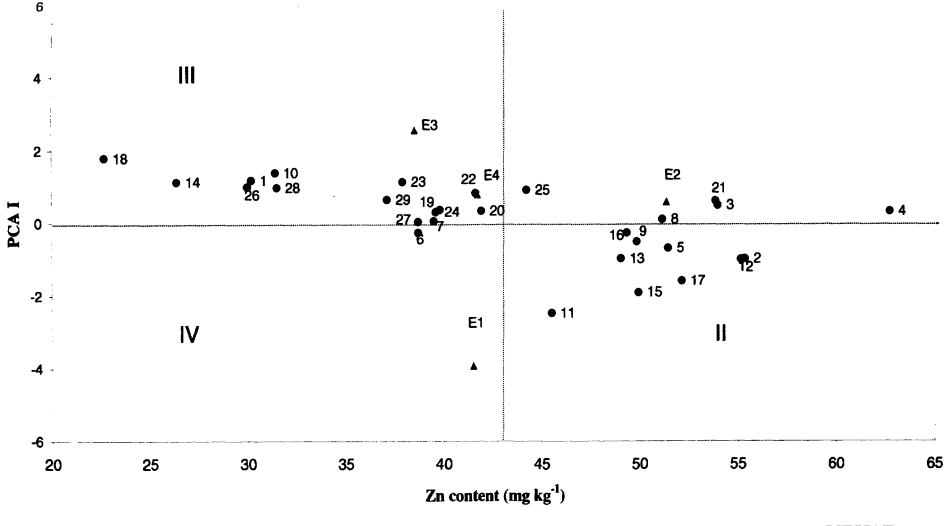


Fig 10. AMMI biplot graph between mean grain Zn content and PCA I scores of 29 entries and four environments, ICRISAT-Patancheru. (E1: summer 2004; E2: rainy 2004; E3: summer 2005; E4: rainy 2005 and the list of 29 entries given in Table 26)

(2.78) and negative PCA (-1.70) scores deviating from zero suggesting differences in their performances in contrasting environments such as E_1 and E_4 environments respectively. Most of the entries (except three) with medium and low Fe content (smaller main effects) also had smaller interaction effects as revealed by their PCA scores nearly zero (-0.76 to 0.61). The environments E_4 (4.20) and E_2 (0.71) had the high positive environmental effects (PCA I), indicating favorable site for better expression of grain Fe, whereas E_1 (-4.74) and E_3 (-0.16) had the negative PCA effect.

Of the 14 entries with high Zn content (higher main effects) occupying in quadrant I and II, seven had smaller interaction effects as revealed by their PCA score nearing zero (Table 26 and Fig. 10). The high grain Zn content in inbred line AIMP 92901 S1-15-1-2-B (62.6 mg kg^{-1}), 863B (53.9 mg kg^{-1}) and IPC 843 (53.8 mg kg^{-1}) were due to larger main effects and smaller interaction effects as these had PCA scores nearly zero (0.32–0.61). Similar were the case with other entries such as EEBC (51.1 mg kg^{-1}), AIMP 92901 S1-183-2-2-B (51.4 mg kg^{-1}), GB 8735 (49.8 mg kg^{-1}) and ICMB 98222 (49.3 mg kg^{-1}) which had high Zn content with smaller interaction effects (-0.69 to 0.10). Six entries (in quadrant II) had higher main effects but larger interaction effects as indicated by their high negative PCA scores indicating that these entries perform well in the environment like E_1, which had negative PCA score (-3.92), but not in the environments with positive PCA score. On the contrary, an inbred line SDMV 90031 S1 84-1-1-2-B (44.2 mg kg^{-1}) had higher main effect but larger positive interaction effect (0.91) indicating its ability to perform well in the environments like E_2 (0.58), E_3 (2.55) and E_4 (0.79) as these also had positive PCA score. Two seed parents, 843B (55.3 mg kg^{-1}) and ICMB 00999 (55.1 mg kg^{-1}) had higher main effects with larger interaction effects (-0.98 to −1.00). Most of the entries (except three) with medium and low Zn content (smaller main effects) also had smaller interaction effects as revealed by their PCA scores nearly zero. Of the 15 entries with medium and low Zn content (smaller main effects), 7 had smaller interaction effects (-0.26 to 0.37), whereas the remaining entries had higher interaction effects as revealed by high PCA scores. The environment E_3 (2.55)

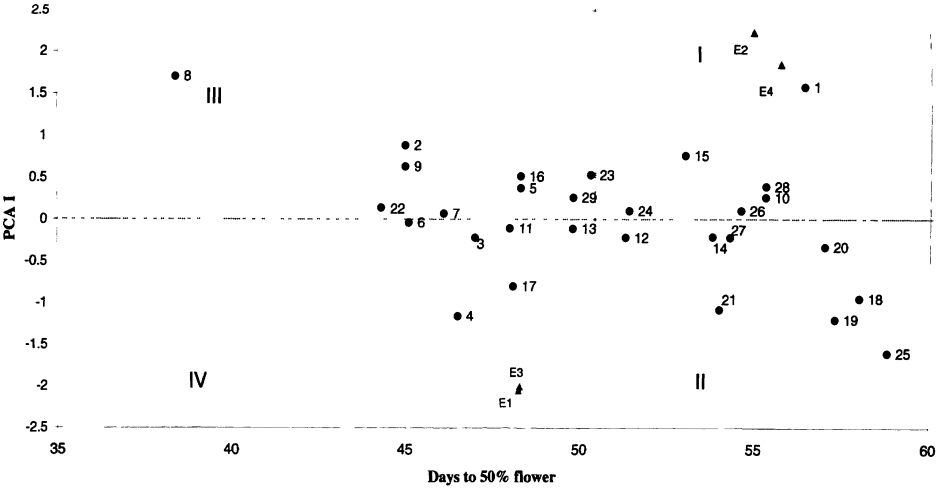


Fig 11. AMMI biplot graph between mean days to 50% flower and PCA I scores of 29 entries and four environments, ICRISAT-Patancheru. (E1: summer 2004; E2: rainy 2004; E3: summer 2005; E4: rainy 2005 and the list of 29 entries given in Table 26)

had the high positive environmental effect, followed by E_4 (0.79) and E_2 (0.58), while E_3 had the main negative effect (-3.92).

For days to 50% flower, the entries had varying main effects but most of them (except seven) had smaller interaction effects (Table 26 and Fig. 11). The summer season environments had negative PCA score and rainy season environments had positive PCA scores both deviating from zero suggesting larger interaction effects. Most of the entries with high Fe and Zn content also showed stability in flowering behavior with their PCA scores nearly zero. Among the high grain Fe and Zn entries, 863B, ICMB 00888 and ICMB 88004 were early-maturing (47–50 days) with PCA I approaching to zero (-0.11 to -0.22), whereas the entries, 843B and GB 8735 were also early-flowering (45 days) but with PCA score deviating from zero (0.63–0.88). Since entries × environments interaction were non-significant for 1000-grain mass, the PCA values were not calculated.

4.6. INHERITANCE STUDIES

A full diallel set of crosses was made among 10 inbred lines that were chosen from the set I trial with four high lines (Fe: 60.4–75.7 mg kg^{-1} and Zn: 55.8–64.8 mg kg^{-1}) and three each of medium (Fe: 41.8–44.8 mg kg^{-1} and Zn: 40.9–47.1 mg kg^{-1}) and low lines (Fe: 30.1–36.3 mg kg^{-1} and Zn: 24.5–33.5 mg kg^{-1}) for Fe and Zn content (Table 27). The 10 parental lines and their 90 F_1 hybrids that included reciprocals were evaluated for grain Fe and Zn content, days to 50% flower and 1000-grain mass during rainy season 2005. The data obtained on the mean performance of entries (parents and hybrids) for the above mentioned traits were analyzed statistically as per randomized complete block design and the diallel analysis was carried out according to Hayman's (1954) and Griffing's (1956) approaches.

4.6.1. Hayman's approach of diallel analysis

Hayman's (1954) numerical and graphical analysis was conducted to understand the nature of inheritance of Fe, Zn, days to 50% flowering and 1000-grain mass.

Table 27. Parental lines used in diallel cross with their levels of grain Fe and Zn content selected from set I trial, 2004 summer and rainy seasons, ICRISAT - Patancheru.

Sl. No.	Inbred line	Class	Fe content (mg kg^{-1})			Zn content (mg kg^{-1})		
			Summer 2004	Rainy 2004	Mean	Summer 2004	Rainy 2004	Mean
1	863B	High	69.0	76.5	72.7	54.0	57.7	55.8
2	ICMB 94111	High	51.7	75.4	63.6	43.7	69.9	56.8
3	ICMB 00888	High	56.7	64.1	60.4	49.3	64.1	56.7
4	AIMP 92901 S1-15-1-2-B	High	74.7	76.6	75.7	61.6	68.0	64.8
5	ICMB 95222	Medium	35.2	48.5	41.8	39.2	55.1	47.1
6	ICMV 93074 S1-9-1-1-1-B	Medium	40.3	46.7	43.5	43.4	47.8	45.6
7	MC 94 C2-S1-46-1-1-B	Medium	40.8	48.9	44.8	40.8	40.9	40.9
8	81B	Low	32.4	36.4	34.4	31.3	32.2	31.7
9	ICMS 8511 S1-17-2-1-1-B	Low	30.8	29.4	30.1	25.0	24.0	24.5
10	ICMV 91059 S1-14-2-4-2-2-B	Low	31.2	41.5	36.3	29.1	38.0	33.5

4.6.1.1. Test of assumptions for the additive-dominance model

Hayman's graphical approach of diallel analysis is based on additive-dominance model. Hence, the first requirement is to test the adequacy of this model by detecting whether non-allelic interaction (epistasis) is present or absent. The model provides different statistics to test the hypothesis. The validity of the additive-dominance model was satisfied as the uniformity test (t^2) was non-significant for grain Fe (t^2 = 0.003) and Zn (t^2 = 0.001), days to 50% flower (t^2 = 0.460) and 1000-grain mass (t^2 = 3.250), indicating the absence of epistasis (Table 28 and 29). The regression of parent-offspring co-variance (Wr) on parental variance (Vr) was significantly different from zero and approaching to unity for grain Fe (0.937±0.123), Zn content (0.921±0.01), 1000-grain mass (0.933±0.159) and days to 50% flower (0.963±0.090), again indicating the absence of non-allelic interaction. The estimated values of Wr, Vr for each of the replications were used to derive (Wr+Vr) and (Wr-Vr) which were analyzed to partition the total variation among those into those due to arrays and due to replicate blocks. The variation among arrays for (Wr+Vr) and (Wr-Vr) were significant for all the traits, except 1000-grain mass as tested by the F' test indicating that either the additive-dominance model does not holds, or that its inadequacy results from non-independent distribution of genes among the parents (Table 30). However, the other tests showed the adequacy of the additive-dominance model.

4.6.1.2. Dominance relationship

When epistasis is non-operating, the additive or dominance gene action is tested by dominance relationship i.e., the overall difference between the hybrids and parental means averaged over all loci $(ML_1-ML_0)^2$. The $(ML_1-ML_0)^2$ values were around zero for grain Zn content (0.02), whereas for other traits it deviated from zero (0.65–8.41) (Table 28 and 29). To understand the nature or degree of dominance, the Wr-Vr graph was constructed.

Table 28. Co-variance (Wr) and variance (Vr) of arrays for grain Fe and Zn content in 10 × 10 diallel trial, rainy season 2005, ICRISAT- Patancheru.

Sl. No.	Parent	Fe content (mg kg^{-1})					Zn content (mg kg^{-1})				
		Wr	Vr	Wr-Vr	Wr+Vr	Yr	Wr	Vr	Wr-Vr	Wr+Vr	Yr
1	863B	103.2	69.8	33.4	173.1	69.9	19.9	19.6	0.3	39.5	41.5
2	ICMB 94111	123.1	101.7	21.5	224.8	68.9	22.2	21.1	1.1	43.3	40.0
3	ICMB 00888	91.3	52.2	39.1	143.5	65.8	24.4	18.8	5.6	43.2	41.4
4	AIMP 92901 S1-15-1-2-B	98.2	65.0	33.3	163.2	68.6	26.5	27.7	-1.3	54.2	40.8
5	ICMB 95222	76.4	40.2	36.2	116.6	47.9	25.5	15.7	9.8	41.3	34.4
6	ICMV 93074 S1-9-1-1-1-B	98.0	81.8	16.2	179.9	54.4	26.7	18.0	8.7	44.7	33.8
7	MC 94 C2-S1-46-1-1-B	70.5	47.3	23.2	117.9	49.1	24.3	16.1	8.2	40.4	31.7
8	81B	75.6	40.3	35.4	115.9	32.2	21.8	14.6	7.2	36.4	25.4
9	ICMS 8511 S1-17-2-1-1-B	36.8	19.5	17.3	56.3	38.0	9.0	7.0	2.0	16.0	27.9
10	ICMV 91059 S1-14-2-4-2-2-B	82.9	54.3	28.6	137.2	42.9	30.2	24.7	5.5	54.9	22.5
	Mean	85.6	57.2	28.4	142.8	53.8	23.0	18.3	4.7	41.4	33.9
	t^2	0.003**					0.001**				
	r(Wr+Vr:Yr)	0.755**					0.412*				
	$(ML_1-ML_0)^2$	8.41					0.02				
	$b_{Wr:Vr}$ (H_0=1)	0.937±0.123					0.921±0.01				

* Significant at 5% and 1% probability levels, respectively.

Table 29. Co-variance (Wr) and variance (Vr) of arrays for days to 50% flower and 1000-grain mass in 10 × 10 diallel trial, rainy season 2005, ICRISAT - Patancheru.

Sl. No.	Parent	Days to 50% flower					1000-grain mass (g)				
		Wr	Vr	Wr-Vr	Wr+Vr	Yr	Wr	Vr	Wr-Vr	Wr+Vr	Yr
1	863B	3.7	4.1	-0.4	7.8	51.7	0.7	0.6	-0.8	0.3	12.4
2	ICMB 94111	2.7	3.2	-0.5	5.9	49.3	1.8	1.8	0.0	3.5	12.7
3	ICMB 00888	2.9	4.6	-1.7	7.4	44.0	0.8	1.1	-0.4	1.7	12.7
4	AIMP 92901 S1-15-1-2-B	4.4	6.4	-2.0	10.8	48.0	1.1	1.0	0.1	2.2	10.9
5	ICMB 95222	9.3	9.8	-0.5	19.2	53.7	1.5	1.4	0.1	2.9	9.8
6	ICMV 93074 S1-9-1-1-1-B	8.1	8.9	-0.8	17.0	51.7	1.4	1.5	-0.1	2.9	9.6
7	MC 94 C2-S1-46-1-1-B	1.6	2.2	-0.1	4.2	47.0	1.5	1.6	-0.1	3.1	10.9
8	81B	1.8	2.1	0.5	4.7	53.0	2.5	2.8	-0.3	5.3	8.1
9	ICMS 8511 S1-17-2-1-1-B	5.5	7.0	-1.5	12.4	54.3	2.0	1.8	0.2	3.8	9.4
10	ICMV 91059 S1-14-2-4-2-2-B	8.0	8.6	-0.5	16.6	52.3	1.9	2.1	-0.2	3.9	8.8
	Mean	4.9	5.7	-0.8	10.6	50.5	1.4	1.5	-0.2	3.0	10.5
	t^2	0.460					3.250				
	r(Wr+Vr:Yr)	0.506**					-0.735**				
	$(ML_1-ML_0)^2$	9.78					0.65				
	$b_{Wr:Vr}$ (H_0=1)	0.963±0.090					0.933±0.159				

** Significant at 5% and 1% probability levels, respectively.

Table 30. Analysis of variance of Wr, Vr estimates in a 10 × 10 diallel set of crosses for grain Fe and Zn content, days to 50% flower and 1000-grain mass, rainy season 2005, ICRISAT- Patancheru.

Character	Source	df	Mean square
Fe content (mg kg^{-1})	Vr array differences	9	12.53
	Wr array differences	9	6.58
	(Vr + Wr) array differences	9	133.19**
	(Vr +Wr) block differences	10	2.98
	(Wr - Vr) array differences	9	488.40**
	(Wr - Vr) block differences	10	8.55
Zn content (mg kg^{-1})	Vr array differences	9	8.31
	Wr array differences	9	3.54
	(Vr + Wr) array differences	9	30.17**
	(Vr + Wr) block differences	10	2.98
	(Wr - Vr) array differences	9	225.66**
	(Wr - Vr) block differences	10	6.40
Days to 50% flower	Vr array differences	9	2.93
	Wr array differences	9	2.65
	(Vr + Wr) array differences	9	1.24**
	(Vr + Wr) block differences	10	0.01
	(Wr - Vr) array differences	9	59.70**
	(Wr - Vr) block differences	10	0.38
1000-grain mass (g)	Vr array differences	9	0.47
	Wr array differences	9	1.28
	(Vr + Wr) array differences	9	0.22
	(Vr + Wr) block differences	10	0.08
	(Wr - Vr) array differences	9	3.64
	(Wr - Vr) block differences	10	0.12

** significant at 1% probability level.

4.6.1.3. Degree of dominance

To understand the average level of dominance the Wr-Vr graph was constructed. The Wr-Vr graph for grain Fe and Zn content, and 1000-grain mass showed the regression line intercepting above the origin on the covariance axis (Figs. 12, 13, 15), indicating partial dominance for these traits. However, for days to 50% flower, the regression line intercepted slightly below the origin on the covariance axis (Fig. 14), implying a small degree of over-dominance, which could result also from non-independent distribution of genes in the parents (in repulsion phase).

4.6.1.4. Parental order of dominance

The distribution of array points in the Wr-Vr graph indicates the parental order of dominance; the parents near to the origin supposed to have maximum dominant alleles and those on the other end having maximum number of recessive alleles. The scattered array points on regression line indicated diversity among the parents for all the four traits studied. Based on distribution of array points on Wr-Vr graph, the parent ICMS 8511-S1-17-2-1-1-B occupied the place nearby the origin (Fig. 12 and 13). However, the parent ICMB 94111 for Fe and parents AIMP 92901 S1-15-1-2-B and ICMV 91059 S1 14-2-4-2-2-B for Zn content were far away from the origin and the remaining parents occupied intermediate position. Same results as observed from Wr-Vr graphs for Fe and Zn were obtained, when parental order of dominance was examined using the size of (Wr+Vr) values, which is inversely proportional to the parental order of dominance (Table 28). Parents such as ICMB 94111, ICMV 93074 S1-9-1-1-B, 863B and AIMP 92901 S1-15-1-2-B for Fe and the parents, AIMP 92901 S1-15-1-2-B and ICMV 91059 S1-14-2-4-2-2-B for Zn content had high (Wr+Vr) values. However, the parent ICMS 8511 S1-17-2-1-1-B had low (Wr+Vr) values for both grain Fe and Zn content.

For 1000-grain mass, the parent 863B occupied a place nearby the origin, whereas the parent 81B was clustered away from the origin and the remaining parents occupied intermediate positions (Fig. 15). For days to 50% flower the parents, MC 94 C2-S1-46-1-1-B and 81B occupied place near to the origin in Wr-Vr graph and the parents,

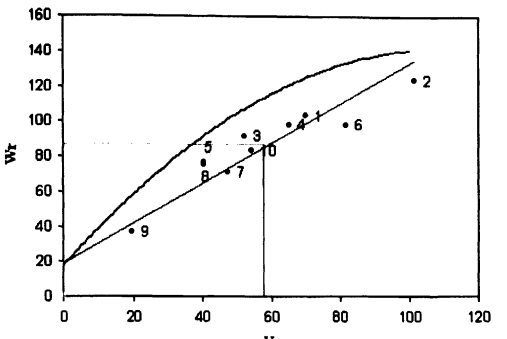


Fig 12. Wr-Vr graph for grain Fe content (mg kg^{-1}) in 10 x 10 diallel trial, rainy season 2005, ICRISAT- Patancheru. (Array points, 1: 863B, 2: ICMB 94111, 3: ICMB 00888, 4: AIMP 92901 S1-15-1-2-B, 5: ICMB 95222, 6: ICMV 93074 S1-9-1-1-1-B, 7: MC 94 C2-S1-46-1-1-B, 8: 81B, 9: ICMS 8511 S1-17-2-1-1-B and 10: ICMV 91059 S1-14-2-4-2-2-B).

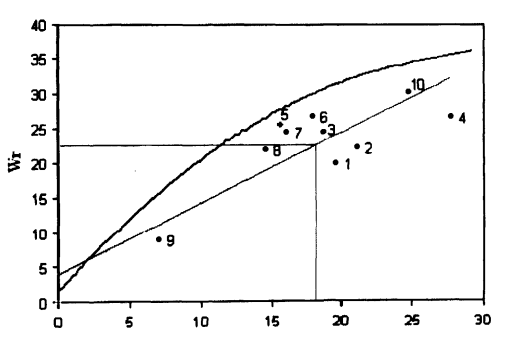


Fig 13. Wr-Vr graph for grain Zn content (mg kg^{-1}) in 10 x 10 diallel trial, rainy season 2005, ICRISAT- Patancheru. (Array points, 1: 863B, 2: ICMB 94111, 3: ICMB 00888, 4: AIMP 92901 S1-15-1-2-B, 5: ICMB 95222, 6: ICMV 93074 S1-9-1-1-1-B, 7: MC 94 C2-S1-46-1-1-B, 8: 81B, 9: ICMS 8511 S1-17-2-1-1-B and 10: ICMV 91059 S1-14-2-4-2-2-B).

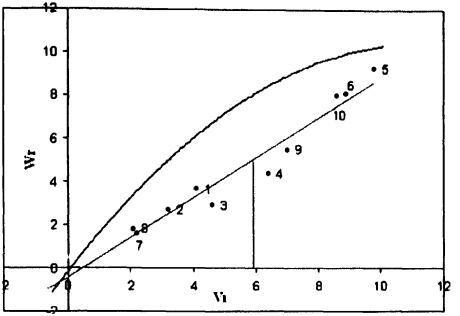


Fig 14. Wr-Vr graph for days to 50% flower in 10 × 10 diallel trial, rainy season 2005, ICRISAT- Patancheru. (Array points, 1: 863B, 2: ICMB 94111, 3: ICMB 00888, 4: AIMP 92901 S1-15-1-2-B, 5: ICMB 95222, 6: ICMV 93074 S1-9-1-1-1-B, 7: MC 94 C2-S1-46-1-1-B, 8: 81B, 9: ICMS 8511 S1-17-2-1-1-B and 10: ICMV 91059 S1-14-2-4-2-2-B).

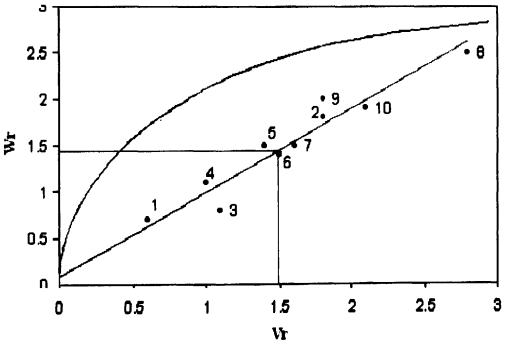


Fig 15. Wr-Vr graph for 1000-grain mass (g) in 10 × 10 diallel trial, rainy season 2005, ICRISAT- Patancheru. (Array points, 1: 863B, 2: ICMB 94111, 3: ICMB 00888, 4: AIMP 92901 S1-15-1-2-B, 5: ICMB 95222, 6: ICMV 93074 S1-9-1-1-1-B, 7: MC 94 C2-S1-46-1-1-B, 8: 81B, 9: ICMS 8511 S1-17-2-1-1-B and 10: ICMV 91059 S1-14-2-4-2-2-B).

ICMB 95222 and ICMV 93074 S1-9-1-1-1-B belonged to high Wr-Vr group, whereas the remaining array points occupied intermediate positions (Fig. 14). Same results as observed from Wr-Vr graphs for 1000-grain mass and days to 50% flowering were obtained, when parental order of dominance was examined using the size of Wr+Vr values, which is inversely proportional to the parental order of dominance (Table 29).

An examination of the correlation ($r_{Yr,(Wr+Vr)}$) between parental measurement, Yr, and the parental order of dominance (Wr+Vr), revealed positive significant association for grain Fe (r=0.755; P <0.01) and Zn content (r=0.412; P <0.01), and days to 50% flower (r=0.506; P <0.01), whereas highly significant negative association for 1000-grain mass (r= -0.735; P <0.01). (Table 28 and 29). The standardized deviation graph of Yr'-(Wr+Vr)' for grain Fe content showed that, the four high Fe parents (1–4) had positive Yr' and (Wr+Vr)' values (Fig. 16). On the contrary low Fe parents (8–10) had negative Yr' and (Wr+Vr)' values. For grain Zn content, the three high parents (ICMB 94111, ICMB 00888 and AIMP 92901 S1-15-1-2-B) had positive Yr' and (Wr+Vr)' values (Fig. 17). One parent had positive (Wr+Vr)' value but negative Yr' value. Of the three low parents, two parents (81B and ICMS 8511 S1-17-1-1-B) having negative Yr' and (Wr+Vr)' values.

For days to 50% flower, the medium to low Fe and Zn parents, ICMB 95222, ICMV 93074 S1-9-1-1-1-B, ICMV 91059 S1-14-2-4-2-2-B and ICMS 8511 S1-17-1-1-B which were in general late flowering had positive Yr' and (Wr+Vr)' values (Fig. 18). The two high (ICMB 00888 and ICMB 94111) and one medium (MC 94 C2-S1-46-1-1-B) Fe and Zn parents had Yr' and (Wr+Vr)' values in negative direction. For 1000-grain mass, of the four high Fe and Zn parents, three had large seed size with positive (Wr+Vr)' values but negative Yr' values (Fig. 19). The three low Fe and Zn parents with smaller seed size had negative Yr' and (Wr+Vr)' values.

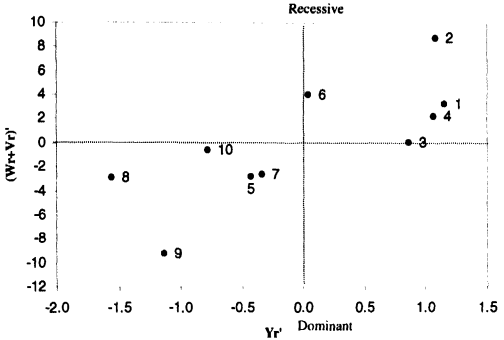


Fig 16. Standardized deviations (Yr: Wr+Vr) graph for grain Fe content in 10 × 10 diallel trial, rainy season 2005, ICRISAT-Patancheru (Array points, 1: 863B, 2: ICMB 94111, 3: ICMB 00888, 4: AIMP 92901 S1-15-1-2-B, 5: ICMB 95222, 6: ICMV 93074 S1-9-1-1-1-B, 7: MC 94 C2-S1-46-1-1-B, 8: 81B, 9: ICMS 8511 S1-17-2-1-1-B and 10: ICMV 91059 S1-14-2-4-2-2-B).

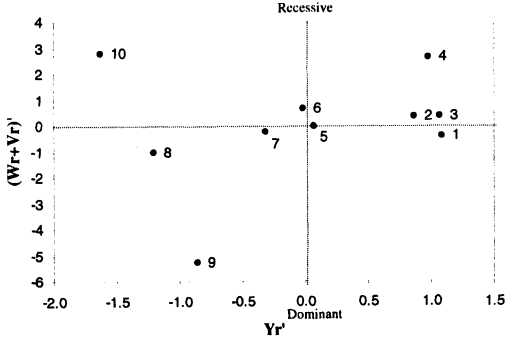


Fig 17. Standardized deviations (Yr: Wr+Vr) graph for grain Zn content in 10 × 10 diallel trial, rainy season 2005, ICRISAT-Patancheru (Array points, 1: 863B, 2: ICMB 94111, 3: ICMB 00888, 4: AIMP 92901 S1-15-1-2-B, 5: ICMB 95222, 6: ICMV 93074 S1-9-1-1-1-B, 7: MC 94 C2-S1-46-1-1-B, 8: 81B, 9: ICMS 8511 S1-17-2-1-1-B and 10: ICMV 91059 S1-14-2-4-2-2-B).

4.6.2. Estimates of genetic component of variation (Hayman's numerical approach)

Under the additive-dominance genetic model, the total sum of squares was partitioned into additive (D) and non-additive (H_1) genetic components. Analysis of variance showed significant additive and non-additive genetic variances for all the traits studied (Table 31). The proportion of additive variance component was higher (183.75±6.16; P <0.05 for Fe; 45.67±2.90; P <0.05 for Zn; 3.09±0.47; P <0.05 for 1000-grain mass) than non-additive component for grain Fe (51.79±13.11; P <0.05) and Zn (20.63±6.16; P <0.05), and for 1000-grain mass (2.69±0.22; P<0.05). However, the additive component of variance for days to 50% flower (9.63±0.54; P <0.05) was slightly lower than non-additive component (10.20±1.16; P <0.05 for days to flower).

The proportion of positive and negative genes (H_2) was significant for all the four traits and near-equality of H_1 and H_2 for grain Zn and days to 50% flower. However, the non-significant F statistic (mean co-variance of additive and non-additive components) was observed for these traits. For grain Fe, the size of H_1 (51.79±13.11; P <0.05) and H_2 (38.58±11.14; P <0.05) differed ($H_1 > H_2$) with the positive significant F statistic (29.09±14.21; P <0.05). In case of 1000-grain mass H_2 was more than H_1, with the negative non-significant F statistic (-0.18±0.51). The overall dominance effect (h^2) was significant for grain Fe (29.99±14.21; P <0.05), days to 50% flower (38.52±0.66; P <0.05) and 1000-grain mass (2.54±0.27; P <0.05), but it was not significant for grain Zn content. The K estimate (h^2/H_2), the number of effective factors or blocks with dominant genes was less than unity for all the traits (0.07 to 0.91) except for days to 50% flower (3.99). The mean degree of dominance $\sqrt{\frac{H_1}{D}}$ was less than unity for grain Fe (0.53) and Zn content (0.67), and for 1000-grain mass (0.93), whereas it was more than unity for days to 50% flower (1.03). The proportion of dominant and recessive genes was ascertained by the ratio of $\{[\sqrt{\langle 4DH_1 \rangle}+F)/\ [\sqrt{\langle 4DH_1 \rangle}-F)\}$, which was around unity for grain Zn content and days to 50% flower (both 1.01). However, this ratio was more than one in case of grain Fe content. For 1000-grain mass the ratio of proportion of dominant and recessive alleles was less than one. Significant environmental component (E) was

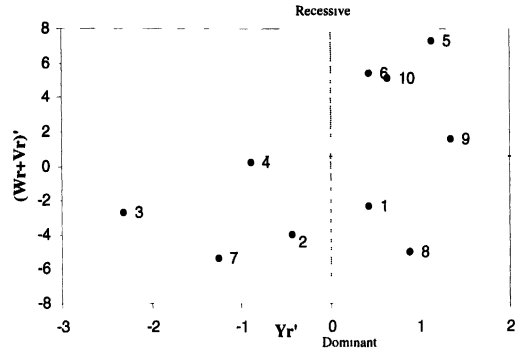
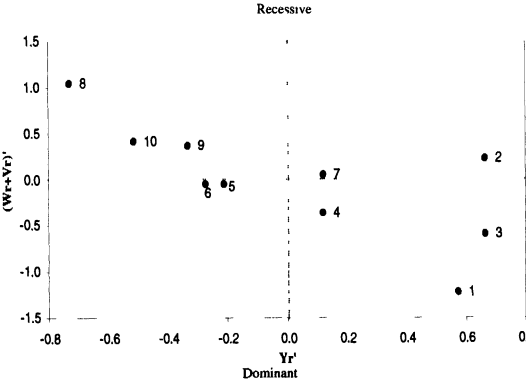


Fig 18. **Standardized deviations (Yr: Wr+Vr) graph for days to 50% flower in 10 x 10 diallel trial, rainy season 2005, ICRISAT-Patancheru** (Array points, 1 863B, 2. ICMB 94111, 3: ICMB 00888, 4: AIMP 92901 S1-15-1-2-B, 5: ICMB 95222, 6: ICMV 93074 S1-9-1-1-1-B, 7 MC 94 C2-S1-46-1-1-B, 8: 81B, 9: ICMS 8511 S1-17-2-1-1-B and 10: ICMV 91059 S1-14-2-4-2-2-B)



g 19. **Standardized deviations (Yr: Wr+Vr) graph for days to 1000-grain mass in 10 x 10 diallel trial, rainy season 2005, ICRISAT-Patancheru** (Array points, 1: 863B, 2: ICMB 94111, 3: ICMB 00888, 4: AIMP 92901 S1-15-1-2-B, 5: ICMB 95222, 6: ICMV 93074 S1-9-1-1-1-B, 7: MC 94 C2-S1-46-1-1-B, 8: 81B, 9: ICMS 8511 S1-17-2-1-1-B and 10: ICMV 91059 S1-14-2-4-2-2-B).

observed for grain Fe (10.17±1.86; P <0.05) and Zn content (3.46±0.87; P <0.05), and days to 50% flower (1.39±0.16; P <0.05), which was much lower than the additive (D) and non-additive (H_1) components. The narrow-sense heritability (h^2_n), which was obtained from the ratio of additive variance to total phenotypic variation were in higher magnitude for grain Fe (80%) and Zn content (77%) and 1000-grain mass (67%). Whereas, relatively lower level of narrow-sense heritability was observed for days to 50% flower (56%).

4.6.3. Analysis of variance for combining ability

The analysis of variance showed existence of significant variation among parents, among crosses, among direct and reciprocal F_1's for all the traits (Table 32). Of the two single degree of freedom comparison, the mean squares due to parents *Vs* crosses were highly significant for all the traits except grain Zn (0.02). Mean squares due to direct and reciprocal crosses (another single degree of freedom comparison) were non-significant for both grain Fe and Zn content suggesting no considerable reciprocal differences, whereas it was significant for days to 50% flower and 1000-grain mass indicating the presence of considerable reciprocal differences. The analysis of variance for combining ability was subsequently estimated. The mean square due to general combining ability (GCA) effects of parents and specific combining ability (SCA) effects of crosses showed significant differences for all the four traits. Reciprocal effects were statistically non-significant for grain Fe and Zn content; however, the reciprocal effects were highly significant for days to 50% flower and 1000-grain mass. Since the reciprocal differences were non-significant for grain Fe and Zn content, the half-diallel analysis of Griffing's (Method II and Model II) was done. The predictability ratio measured by $2\sigma^2gca/(2\sigma^2gca + \sigma^2sca)$ was 0.89 for both grain Fe and Zn content, and 0.78 for 1000-grain mass, whereas the ratio was deviating more from unity for days to 50% flower (0.62).

Table 31. Estimates of genetic parameters for grain Fe and Zn content, days to 50% flower and 1000-grain mass in 10 × 10 diallel trial, rainy season 2005, ICRISAT- Patancheru.

Statistic	Fe content (mg kg^{-1})	Zn content (mg kg^{-1})	Days to 50% flower	1000-grain mass (g)
D	183.75±6.16*	45.67±2.90*	9.63±0.54*	3.09±0.47*
H_1	51.79±13.11*	20.63±6.16*	10.20±1.16*	2.69±0.22*
H_2	38.58±11.14*	15.38±5.24*	9.66±0.98*	2.80±0.40*
F	29.09±14.21*	0.56±6.68	0.12±1.25	-0.18±0.51
h^2	29.99±7.46*	1.15±3.51	38.52±0.66*	2.54±0.27*
E (Comp.)	10.17±1.86*	3.46±0.87*	1.39±0.16*	0.12±0.07
$\sqrt{\frac{H_1}{D}}$	0.53	0.67	1.03	0.93
K (h^2/H_2)	0.78	0.07	3.99	0.91
$[\sqrt{(4DH_1)}+F)/ [\sqrt{(4DH_1)}-F)]$	1.18	1.01	1.01	0.94
h^2_n	80	77	56	67

* Significant at 5% probability level.

Table 32. Analysis of variance of combining ability estimates for grain Fe and Zn content, days to 50% flower and 1000-grain mass in 10 × 10 diallel trial, rainy season 2005, ICRISAT - Patancheru.

Source	df	Mean squares			
		Fe content (mg kg^{-1})	Zn content (mg kg^{-1})	Days to 50% flower	1000-grain mass (g)
Diallel entries	99	272.97**	94.22**	23.08**	7.83**
Parents	9	581.74**	143.65**	33.06**	8.44**
Parents *vs* Crosses	1	342.26**	0.02	255.15**	21.50**
Crosses	89	240.97**	90.28**	19.47**	7.62**
F1's	44	243.16**	80.34**	18.32**	7.33**
Reciprocals	44	243.56**	102.20**	20.10**	7.98**
F1 *vs* Reciprocals	1	30.81	2.50	42.40**	4.85**
Error	198	30.34	10.87	3.71	0.34
Total	299	110.75	38.88	10.38	2.84
Combining ability estimates					
GCA	9	834.94**	278.97**	50.37**	15.90**
SCA	45	20.62**	7.06**	4.25**	1.52**
Reciprocal	45	12.57	6.24	2.61**	1.04**
Error	198	10.11	3.62	1.24	0.11
σ^2_g		41.24	13.77	2.46	1.41
σ^2_s		10.51	3.44	3.01	0.79
$2\sigma^2_g/(2\sigma^2_g+\sigma^2_s)$		0.89	0.89	0.62	0.78

** Significant at 5% and 1% probability levels, respectively.

4.6.4. Mean performance of parents and hybrids

4.6.4.1. Grain Fe content

Grain Fe content among parents ranged from 32.2 (81B) to 69.9 mg kg^{-1} (863B) and among the hybrids it ranged from 37.7 to 70.0 mg kg^{-1} (Table 33). The high parents for Fe identified from the previous studies had higher Fe content of 65.8 to 69.9 mg kg^{-1} than medium parents (40.5 to 49.1 mg kg^{-1}) and low parents (32.2 to 42.9 mg kg^{-1}). All the high × high hybrids had >60 mg kg^{-1} grain Fe content.

4.6.4.2. Grain Zn content

For grain Zn content, the parental mean ranged from 22.5 (ICMS 8511 S1-17-2-1-1-B) to 41.5 mg kg^{-1} (863B) and the hybrid mean varied from 25.0 to 44.4 mg kg^{-1} (Table 34). The high parents identified from the previous study had higher Zn content (40.0 to 41.5 mg kg^{-1}) compared to the medium parents (31.7 to 34.4 mg kg^{-1}) and low parents (22.5 to 29.0 mg kg^{-1}) Zn content. All the high × high hybrids had >40 mg kg^{-1} of grain Zn content.

4.6.4.3. Days to 50% flower

The time to flower among parents ranged from 44 (ICMB 00888) to 54 days (ICMS 8511 S1-17-2-1-1-B), whereas among hybrids it varied from 42 to 53 days with the mean of 47 days (Table 35). All the high parents and a medium parent flowered below 50 days, and all the low parents and two medium parents flowered above 50 days (52–54 days). The high × high Fe and Zn crosses were early flowering (42–48 days) compared to low × low Fe and Zn content crosses (49–52 days). Some of the early-flowering hybrids were 863B × ICMB 94111, ICMB 00888 × ICMV 93074 S1-9-1-1-1-B, ICMB 00888 × AIMP 92901-S1-15-1-2-B and ICMB 00888 × MC 94 C2-S1-46-1-1-B (42–43 days).

4.6.4.4. 1000-grain mass (g)

The 1000-grain mass in parents varied from 8.1 g (81B) to 13.1 g (863B) and the hybrid mean ranged between 8.3 and 14.7 g (Table 36). High Fe and Zn parents had higher 1000-grain mass (12.0–13.1 g) compared to medium (9.6–10.9 g) and low (8.1–9.4g) Fe and Zn parents. Twenty hybrids had >13.0 g 1000-grain mass and a hybrid, ICMB 94111 × 863B (14.7 g) had highest 1000-grain mass followed by ICMB 94111 ×

Table 33. Grain Fe content (mg kg^{-1}) in parents and hybrids (direct crosses) of 10 × 10 diallel trial, rainy season 2005, ICRISAT - Patancheru.

Parents/Hybrids	863B	ICMB 94111	ICMB 00888	AIMP 92901 S1-15-1-2-B	ICMB 95222	ICMV 93074 S1-9-1-1-1-B	MC 94 C2-S1-46-1-1-B	81B	ICMS 8511 S1-17-2-1-1-B	ICMV 91059 S1-14-2-4-2-2-B
863B	**69.9**	70.0	64.1	69.6	52.6	63.8	56.5	53.4	50.5	58.3
ICMB 94111		**68.9**	60.5	62.8	51.2	52.5	56.8	47.2	42.1	50.8
ICMB 00888			**65.8**	62.3	48.2	49.8	53.1	44.1	45.1	47.7
AIMP 92901 S1-15-1-2-B				**68.6**	53.3	56.0	53.7	48.5	47.9	51.1
ICMB 95222					**47.9**	40.8	47.8	37.7	38.9	39.0
ICMV 93074 S1-9-1-1-1-B						**40.5**	52.7	42.6	42.6	45.5
MC 94 C2-S1-46-1-1-B							**49.1**	39.4	38.0	55.9
81B								**32.2**	39.5	37.9
ICMS 8511 S1-17-2-1-1-B									**38.0**	38.9
ICMV 91059 S1-14-2-4-2-2-B										**42.9**

Diagonal values: parents: above diagonal: direct crosses.

Table 34. Grain Zn content (mg kg^{-1}) in parents and hybrids (direct crosses) of 10 × 10 diallel trial, rainy season 2005, ICRISAT - Patancheru.

Parents/Hybrids	863B	ICMB 94111	ICMB 00888	AIMP 92901 S1-15-1-2-B	ICMB 95222	ICMV 93074 S1-9-1-1-1-B	MC 94 C2-S1-46-1-1-B	81B	ICMS 8511 S1-17-2-1-1-B	ICMV 91059 S1-14-2-4-2-2-B
863B	**41.5**	43.8	42.3	44.4	36.4	42.2	35.1	37.5	36.0	39.5
ICMB 94111		**40.0**	38.3	38.4	37.3	35.8	35.5	32.7	27.5	32.3
ICMB 00888			**41.4**	44.3	34.0	37.7	36.1	34.0	31.8	31.5
AIMP 92901 S1-15-1-2-B				**40.8**	37.6	37.9	36.4	33.8	33.2	34.8
ICMB 95222					**34.4**	31.1	34.1	27.7	27.7	27.8
ICMV 93074 S1-9-1-1-1-B						**33.8**	34.5	30.8	31.2	32.3
MC 94 C2-S1-46-1-1-B							**31.7**	25.9	25.0	30.1
81B								**25.4**	28.4	27.7
ICMS 8511 S1-17-2-1-1-B									**22.5**	25.2
ICMV 91059 S1-14-2-4-2-2-B										**29.0**

Diagonal values: parents; above diagonal: direct crosses.

ICMB 00888 (14.3 g). The crosses with high × high, high × medium and high × low Fe and Zn content had larger seed size (10.2 to 14.7 g) compared to the crosses with low × low Fe and Zn (8.3 to 10.1 g).

4.6.5. General combining ability effects

The parents with high Fe and Zn content (863B, AIMP 92901 S1-15-1-2-B, ICMB 94111 and ICMB 00888) exhibited significant positive *gca* effects for both grain Fe (3.47–10.29; P <0.05) and Zn content (1.62–5.19; P <0.05) and the parents with low Fe and Zn content (-8.44 to -3.79; P <0.05 for Fe and -4.29 to -3.64; P <0.05 for Zn) had significant negative *gca* effects (Table 37). Among the medium parents, ICMB 95222 (-4.85; P <0.05) had significant negative *gca* effect for grain Fe and MC 94 C2-S1-46-1-1-B (-2.32; P <0.05) for Zn content.

For days to 50% flower, the parents with high Fe and Zn showed significant negative *gca* effects (-2.98 to -0.63) except 863B (-0.08), whereas the parents with low Fe and Zn showed positive significant *gca* effects (0.78–2.17; P <0.05). Among the parents with medium Fe and Zn, ICMB 95222 (1.34; P <0.05) and MC 94 C2-S146-1-1-B (-1.55; P <0.05) exhibited significant positive and negative *gca* effects respectively.

For 1000-grain mass all the parents showed non-significant *gca* effects except the parent with high Fe and Zn content 863B (1.27; P <0.05), that had positive significant *gca* effect and the parent with low Fe and Zn, ICMV 91059 S1-14-2-4-2-2-B (-1.40; P <0.05) had significant negative *gca* effect. The correlation co-efficients between mean performance of parents and *gca* effects were highly significant and positive for all the four traits (r= 0.93 to 0.96; P <0.01).

4.6.6. Specific combining ability effects

Five hybrids exhibited significant positive *sca* effects for grain Fe (4.62–9.54; P <0.05), and of these only two, 863B × ICMB 94111 (high × high) and 863B × ICMV 93074 S1-9-1-1-1-B (high × medium) had higher Fe content (70.0 and 63.8 mg kg^{-1}) (Table 38). One cross, ICMB 94111 × AIMP 92901 S1-15-1-2- B (62.8 mg kg^{-1}) exhibiting negative significant *sca* effect also had higher grain Fe content (62.8 mg kg^{-1}). Seven hybrids showed significant positive *sca* effect for Zn content (2.65–4.86; P <0.05),

Table 35. **Mean performance of days to 50% flower in parents and hybrids of 10 × 10 diallel trial, rainy season 2005, ICRISAT - Patancheru.**

Parents/Hybrids	863 B	ICMB 94111	ICMB 00888	AIMP 92901 S1-15-1-2-B	ICMB 95222	ICMV 93074 S1-9-1-1-1-B	MC 94 C2-S1-46-1-1-B	81 B	ICMS 8511 S1-17-2-1-1-B	ICMV 91059 S1-14-2-4-2-2-B
863 B	[illegible]	42	44	47	48	46	45	[illegible]	[illegible]	[illegible]
ICMB 94111	[illegible]	[illegible]	45	45	47	44	46	[illegible]	[illegible]	[illegible]
ICMB 00888	[illegible]	[illegible]	[illegible]	43	44	42	43	[illegible]	[illegible]	[illegible]
AIMP 92901 S1-15-1-2-B	[illegible]	[illegible]	[illegible]	[illegible]	46	45	47	[illegible]	[illegible]	[illegible]
ICMB 95222	48	48	48	52	[illegible]	[illegible]	[illegible]	51	51	52
ICMV 93074 S1-9-1-1-1-B	45	47	44	47	[illegible]	[illegible]	[illegible]	50	49	48
MC 94 C2-S1-46-1-1-B	46	44	44	43	[illegible]	[illegible]	[illegible]	48	47	45
81 B	[illegible]	[illegible]	[illegible]	[illegible]	48	50	49	[illegible]	[illegible]	[illegible]
ICMS 8511 S1-17-2-1-1-B	[illegible]	[illegible]	[illegible]	[illegible]	50	48	48	[illegible]	[illegible]	[illegible]
ICMV 91059 S1-14-2-4-2-2-B	[illegible]	[illegible]	[illegible]	[illegible]	49	52	46	[illegible]	[illegible]	[illegible]

Diagonal values: parents; above diagonal: direct crosses; below diagonal: reciprocal crosses.

Table 36. Grain mass (g 1000^{-1}) in parents and hybrids of 10 × 10 diallel trial, rainy season 2005, ICRISAT - Patancheru.

Parents/Hybrids	863B	ICMB 94111	ICMB 00888	AIMP 92901 S1-15-1-2-B	ICMB 95222	ICMV 93074 S1-9-1-1-1-B	MC 94 C2-S1-46-1-1-B	81B	ICMS 8511 S1-17-2-1-1-B	ICMV 91059 S1-14-2-4-2-2-B
863B	**13.1**	12.9	10.8	13.9	11.7	13.5	11.5	13.0	11.5	13.7
ICMB 94111	14.7	**12.7**	14.3	11.6	11.8	11.5	12.4	11.7	12.8	10.2
ICMB 00888	12.8	13.7	**12.7**	12.8	12.6	11.7	13.4	13.2	13.1	11.7
AIMP 92901 S1-15-1-2-B	11.7	12.6	13.4	**12.0**	11.6	11.9	12.8	12.1	11.7	10.1
ICMB 95222	13.4	11.7	11.6	11.7	**9.8**	10.1	11.3	11.7	9.8	9.0
ICMV 93074 S1-9-1-1-1-B	13.1	10.7	9.6	11.8	10.1	**9.6**	10.8	8.5	10.5	8.5
MC 94 C2-S1-46-1-1-B	13.1	11.4	13.6	13.5	13.0	9.4	**10.9**	11.0	13.0	9.5
81B	13.6	10.4	11.7	12.1	10.1	11.2	10.5	**8.1**	9.8	8.3
ICMS 8511 S1-17-2-1-1-B	12.2	13.0	9.8	12.2	8.9	10.7	9.8	8.4	**9.4**	9.6
ICMV 91059 S1-14-2-4-2-2-B	11.8	9.2	12.9	9.4	9.7	9.4	9.8	8.9	9.4	**8.8**

Diagonal values: parents; above diagonal: direct crosses; below diagonal: reciprocal crosses.

and of these only three high × high crosses, 863B × AIMP 92901 S1-15-1-2-B (3.95; P <0.05), ICMB 00888 × AIMP 92901 S1-15-1-2-B (4.36; P <0.05) and 863B × ICMB 94111(2.77; P <0.05) had higher Zn content ranged from 43.8 to 44.4 mg kg^{-1}. In general, there was no correlation between performance of the hybrids and *sca* effects (r=0.233 for Fe and r= 0.290 for Zn).

Of the 14 crosses showing significant *sca* effects for days to 50% flower, 11 hybrids were in negative side with flowering ranging from 42 to 45 days (Table 39). Of these 11 crosses, three hybrids had high Fe and Zn content, two crosses had only high Fe (50.5–55.9 mg kg^{-1}) and one had high Zn content (34.0 mg kg^{-1}). The correlation between *sca* effects of crosses and days to 50% flower of crosses was positive and highly significant (r=0.530; P <0.01). Among the 20 crosses showing significant positive *sca* effects for 1000-grain mass, two hybrids had high Fe (63.8 mg kg^{-1}) and Zn (38.3 mg kg^{-1}) content. There was no correlation between *sca* effects of crosses and mean 1000-grain mass of crosses (r=0.047) and in all 20 hybrids recorded significant positive *sca* effects.

4.6.7. Heterosis

4.6.7.1. Grain Fe and Zn content

The average mid-parent heterosis for grain Fe content was negative (-5.66%), ranging from -30.53 to 20.28%; and over better-parent it varied from -46.13 to 12.69% (Appendix VIII). Based on the range of mid-parent and better-parent heterosis observed in each trait, the hybrids derived from different parental groups (high, medium and low for grain Fe and Zn) were classified into various heterotic classes to identify the parental groups producing higher frequency of heterotic hybrids. Based on the range, frequency distribution of heterosis for all the 45 hybrids was done in 6 class intervals for mid-parent heterosis and 7 class intervals for better-parent heterosis for grain Fe and 6 class intervals each for mid-parent and better-parent heterosis for grain Zn content (Table 40). Frequency distribution of hybrids for mid-parent heterosis of grain Fe content showed 11 hybrids (24%) possessing positive heterosis (>0–30%) indicating lesser frequency of

Table 37. General combining ability (*gca*) effects of parents for grain Fe and Zn content, days to 50 % flower and 1000-grain mass in 10 × 10 diallel trial, rainy season 2005, ICRISAT - Patancheru.

Sl. No.	Parent	Fe content (mg kg^{-1})		Zn content (mg kg^{-1})		Days to 50% flower		1000-grain mass (g)	
		Mean	*gca* effects	Mean	*gca* effects	Mean	*gca* effects	Mean	*gca* effects
1	863B	69.9	10.29*	41.5	5.19*	52	-0.08	12.4	1.27*
2	ICMB 94111	68.9	5.69*	40.0	1.62*	49	-0.63*	12.7	0.82
3	ICMB 00888	65.8	3.47*	41.4	3.48*	44	-2.98*	12.7	0.98
4	AIMP 92901 S1-15-1-2-B	68.6	6.78*	40.8	4.25*	48	-0.69*	10.9	0.54
5	ICMB 95222	47.9	-4.85*	34.4	-0.45	54	1.34*	9.8	-0.40
6	ICMV 93074 S1-9-1-1-1-B	40.5	-0.52	33.8	-0.01	52	-0.30	9.6	-0.72
7	MC 94 C2-S1-46-1-1-B	49.1	-0.29	31.7	-2.32*	47	-1.55*	10.9	0.26
8	81B	32.2	-8.35*	25.4	-3.84*	53	2.17*	8.1	-0.77
9	ICMS 8511 S1-17-2-1-1-B	38.0	-8.44*	22.5	-4.29*	54	1.92*	9.4	-0.58
10	ICMV 91059 S1-14-2-4-2-2-B	42.9	-3.79*	29.0	-3.64*	52	0.78*	8.8	-1.40*
	Correlation (r) between mean and *gca* effects	0.96**		0.95**		0.93**		0.94**	

*, ** Significant at 5% and 1% probability levels, respectively.

Table 38. Crosses with significant *sca* effects for grain Fe and Zn content in 10 × 10 diallel trial, rainy season 2005, ICRISAT-Patancheru.

Sl. No.	Character	Hybrids	Mean	*sca* effects
1	**Fe content**	863B × ICMB 94111	70.0	4.82*
2	**(mg kg^{-1})**	863B × ICMV 93074 S1-9-1-1-1-B	63.8	6.22*
3		863B × ICMS 8511 S1-17-2-1-1-B	50.5	-4.08*
4		ICMB 94111 × AIMP 92901 -S1-15-1-2-B	62.8	-4.24*
5		ICMB 94111 × ICMS 8511 S1-17-2-1-1-B	42.1	-9.44*
6		AIMP 92901 -S1-15-1-2-B × MC 94 C2-S1-46-1-1-B	53.7	-4.75*
7		ICMB 95222 × ICMV 93074 S1-9-1-1-1-B	40.8	-7.52*
8		ICMV 93074 S1-9-1-1-1-B × MC 94 C2-S1-46-1-1-B	52.7	4.62*
9		MC 94 C2-S1-46-1-1-B × ICMV 91059 S1 -14-2-4-2-2-B	55.9	9.54*
10		81B × ICMS 8511 S1-17-2-1-1-B	39.5	7.31*
		Correlation between mean and *sca* effects		0.233
1	**Zn content**	863B × ICMB 94111	43.8	2.77*
2	**(mg kg^{-1})**	863B × AIMP 92901 -S1-15-1-2-B	44.4	3.95*
3		863 B × MC 94 C2-S1-46-1-1-B	35.1	-3.13*
4		863B × ICMV 91059 S1-14-2-4-2-2-B	39.5	4.86*
5		ICMB 94111 × ICMB 00888	38.3	-3.02*
6		ICMB 94111 × ICMS 8511 S1-17-2-1-1-B	27.5	-4.68*
7		ICMB 00888 × AIMP 92901 -S1-15-1-2-B	44.3	4.36*
8		ICMB 00888 × ICMB 95222	34.0	-2.51*
9		AIMP 92901-S1-15-1-2-B × ICMS 8511 S1-17-2-1-1-B	33.2	-3.28*
10		ICMB 95222 × MC 94 C2-S1-46-1-1-B	34.1	2.65*
11		ICMV 93074 S1-9-1-1-1-B × MC 94 C2-S1-46-1-1-B	34.5	-2.63*
12		MC 94 C2-S1-46-1-1-B × 81B	25.9	-2.53*
13		81B × ICMS 8511 S1-17-2-1-1-B	28.4	3.38*
14		ICMS 8511 S1-17-2-1-1-B × ICMV 91059 S1-14-2-4-2-2-B	25.2	3.01*
		Correlation (r) between mean and *sca* effects		0.290

* Significant at 5% probability level.

Table 39. Crosses with significant *sca* effects for days to 50% flower and 1000-grain mass (g) in 10 × 10 diallel trial, rainy season 2005, ICRISAT-Patancheru.

Sl. No.	Character	Hybrids	Mean	*sca* effect
1	**Days to 50% flower**	863B × ICMS 8511 S1-17-2-1-1-B	47	-2.84*
2		ICMB 94111 × ICMV 93074 S1-9-1-1-1-B	44	-2.73*
3		ICMB 94111 × ICMV 91059 S1-14-2-4-2-2-B	46	-1.48*
4		ICMB 00888 × ICMB 95222	44	-2.37*
5		ICMB 00888 × ICMV 93074 S1-9-1-1-1-B	42	-2.73*
6		ICMB 00888 × 81B	49	2.47*
7		AIMP 92901-S1-15-1-2-B × ICMB 95222	46	-2.31*
8		AIMP 92901-S1-15-1-2-B × ICMV 93074 S1-9-1-1-1-B	45	-1.67*
9		AIMP 92901 -S1-15-1-2-B × MC 94 C2-S1-46-1-1-B	47	1.91*
10		AIMP 92901-S1-15-1-2-B × ICMS 8511 S1-17-2-1-1-B	52	3.11*
11		AIMP 92901-S1-15-1-2-B × ICMV 91059 S1-14-2-4-2-2-B	45	-2.76*
12		ICMB 95222 × ICMV 91059 S1-14-2-4-2-2-B	52	2.55*
13		MC 94 C2-S1-46-1-1-B × ICMV 91059 S1 -14-2-4-2-2-B	45	-2.28*
14		81B × ICMS 8511 S1-17-2-1-1-B	50	-1.76*
		Correlation between mean and *sca* effects		0.530
	1000-grain mass (g)	Direct crosses		
1		863B × ICMB 00888	9.7	-2.31*
2		863B × ICMV 93074 S1-9-1-1-1-B	13.5	1.39*
3		863B × 81B	13.0	1.49*
4		863B × ICMV 91059 S1-14-2-4-2-2-B	13.7	1.56*
5		ICMB 94111 × ICMB 00888	14.3	0.90*
6		ICMB 94111 × ICMS 8511 S1-17-2-1-1-B	12.8	1.34*
7		ICMB 94111 × ICMV 91059 S1-14-2-4-2-2-B	10.2	-1.07*
8		ICMB 00888 × ICMV 93074 S1-9-1-1-1-B	11.7	-0.94*
9		ICMB 00888 × MC 94 C2-S1-46-1-1-B	13.4	0.94*
10		ICMB 00888 × 81B	13.2	0.93*
11		ICMB 00888 × ICMV 91059 S1-14-2-4-2-2-B	11.7	1.41*
12		AIMP 92901 -S1-15-1-2-B × MC 94 C2-S1-46-1-1-B	12.8	1.02*
13		ICMB 95222 × MC 94 C2-S1-46-1-1-B	11.3	0.96*
14		ICMB 95222 × 81 B	11.7	0.77*
15		ICMB 95222 × ICMS 8511 S1-17-2-1-1-B	9.8	-0.99*
16		ICMV 93074 S1-9-1-1-1-B × MC 94 C2-S1-46-1-1-B	10.8	-0.75*
17		81B × ICMS 8511 S1-17-2-1-1-B	9.8	-0.90*
18		ICMV 91059 S1-14-2-4-2-2-B × 863B	11.8	0.94*
		Reciprocal crosses		
19		ICMB 00888 × 81 B	12.8	-1.52*
20		AIMP 92901 S1-15-1-2-B × 863B	11.7	1.06*
21		AIMP 92901 S1-15-1-2-B × ICMB 00888	13.4	-0.93*
22		ICMB 95222 × 863B	10.9	1.27*
23		ICMV 93074 S1-9-1-1-1-B × ICMB 00888	9.6	1.08*
24		MC 94 C2-S1-46-1-1-B × 863B	13.1	-0.80*
25		MC 94 C2-S1-46-1-1-B × ICMB 95222	13.0	-0.81*
26		81B × ICMB 00888	11.7	0.75*
27		81B × AIMP 92901 S1-15-1-2-B	10.6	0.75*
28		81B × ICMB 95222	10.1	0.80*
29		ICMS 8511 S1-17-2-1-1-B × ICMB 00888	9.5	1.78*
30		ICMS 8511 S1-17-2-1-1-B × MC 94 C2-S1-46-1-1-B	9.8	1.60*
		Correlation (r) between mean and *sca* effects		0.047

* Significant at 5% probability level.

hybrids with positive mid-parent heterosis compared to those having negative mid-parent heterosis (76%). Of the 11 hybrids, 6 (2 each produced from high × high, high × medium and high × low crosses) had mid-parent heterosis not exceeding 20% for grain Fe content. Of these 6 hybrids, 5 had heterosis only up to 10%, indicating the parents with high Fe content produced hybrids with lower level of positive mid-parent heterosis in lower frequency. This was also true based on the observation that about 50% of the hybrids showing negative heterosis had one parent with high Fe content. Among the remaining five hybrids (out of 11) showing positive heterosis, 3 were produced from medium × low crosses and 1 each from medium × medium and low × low crosses. The maximum mid-parent heterosis (20–30%) was observed in a cross between medium × low parents.

Five hybrids (11%) showed positive heterosis over better-parent for grain Fe content. Of the five, 2 hybrids were produced using high × high crosses and one each using medium × medium, medium × low and low × low crosses. About 62 % of the hybrids (28) having negative heterosis for grain Fe content were produced using at least one high parent. Again the maximum better-parent heterosis was observed from a cross involving medium × low parent. None of the hybrids had significant positive mid-parent heterosis except for the hybrid MC 94 C2-S1-46-1-1-B × ICMV 91059 S1-14-2-4-2-2-B (20.28%) (Table 41). None of the hybrids showed positive significant better-parent heterosis. The correlation between mid-parent value and hybrid *per se* performance was highly significant ($r= 0.87$; $P <0.01$) (Fig 20), however there was no correlation between mid-parent value and mid-parent heterosis for grain Fe ($r = -0.009$) (Fig 21).

The average heterosis for grain Zn content was almost negligible (0.72%) (Appendix VIII). The heterosis over mid-parent ranged from -21.18 to 26.43% and the better-parent heterosis varied from -33.11 to 14.29%. Frequency distribution of different heterotic classes showed 24 hybrids (53%) had positive mid-parent heterosis (>0–30%) indicating almost equal frequency of hybrids with positive and negative mid-parent heterosis (Table 40). Out of 24 hybrids with positive mid-parent heterosis, 17 hybrids (71%) based on at least one high parent had positive mid-parent heterosis, indicating the

Table 40. Frequency distribution of mid-parent and better-parent heterosis for grain Fe and Zn content in 10 × 10 diallel trial, rainy season 2005, ICRISAT-Patancheru.

Micronutrient	Cross type	Mid-parent heterosis (%)					
		-30 to -20	-20 to -10	-10 to 0	0 to 10	10 to 20	20 to 30
Fe content (mg kg^{-1})	High × High	0	2	2	2	0	0
	High × Medium	0	4	6	1	1	0
	High × Low	1	3	6	2	0	0
	Medium × Medium	1	0	1	1	0	0
	Medium × Low	0	2	4	1	1	1
	Low × Low	0	0	2	0	1	0
Zn content (mg kg^{-1})	High × High	0	2	0	2	2	0
	High × Medium	0	0	6	5	0	1
	High × Low	1	1	3	5	1	1
	Medium × Medium	0	1	1	1	0	0
	Medium × Low	0	2	4	3	0	0
	Low × Low	0	0	0	0	3	0

Micronutrient	Cross type	Better-parent heterosis (%)						
		-50 to -40	-40 to -30	-30 to -20	-20 to -10	-10 to 0	0 to 10	10 to 20
Fe content (mg kg^{-1})	High × High	0	0	0	3	1	2	0
	High × Medium	0	0	6	4	2	0	0
	High × Low	1	4	6	1	0	0	0
	Medium × Medium	0	0	1	0	1	1	0
	Medium × Low	0	0	3	5	0	0	1
	Low × Low	0	0	0	2	0	1	0
Zn content (mg kg^{-1})	High × High	0	0	0	2	0	2	2
	High × Medium	0	0	0	5	7	0	0
	High × Low	0	1	3	7	1	0	0
	Medium × Medium	0	0	0	1	2	0	0
	Medium × Low	0	0	0	7	2	0	0
	Low × Low	0	0	0	0	0	3	0

Table 41. Crosses with significant mid-parent and better-parent heterosis for grain Fe (mg kg^{-1}) content in 10 × 10 diallel trial, rainy season 2005, ICRISAT-Patancheru.

Sl. No.	Hybrid	Mean	Mid heterosis	Better heterosis
1	863B × ICMB 95222	54.7	-7.13	-21.75**
2	863 B × MC 94 C2-S1-46-1-1-B	57.3	-3.67	-17.98**
3	863B × 81B	53.7	5.23	-23.18**
4	863B × ICMS 8511 S1-17-2-1-1-B	48.1	-10.75	-31.14**
5	863B × ICMV 91059 S1-14-2-4-2-2-B	56.6	0.38	-18.98**
6	ICMB 94111 × ICMB 00888	56.5	-16.11**	-18.04**
7	ICMB 94111 × AIMP 92901 -S1-15-1-2-B	58.4	-15.10**	-15.28*
8	ICMB 94111 × ICMB 95222	50.5	-13.55*	-26.74**
9	ICMB 94111 × ICMV 93074 S1-9-1-1-1-B	54.9	-10.94	-20.31**
10	ICMB 94111 × MC 94 C2-S1-46-1-1-B	54.5	-7.68	-20.94**
11	ICMB 94111 × 81B	44.3	-12.36	-35.74**
12	ICMB 94111 × ICMS 8511 S1-17-2-1-1-B	37.1	-30.53**	-46.13**
13	ICMB 94111 × ICMV 91059 S1-14-2-4-2-2-B	51.8	-7.39	-24.85**
14	ICMB 00888 × ICMB 95222	47.2	-16.89*	-28.18**
15	ICMB 00888 × ICMV 93074 S1-9-1-1-1-B	54.6	-9.15	-16.98*
16	ICMB 00888 × MC 94 C2-S1-46-1-1-B	53.3	-7.22	-18.96**
17	ICMB 00888 × 81B	45.4	-7.22	-30.92**
18	ICMB 00888 × ICMS 8511 S1-17-2-1-1-B	46.8	-9.70	-28.79**
19	ICMB 00888 × ICMV 91059 S1-14-2-4-2-2-B	50.2	-7.57	-23.62**
20	AIMP 92901-S1-15-1-2-B × ICMB 95222	55.4	-4.86	-19.23**
21	AIMP 92901 -S1-15-1-2-B × MC 94 C2-S1-46-1-1-B	52.4	-10.95	-23.60**
22	AIMP 92901 S1-15-1-2-B × 81B	49.2	-2.31	-28.27**
23	AIMP 92901-S1-15-1-2-B × ICMS 8511 S1-17-2-1-1-B	47.4	-11.07	-30.94**
24	AIMP 92901-S1-15-1-2-B × ICMV 91059 S1-14-2-4-2-2-B	51.2	-8.22	-25.40**
25	ICMB 95222 × ICMV 93074 S1-9-1-1-1-B	40.7	-20.39**	-25.17**
26	ICMB 95222 × 81 B	38.2	-4.58	-20.25*
27	ICMB 95222 × ICMV 91059 S1-14-2-4-2-2-B	38.8	-14.50	-18.93*
28	ICMV 93074 S1-9-1-1-1-B × ICMS 8511-S1-17-2-1-1-B	42.2	-8.73	-22.54**
29	ICMV 93074 S1-9-1-1-1-B × ICMV 91059 S1-14-2-4-2-2-B	45.8	-5.92	-15.86*
30	MC 94 C2-S1-46-1-1-B × ICMS 8511 S1-17-2-1-1-B	37.6	-13.59	-23.41**
31	MC 94 C2-S1-46-1-1-B × ICMV 91059 S1-14-2-4-2-2-B	55.4	20.28*	12.69
	Mean	50.6	-5.66	-17.87
	Minimum	37.7	-30.53	-46.13
	Maximum	70.0	20.28	12.69
	LSD ($P = 0.05$)	8.2		
	CV (%)	10.3		
Correlation (r) between mid-parent value and hybrid performance		0.87**		

* Significant at 5% and 1% probability levels, respectively.

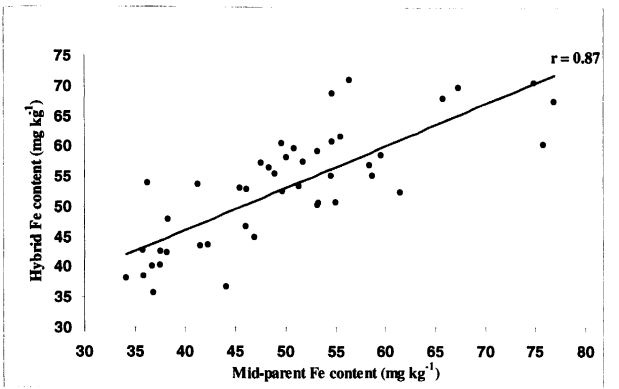


Fig 20. Relationship between mid-parent Fe content and hybrid *per se* performance for grain Fe content

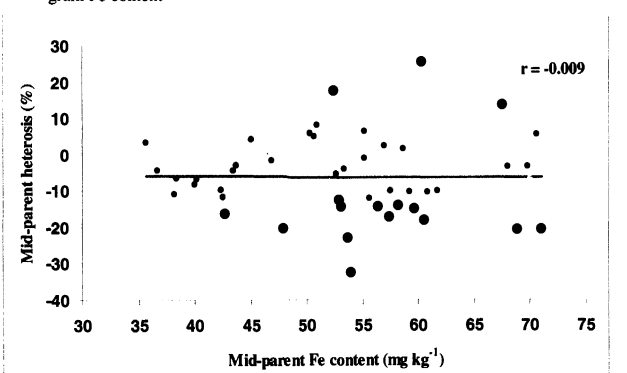


Fig 21. Relationship between mid-parent Fe content and hybrid performance *per se* for grain Fe content (● significant mid-parent heterotic hybrids)

parents with high Zn content produced hybrids with higher level of positive heterosis. The hybrids produced from high × medium and high × low parents (each 1) had maximum (20–30%) mid-parent heterosis for grain Zn content. Among the remaining seven hybrids (out of 24) showing positive mid-parent heterosis, 3 each were produced from medium × low and low × low and one hybrid produced from medium × medium cross.

Seven hybrids (16%) had positive better-parent heterosis for grain Zn content, of these, 4 hybrids was based on high × high crosses and 3 based on low × low crosses. About 58% of the hybrids (26) having negative heterosis for grain Zn content were had at least one high parent. The maximum better-parent heterosis (2 hybrids) was observed from crosses between high × high cross (10–20%). Highly significant positive mid-parent heterosis was observed in the high × high crosses, 863B × ICMV 91059 S1-14-2-4-2-2-B (26.43%) and 863B × AIMP 92901 S1-15-1-2-B (15.26%) and a hybrid 863B × AIMP 92901 S1-15-1-2-B (14.29%) had significant positive better-parent heterosis (Table 42). Twenty-two hybrids had significant negative better-parent heterosis and three hybrids had significant negative mid-parent heterosis. The correlation between mid-parent value and hybrid *per se* performance was highly significant ($r= 0.89$; $P <0.01$) (Fig 22), whereas no correlation was observed between mid-parent value and mid-parent heterosis for Zn content ($r = -0.02$) (Fig 23).

4.6.7.2. Days to 50% flower and 1000-grain mass

The average heterosis for days to 50% flower was negative (-6.07%) and the mid-parent heterosis ranged from -12.89 to 1.72% and better-parent heterosis varied between -19.35 and -1.39% (Appendix IX). Based on frequency distribution of heterosis, all the 90 hybrids for days to 50% flower were classified into three class intervals for mid-parent heterosis and two class intervals for better-parent heterosis for days to 50% flower and seven class intervals each for mid-parent and better-parent heterosis for 1000-grain mass (Table 43). Frequency distribution of mid-parent heterosis for days to 50% flower showed 97% hybrids possessing negative heterosis (up to -20%) indicating higher frequency of hybrids with negative mid-parent heterosis. Among the hybrids showing negative mid-

Table 42. Crosses with significant mid-parent and better-parent heterosis for grain Zn (mg kg^{-1}) content in 10 × 10 diallel trial, rainy season 2005, ICRISAT-Patancheru.

Sl. No.	Hybrid	Mean	Mid heterosis	Better heterosis
1	863B × AIMP 92901 -S1-15-1-2-B	47.5	15.26**	14.29**
2	863 B × MC 94 C2-S1-46-1-1-B	33.8	-7.64	-18.54**
3	863B × 81B	36.1	7.77	-13.16*
4	863B × ICMS 8511 S1-17-2-1-1-B	33.5	-3.46	-19.34**
5	863B × ICMV 91059 S1-14-2-4-2-2-B	40.5	26.43**	-2.49
6	ICMB 94111 × ICMB 00888	36.2	-11.10*	-12.64*
7	ICMB 94111 × AIMP 92901 -S1-15-1-2-B	34.8	-13.86*	-14.78*
8	ICMB 94111 × MC 94 C2-S1-46-1-1-B	33.7	-5.90	-15.60*
9	ICMB 94111 × 81B	31.9	-2.40	-20.18**
10	ICMB 94111 × ICMS 8511 S1-17-2-1-1-B	26.7	-21.18**	-33.11**
11	ICMB 94111 × ICMV 91059 S1-14-2-4-2-2-B	33.5	7.20	-16.18*
12	ICMB 00888 × ICMB 95222	34.6	-8.67	-16.43*
13	ICMB 00888 × MC 94 C2-S1-46-1-1-B	35.6	-2.73	-14.09*
14	ICMB 00888 × 81B	34.6	3.59	-16.43*
15	ICMB 00888 × ICMS 8511 S1-17-2-1-1-B	34.3	-1.06	-17.23**
16	ICMB 00888 × ICMV 91059 S1-14-2-4-2-2-B	32.4	1.46	-21.66**
17	AIMP 92901 S1-15-1-2-B × 81B	33.8	2.16	-17.14**
18	AIMP 92901-S1-15-1-2-B × ICMS 8511 S1-17-2-1-1-B	30.8	-10.43	-24.65**
19	ICMB 95222 × 81 B	27.5	-7.86	-19.88*
20	ICMB 95222 × ICMV 91059 S1-14-2-4-2-2-B	28.4	-0.29	-17.46*
21	ICMV 93074 S1-9-1-1-1-B × ICMS 8511-S1-17-2-1-1-B	28.5	-7.46	-15.58*
22	MC 94 C2-S1-46-1-1-B × 81B	25.4	-11.09	-19.96*
23	MC 94 C2-S1-46-1-1-B × ICMS 8511 S1-17-2-1-1-B	26.1	-12.30	-17.65*
	Mean	34.1	0.72	-10.19
	Minimum	25.0	-21.18	-33.11
	Maximum	44.4	26.43	14.29
	LSD ($P = 0.05$)	5.6		
	CV (%)	10.3		
	Correlation (r) between mid-parent value and hybrid performance		0.89**	

*, ** Significant at 5% and 1% probability levels, respectively.

parent heterosis, 57 hybrids (67%) had at least one high Fe and Zn parent indicating the parents with high Fe and Zn content produced hybrids with higher level of negative heterosis for days to 50% flower. In general, the high × medium, high × low and medium × low Fe and Zn crosses and their reciprocals produced maximum number of hybrids with negative mid-parent heterosis.

All the hybrids had negative better-parent heterosis (up to -20%) for days to 50% flower. About 67% of the hybrids (60) having negative better-parent heterosis for days to 50% flower had at least one of the high Fe and Zn content parent, indicating that the high parents produced higher frequency of hybrids with negative better-parent heterosis. The high × medium, high × low and medium × low Fe and Zn crosses and their reciprocals produced maximum number of hybrids with negative better-parent heterosis.

Totally 55 hybrids showed significant negative mid-parent heterosis and 75 hybrids exhibited significant negative better-parent heterosis for days to 50% flower (Appendix IX). Of the crosses showing significant negative mid and better-parent heterosis for days to 50% flower, a hybrid, MC 94 C2-S1-46-1-1-B × ICMV 91059 S1-14-2-4-2-2-B (20.28%) showed significant positive mid-parent heterosis and positive better-parent heterosis (12.69%) for grain Fe content, similarly, a hybrid 863B × ICMV 91059 S1-14-2-4-2-2-B (26.43%) exhibited significant better-parent heterosis for Zn content. Non-significant correlation co-efficient (r= 0.23) observed between mid-parent value and hybrid performance, whereas highly significant positive correlation observed between mid-parent value and mid-parent heterosis (r=0.52; $P<0.01$) for days to 50% flower.

For 1000-grain mass, the average heterosis was positive (8.55%) and the heterosis over mid-parent ranged from -22.54 to 32.98%, and the better-parent heterosis varied from -27.65 to 23.55% (Appendix IX). Frequency distribution of mid-parent heterosis for 1000-grain mass showed 70 hybrids (78%) had positive mid-parent heterosis (>0–40%) indicating high frequency of hybrids with positive mid-parent heterosis (Table 43).

Table 43. Frequency distribution of mid-parent and better-parent heterosis for days to 50% flower and 1000-grain mass, classified based on Fe and Zn content in 10 × 10 diallel trial, rainy season 2005, ICRISAT - Patancheru.

Days to 50% flower	Cross type	Mid-parent heterosis (%)			Better-parent heterosis (%)	
		-20 to -10	-10 to 0	0 to 10	-20 to -10	-10 to 0
Direct	High × High	0	6	0	2	4
	High × Medium	4	8	0	9	3
	High × Low	2	8	2	6	6
	Medium × Medium	0	3	0	2	1
	Medium × Low	1	8	0	3	6
	Low × Low	0	3	0	0	3
Reciprocal	High × High	0	6	0	5	1
	Medium × High	1	10	1	3	9
	Low × High	1	11	0	2	10
	Medium × Medium	0	3	0	1	2
	Low × Medium	1	8	0	5	4
	Low × Low	0	3	0	1	2

1000-grain mass	Cross type	Mid-parent heterosis (%)							Better-parent heterosis (%)						
		-30 to -20	-20 to -10	-10 to 0	0 to 10	10 to 20	20 to 30	30 to 40	-30 to -20	-20 to -10	-10 to 0	0 to 10	10 to 20	20-30	30-40
Direct	High × High	1	0	1	2	2	0	0	1	0	1	2	2	0	0
	High × Medium	0	0	1	4	5	2	0	0	0	6	5	1	0	0
	High × Low	0	0	1	3	4	4	0	1	0	4	5	2	0	0
	Medium × Medium	0	0	0	3	0	0	0	0	0	1	2	0	0	0
	Medium × Low	0	0	4	1	2	1	1	0	3	1	2	3	0	0
	Low × Low	0	0	1	1	1	0	0	0	0	1	2	0	0	0
Reciprocal	High × High	0	0	0	4	2	0	0	0	0	2	3	1	0	0
	Medium × High	0	1	2	2	6	1	0	1	3	2	5	0	1	0
	Low × High	0	2	1	1	5	2	1	2	2	4	3	1	0	1
	Medium × Medium	0	0	1	1	0	1	0	0	1	0	1	1	0	0
	Low × Medium	0	0	3	2	3	1	0	0	1	5	1	2	0	0
	Low × Low	0	0	1	2	0	0	0	0	1	0	2	0	0	0

High, medium and low for grain Fe and Zn content.

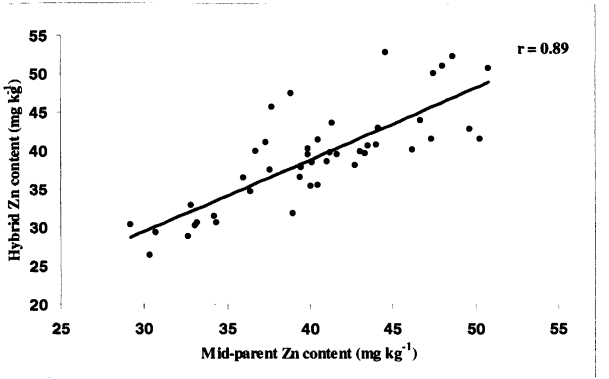


Fig 22. Relationship between mid-parent Zn content and hybrid *per se* performance for grain Zn content

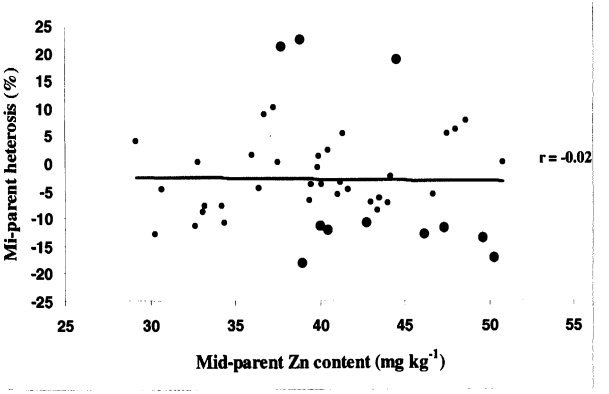


Fig 23. Relationship between mid-parent Zn content and mid-parent heterosis for grain Zn content (● significant mid-parent heterotic hybrids)

Among the 70 hybrids (36 direct and 34 reciprocal crosses), 50 hybrids were based on at least one high Fe and Zn parent, indicating the parents with high Fe and Zn content produced hybrids with higher levels of positive heterosis for grain mass. The maximum mid-parent heterosis (>30–40%) was from crosses involving medium × low and low × high Fe and Zn parents (each 1) for 1000-grain mass. In general, the high × medium and high × low Fe and Zn crosses and their reciprocals produced maximum number of hybrids with positive mid-parent heterosis (direct: 22 and reciprocal: 18).

In total, 57 hybrids (63%) out of 90 had positive better-parent heterosis for 1000-grain mass. About 56% of the hybrids (32) having positive heterosis for 1000-grain mass had at least one of the high Fe and Zn parent. The maximum better-parent heterosis was observed from a cross involving low × high Fe and Zn parent (30–40%). The high × medium and high × low Fe and Zn crosses and their reciprocals produced maximum number of hybrids with positive better-parent heterosis (direct: 13 and reciprocal: 11).

Totally 46 hybrids showed significant positive mid-parent heterosis for 1000-grain mass (Appendix IX). Of these two hybrids showed significant positive mid-parent heterosis for Zn content (15.26–26.43%) and none for grain Fe content. Eighteen hybrids exhibited positive significant better-parent heterosis for 1000-grain mass. Of these, a hybrid 863B × AIMP 92901 S1-15-1-2-B showed positive significant better-parent heterosis for grain Zn content (14.29%). The correlation between mid-parent value and hybrid performance was highly significant ($r= 0.69$; $P <0.01$), whereas no correlation observed between mid-parent value and mid-parent heterosis for 1000-grain mass ($r= 0.06$).

DISCUSSION

CHAPTER 5

DISCUSSION

Pearl millet is a major warm-season cereal crop, grown on more than 26 million ha annually in some of the most marginal environments of Asia and Africa (FAO and ICRISAT, 1996). In developing countries, poorer people tend to derive even a greater proportion of their daily calories (80–90%) from pearl millet (Kumar, 1994). Pearl millet grain has higher nutritional value of protein, energy and minerals when compared to major cereals and coarse grains (Maiti and Bidinger, 1981). The mineral profile of grain Fe and Zn content in pearl millet is relatively superior (Chauhan *et al.*, 1986) and has large genetic variability (Deosthale *et al.*, 1971; Jambunathan and Subramanian, 1988; Abdalla *et al.*, 1998). To formulate effective breeding strategy for enhancement of Fe and Zn content in pearl millet grain, it is necessary to identify germplasm that has useful genetic variation to exploit it commercially. Such variation with high heritability would be used to develop nutritionally improved cultivars and assist in identifying genetic and physiological basis for variation.

Studies that specifically deal with genetic variation for the grain Fe and Zn content are limited and some of the earlier reports of Hulse *et al.* (1980) and Jambunathan and Subramanian (1988) showed that genetic variation for grain Fe and Zn content in pearl millet appears to be much higher than those in other cereal crops. Hence, a comprehensive study was formulated to assess the magnitude of genetic variation for grain Fe and Zn content among the different classes of breeding materials and germplasm accessions. Since environments, especially soil and climate, seem to have strong influence on grain mineral content, it is necessary to test the stability of grain Fe and Zn content in the lines or varieties over different environments to make the most valid comparisons of the genetically controlled variation. Hence, the stability performance of genotypes was tested over four different year × season environments at ICRISAT to assess their magnitude of genotype × environment interaction for grain Fe and Zn content, realizing the limitations of this study at just one location, though

as wide range of soil Fe and Zn levels. When the potentially best and stable material is identified, it would be necessary to determine the mode of inheritance of these traits and their combining ability, as these will, in turn, define the breeding methods to be employed and efficiency of progress expected towards increasing the micronutrient content. In the present study, inheritance pattern of grain Fe and Zn content were examined through diallel analysis.

To combine various desirable characters in a line or variety, the knowledge on association of grain Fe and Zn with agronomically important traits is essential. The grain Fe and Zn traits needs to be combined with each other and also with farmers-preferred traits such as bold grain and early-maturity to ensure farmers chose the improved lines. Finally, the cost effectiveness of the screening procedure is also important because chemical analyses (AAS) are costly and time consuming and pose problems when dealing with a large numbers of samples and limited seed quantity as in the screening of breeding lines and germplasm accessions. This study also aimed to examine some cost-effective selection procedures for grain Fe and Zn content.

5.1. GENETIC VARIABILITY FOR GRAIN IRON AND ZINC CONTENT

The success of any crop improvement program largely depends upon the genetic variability and the heritability of desirable traits. The magnitude and type of genetic variability will help the breeder to determine the selection criteria and breeding schemes to be used for improvement purposes. Estimation of genetic variability for cryptic quality characters, such as grain Fe and Zn, in a whole range of materials right from germplasm accessions to improved and elite breeding materials, is very important and a preliminary step before exploitation of it. Selecting micronutrient-dense lines among the existing germplasm accessions, breeding lines and varieties is the simplest approach to improve the micronutrient levels in the crop plants. In order to have immediate impact on the food systems to overcome the micronutrient malnutrition, the easiest approch is to identify the micronutrient-dense high-yielding cultivated varieties, which can be transferred to the local farmers of the malnutrition-

affected areas for cultivation. In this study, to have long-, medium- and short-term, and immediate impact, various classes of breeding materials such as hybrid parents, inbred lines, improved populations (with adaptation to both Asia and Africa) along with germplasm accessions were screened for micronutrient content to study the existing genetic variability in two sets of trials.

Highly significant genetic variation and medium to high broad sense heritability was observed for grain Fe and Zn content among the entries of set I trial consisting of inbred lines, improved populations and germplasm accessions, and set II trial consisting of a wide range of improved populations in both summer and rainy seasons. The significant genetic variation and high heritability among entries indicated that there is large genetic variation for effective selection for both grain Fe and Zn content. The magnitude of variability was approximately three-fold for both grain Fe (29.4–102.0 mg kg^{-1}) and Zn (24.0–80.8 mg kg^{-1}) in set I trial, and two-fold in set II trial for grain Fe (42.0–79.9 mg kg^{-1}) and Zn content (27.2–50.2 mg kg^{-1}) suggesting that there is a good potential for genetic improvement for grain Fe and Zn in pearl millet. Some of the earlier studies with limited number of entries revealed higher variability for grain Fe (70–180 mg kg^{-1}) and Zn (28–70 mg kg^{-1}) content in pearl millet (Abdalla *et al.*, 1998; Malik, 1999). In a study with 27 genotypes, Jambunathan and Subramanian (1988) found the highest variability for grain Fe (40–580 mg kg^{-1}), but the information of on the line having highest Fe content and the method of Fe estimation was not reported. The variability for grain Fe and Zn in about 1140 entries of rice (6.3–24.4 mg kg^{-1} Fe and 3.5–58.4 mg kg^{-1} Zn) (Gregorio *et al.*, 2000), about 2000 entries of wheat (25–99 mg kg^{-1} Fe and 22–65 mg kg^{-1} Zn) (Monasterio and Graham, 2000), about 1800 entries of maize (9.6–63.2 mg kg^{-1} Fe and 12.9–57.6 mg kg^{-1} Zn) (Banziger and Long, 2000) and in 84 entries of sorghum (20–37 mg kg^{-1} Fe and 13–31 mg kg^{-1} Zn) (Reddy *et al.*, 2005) was less than the variability observed in this pearl millet study. These results suggest that the genetic variability observed in pearl millet is higher than those found in crops like rice, wheat, maize and sorghum.

The analysis of data from the two seasons showed significant entries × environment interaction for both grain Fe and Zn among different classes of breeding materials and improved populations, but the correlation of micronutrient content of entries between the two seasons was highly significant both for Fe ($r = 0.57$ to 0.66; $P < 0.01$) and Zn ($r = 0.44$ to 0.69; $P < 0.01$), indicating high levels of consistency of the rankings of entries across the two seasons both for Fe and Zn content.

The mean grain Fe and Zn content among the different classes of materials (set I) was comparable both in the summer season (41.5 mg kg^{-1} Fe and 40.1 mg kg^{-1} Zn) and in the rainy season (49.5 mg kg^{-1} Fe and 47.7 mg kg^{-1} Zn), whereas among the improved populations (set II) the average grain Fe content was higher in both summer (57.6 mg kg^{-1}) and rainy season (52.1 mg kg^{-1}) than Zn content (35.7 mg kg^{-1} in rainy and 40.2 mg kg^{-1} in summer season). On an average, the grain Fe was higher than the grain Zn content in pearl millet. The average grain Fe and Zn contents in the rainy season field were 11–20% higher than those in the summer season field in set I trial, which might have largely to do with the Fe and Zn levels in the soil, which had 38–155% more Fe and Zn in the field used during the rainy season than the one used during the summer season. However, in set II trial, the average grain Fe content in rainy season was 30% higher than summer season with respect to 68% increased soil Fe content, whereas summer season had higher grain Zn content than rainy season with no changes in soil Zn content between the seasons.

The range of grain Fe and Zn also varied among the different classes of breeding materials and germplasm accessions. Larger variability for grain Fe and Zn content was in advanced population progeny lines (30.1–75.7 mg kg^{-1} Fe and 24.5–64.8 mg kg^{-1} Zn) followed by seed parents (34.4–72.7 mg kg^{-1} Fe and 27.3–59.6 mg kg^{-1} Zn) compared to open-pollinated varieties (32.4–62.7 mg kg^{-1} Fe; 33.7–53.1 mg kg^{-1} Zn), pollinators (34.9–55.7 mg kg^{-1} Fe and 31.7–55.2 mg kg^{-1} Zn) and germplasm accessions (34.5–54.4 mg kg^{-1} Fe; 35.4–49.3 mg kg^{-1} Zn). In all the classes of breeding materials, the mean grain Fe content was slightly higher than the Zn content, while both had similar values in germplasm

accessions. The maximum variability in seed parents and inbred lines could be used either for production of commercially exploitable hybrids or for further improvement of hybrid parents. All the pollinators evaluated in this study were the pollen parents of either released hybrids or the designated parents from ICRISAT breeding program. The variability in these pollen parents was not encouraging compared to the seed parents, suggesting the need for screening more number of pollinators or identification of new set of pollinators with high Fe and Zn content. The group of inbred lines having large genetic variability in this study would also make good pollinators, but the combining ability and downy mildew resistance of these need to be evaluated. The fact that the variability in the open-pollinated varieties was more than that of randomly selected germplasm, indicated presence of ready-to-use variability in the form of varieties or improved populations. However, this also suggested that there is a necessity of screening diverse germplasm, especially core collection to understand the pattern and magnitude of genetic variability for grain Fe and Zn content.

The variability available for grain Fe and Zn contents in the present study gave a good scope to identify the sources of high, medium and low Fe and Zn contents in different classes of breeding materials that could be used in both strategic and applied research, that is, for pre-breeding, assessing G × E interaction and stability, inheritance studies and for direct utilization as hybrid parents and open pollinated varieties.

Among the 40 hybrid parents, 8 seed parents had 56 to 111% higher mean Fe and 59 to 88% higher mean Zn than the check 81B (34.4 mg kg^{-1} Fe and 31.7 mg kg^{-1} Zn), and 1 pollinator (MIR 97171) had 43% higher mean Fe and 32% higher mean Zn with significant superiority over the check ICMP 451 (39.0 mg kg^{-1} Fe and 35.4 mg kg^{-1} Zn). The hybrid parents with high Fe and Zn content were either direct derivatives of the *iniadi* germplasm, such as 863B, ICMB 98222 and ICMB 88004, or involved a large proportion of the *iniadi* germplasm in their parentage such as ICMB 94111, ICMB 00888, ICMB 00999, 843B and ICMB 94555. All the selected 9 hybrid parents were early-flowering (42-47 days) compared to the check 81B (57 days). Further, the mean 1000-grain mass of 7 hybrid parents was higher

(9.1–12.2 g) than 81B (7.4 g), indicating that the lines with high levels of grain Fe and Zn were available in early-maturing and bold-seeded background. The seed parents at ICRISAT are designated only if they are resistant to at least two of the five diverse pathotypes of downy mildew. Hence, the seed parents identified are also downy mildew resistant, especially the seed parent 863B, which is resistant to all known five downy mildew pathotypes.

Typical characteristics of the *iniadi* germplasm are large seed size (15–18 g 1000^{-1} seed) of globular shape and dark gray color with bright outer exposed surface, starchy endosperm, compact and conical panicles, relatively less photoperiod-sensitive earliness (70–85 days), rapid grain filling (23–25 days relative to 25–32 days for others), downy mildew resistance and good combining ability. A majority of hybrids in India include some degree of *iniadi* germplasm in their parental lines (Rai *et al.*, 1999).

Among the seed parents, 863B had the highest grain Fe content and was fifth highest for Zn content, and it is the female parent of three commercially released hybrids in India. This medium-height male-sterile line also has thick-panicle and large seed with dark gray color. Similarly, another seed parent ICMB 88004, which is a high-tillering, early-maturing line with gray color seed, is the female parent of two hybrids, including ICMH 356 which is an early-maturing, drought-escaping ICRISAT-bred hybrid largely adopted by the farmers in India. The seed parent 843B, that showed highest Zn content is an earliest-maturing male-sterile line in the world and it is a parent of an earliest maturing hybrid, HHB 67 that was released in India. Another male-sterile line, ICMB 94555 was used as seed parent in a recently released dual-purpose hybrid GHB 558 in Gujarat. However, the pollen parents of hybrids, especially ICMH 356 (ICMR 356: 38.4 mg kg^{-1} Fe and 45.1 mg kg^{-1} Zn) and HHB 67 (H 77/833-2: 34.9 mg kg^{-1} Fe and 37.7 mg kg^{-1} Zn) had low Fe and Zn content making these hybrids as crosses between high × low parents and prompts for generating grain Fe and Zn data of hybrids. Nevertheless, these results indicate the possibility of producing commercially acceptable hybrids with high Fe and Zn content using parents with high Fe and Zn content. These results also guide a fast track approach to evaluate commercial hybrids for

grain Fe and Zn content to identify high-yielding and micronutrient-dense hybrids and pass on them to the farmers for large-scale cultivation, which can make immediate impact on the food system.

Among the 20 advanced population progenies evaluated for grain Fe and Zn, 7 had 38-94% higher grain Fe and 36-83% higher grain Zn content than the check ICMP 451. These progenies had similar maturity duration (45–48 days) as ICMP 451 (47 days) and also had almost similar 1000-grain mass (9.8–10.8 g) as ICMP 451 (10.3 g), except a progeny that had higher 1000-grain mass (12.2 g). A critical examination of grain Fe and Zn content in the population progenies indicated that two S_4 progenies derived from an open-pollinated variety (AIMP 92901) had the highest levels of both grain Fe and Zn, which exceeded the grain Fe and Zn content of the original population AIMP 92901 (Fe 50.8 and Zn 42.0 mg kg^{-1}) by 46% and 52%, respectively. AIMP 92901 was developed from a bold-seeded Early Composite that was constituted largely from the *iniadi* germplasm (Witcombe *et al.*, 1997). A S_5 progeny derived from another variety SDMV 90031 (largely based on the *iniadi* germplasm) also had high Fe content, which was 44% more than that of the original population (47.8 mg kg^{-1}). As these results suggested the prevalence of intra-population variability for both grain Fe and Zn, about 65 population progenies were evaluated to study the magnitude of such variability in the set IV trial, and to select lines with still higher levels of Fe and Zn. Further, these inbreds with very high levels of grain Fe and Zn could be used as pollen parents and seed parent of hybrids upon testing their combining ability for grain yield and could also be used to constitute random mating populations for recurrent selection.

Among the 30 open-pollinated varieties and 20 germplasm accessions evaluated, five had 30–50% higher Fe and 24–34% higher Zn than the check WC-C 75 (41.9 mg kg^{-1} Fe and 39.5 mg kg^{-1} Zn). Among the open-pollinated varieties with high Fe and Zn contents, some important entries were: EEBC a photoperiod-insensitive, early-flowering (38 days) composite with large seed size (9.8 g 1000^{-1} seed) and resistance to downy mildew (Rai *et al.*, 1998), followed by ICMV 221 and GB 8735, also early-flowering (43 days) with large seed size

(11.3–12.5 g 1000^{-1} seed), and a germplasm accession, IP 8964 that was a late flowering type (61 days to 50% flower). Among the open-pollinated varieties, GB 8735, developed largely from the *iniadi* germplasm had the highest grain Fe and Zn content. This early-maturing and large-seeded variety, developed by ICRISAT in partnerships with the national programs in the western and central African region has been released in Chad, Mauritiana, Nigeria and Benin. HHVDBC and ICMV 221 consisting of a large proportion of *iniadi* germplasm in their parentage also had high levels of Fe and Zn contents. HHVDBC is a dwarf population (110 cm plant height) of mid-late maturity (90 days to mature), large seed size (13.5g 1000^{-1}) and thick panicles (40 mm diameter) and it has been extensively used at ICRISAT for seed parents breeding. Similarly, ICMV 221 has been released for cultivation in India, Kenya and Eritrea.

Among the improved populations evaluated in the set II trial, an improved high yielding variety ICTP 8203, derived from *iniadi* landrace recorded significantly higher grain Fe and Zn content with early-maturity, resistance to downy mildew, escape from drought and large seed size (Rai *et al.*, 1990). The other populations with high Fe and Zn content such as CGP, GGP bulk, PVGGT-4, PVGGT-5 originating from Togo (West Africa) are early-maturing (42–47 days to 50% flower) with larger seed size (9.6-13.3 g 1000^{-1} seed) compared to the check WC-C75 (46 days to 50% flower and 9.4g 1000^{-1} seed). These populations could be used either as varieties upon multi-location testing or sources to derive inbred lines with high levels of grain Fe and Zn and good combining ability. The inbred lines with high Fe and Zn, could also be source lines that could be used in backcross breeding to transfer the traits into identified good combining lines. However, due to the high cost of Fe and Zn estimation, marker-assisted breeding appears to be a good route for this purpose, which in pearl millet is yet to take-off.

The results of present study showed that the highest levels of grain Fe and Zn content were observed in well-adapted commercial varieties and their progenies, and in the parental lines of hybrids, which had largely *iniadi* germplasm in their parentage. The variability observed in the different classes of genetic materials gave good indications to design

strategies for improvement of grain Fe and Zn content. An intensive exploitation of *iniadi* germplasm is likely to lead to the identification of lines with still higher Fe and Zn content in relatively elite agronomic backgrounds to enable rapid development of open-pollinated varieties. Results also suggested that selection within improved populations, especially those with the predominantly *iniadi* germplasm, provide the greatest opportunities to develop pearl millet varieties and hybrid parents with significantly improved grain Fe and Zn content. *Iniadi* landrace has been observed as a promising germplasm with several positive attributes (Andrews and Anand Kumar, 1996).

The potential for nutritional enhancement through deliberate selection within an improved population or high-yielding cultivar is much greater than by selection within germplasm. Evaluation of just 2–3 progenies from an open-pollinated variety AIMP 92901 in set I trial revealed large within-population variability, this laid foundation for evaluation of progenies ($S_{2:3}$) derived from high Fe and Zn varieties (AIMP 92901 and GB 8735) that might lead to identification of progenies with still higher Fe and Zn content. Larger within-population variability (three-fold) was observed in about 65 (S_2/S_3) progenies of AIMP 92901 for grain Fe (40.9 to 118.9 mg kg^{-1}) and Zn (31.8 to 82.7 mg kg^{-1}) content and in GB 8735 for grain Fe (45.5 to 108.3 mg kg^{-1}) and Zn content (33.8–70.5 mg kg^{-1}) with higher broad-sense heritability indicating that there is potential for effective selection in these open-pollinated varieties for grain Fe and Zn. The progenies with higher Fe (> 80 mg kg^{-1}) and Zn (> 60 mg kg^{-1}) content could be used for random mating to further enhance their Fe and Zn levels through recurrent selection. The progenies with higher Fe and Zn content with good agronomic traits, good combining ability and downy mildew resistance would be used for breeding hybrid parents to produce high-yielding hybrids.

Another study conducted to assess whether selection for grain yield via recurrent selection has any associated changes in grain Fe and Zn content in initial and advanced generation recurrent cycle composite bulks, revealed non-significant differences between initial/original and their advanced generation recurrent cycle composite bulks for grain Fe and

Zn content. This implied that the selection for grain yield during recurrent selection cycles did not cause significant changes in the grain Fe and Zn content. The available bulks of different selection cycles and of different composites were used in this study to arrive at this conclusion. Recurrent selection for high grain Fe and Zn in minimum two-three high-yielding, widely grown open-pollinated varieties such ICTP 8203 or GB 8735 or AIMP 92901 need to be done at least two-three cycles to assess the effect of selection for grain Fe and Zn on these micronutrients and grain yield.

The correlation of grain Fe with Zn and the relationship of both with other agronomic traits are important to decide selection scheme and breeding strategies. In pearl millet days to 50% flower and 1000-grain mass are likely to influence grain Fe and Zn, because it is assumed that longer the maturity duration more is the time available for micronutrient absorption and accumulation, and larger the seed size more is the endosperm and aleurone layer accommodating more micronutrients. Further, early-maturity and larger seed size are important farmers-preferred traits. Hence, in this study correlations of Fe and Zn with days to 50% flower and 1000-grain mass were estimated.

Highly significant and positive correlation was observed between the grain Fe and Zn content in all the experiments, indicating either genetic factors for Fe and Zn content are linked or there are inter connected physiological mechanisms for the accumulation of these micronutrients. The direction and intensity of correlation suggested a possibility of simultaneous genetic improvement for the elevated levels of both micronutrients in common agronomic background. Similar correlation between grain Fe and Zn content has been observed in other cereal crops such as maize (Maziya-Dixon *et al.*, 2000), wheat (Graham *et al.*, 1999) and sorghum (Reddy *et al.*, 2005).

In general, there was negative correlation of days to 50% flower with grain Fe and Zn indicating possibility of breeding micronutrient-dense Fe and Zn lines in early-maturity background, which was also supported by the negative and significant correlation in set I and set II trials of rainy season. Similar negative relationship of grain Fe and Zn content with days

to flower were observed in sorghum (Reddy *et al.*, 2005). Early-maturity is a preferred trait in drought prone areas as it helps in drought escape. Early-maturing cultivars also fit in different cropping systems. Hence, combining early-maturity with high Fe and Zn in desirable genetic background would be possible due to the negative association of days to 50% flower with grain Fe and Zn content, which allows simultaneous selection for these traits.

Significant positive but weak correlations of 1000-grain mass with Fe and Zn in set I and set II trials indicated that breeding for the higher levels of these micronutrients should not reduce the prospects of combining it with large seed size. The strong association between Fe and Zn content and in turn both with 1000-grain mass provide opportunities to select micronutrient-dense lines with bold seeds, which are preferred in some areas. In addition, large grain size (mass) improves processing quality of the grain, increasing the ease of decortications and improving flour yield with both commercial milling and hand-pounding milling methods (Rooney and McDonough, 1987). Also, large grain mass is advantageous in crop establishment, conferring improved rates of seedling emergence, plant stands, faster initial seedling growth and early crop growth (Lawan *et al.*, 1985). Overall, the results suggested that it is possible to deliver high Fe and Zn content in cultivars with farmer's preferred traits such as early-maturity and bold grain.

5.2. STABILITY OF GRAIN IRON AND ZINC CONTENT

Genotype × Environment (G × E) interaction is an important component of variability plant breeding programs. Pearl millet is grown in areas with unpredictable environments of semi-arid regions. Under these variable conditions, the significance of genotype × environment interaction is large. The soil type, soil fertility, seasonal fluctuations and their interactions highly influence the performance of entries in relation to their grain yield and probably grain quality traits. Hence, the stability of grain Fe and Zn content in pearl millet were studied in four different year × season environments at ICRISAT, Patancheru.

A number of biometrical methods are available for assessing the stability of performance of the genotypes. Among them, Eberhart and Russell (1966) and Additive Main

effects and Multiplicative Interaction (AMMI) (Zobel *et al.*, 1988) models are most widely used by plant breeders. The statistical analysis applied to Eberhart and Russell model is applying only analysis of variance, which is based on linear model with additive main effects and interactions. Though it identifies the G × E interaction, it provides no insight into the particular patterns of entries or environment that give rise to G × E interaction. In the AMMI model, the additive portion is separated from interaction by analysis of variance, and then the principal component analysis (PCA) provides a multiplicative model that is applied to analyze the interaction effect from the additive ANOVA model.

Eberhart and Russell model emphasizes that a desired genotype is one with high mean performance, desired linear response (b_i) and low non-linear sensitivity coefficients ($\overline{S}_d^2$). In an AMMI biplot, the entries PCA scores plotted against mean values provide visual inspection and interpretation of the G × E interaction components. Integrating biplot display and genotypic stability statistics enables easy interpretation. If a genotype has high mean and PCA I score of nearly zero it has small interaction effect and is considered as stable variety over wide range of environments. However, the entries with high mean performance with large PCA I scores are considered as having specific adaptability to the environments (Crossa *et al.*, 1990).

In this study, 29 entries were tested in four test environments during 2004 summer (E_1) and rainy (E_2), and 2005 summer (E_3) and rainy seasons (E_4) and data were analyzed following both Eberhart and Russell and AMMI stability models.

The pooled analysis of variance estimated from both the models showed mean squares due to additive main effects such as entries and environments were highly significant for both Fe and Zn, days to 50% flower and 1000-grain mass, indicating the presence of substantial genetic variability in the mean performance of the entries over environments and in the environmental means over test entries. Another additive main effect, the G × E interaction was highly significant for grain Fe and Zn content and days to 50% flower, indicating differential response of entries under different environments. Such statistical interactions

resulted from the changes in the magnitudes of differences between entries from one environment to another but not from the changes in the relative ranking of the entries. This is because of the positive and highly significant correlations of grain Fe and Zn, and days to 50% flower among all seasons. Significant G × E interaction for grain Fe and Zn content have also been observed in crops like wheat (Monasterio and Graham, 2000), rice (Graham *et al.*, 1999), maize (Maziya-Dixon *et al.* 2000) and Bean (Beebe *et al.*, 2000). The G × E interaction was non-significant for 1000-grain mass suggesting the response of entries did not change significantly with the change in the environments.

According to Eberhart and Russell model, the genotype × environment (G × E) interaction was further partioned into environment (linear) and G × E (linear) and non-linear (pooled deviation) components. The highly significant environment (linear) component of G × E variation for grain Fe and Zn, and days to 50% flower indicated variation among the environment was linear. That means there is a unit change in the environmental index for the unit change in the environmental conditions. Since environmental index depends on the performance of the entries in each environment, the significant environment (linear) implied apparently that change in the environmental index linearly commensurated with the changes in the performance of the entries in each environment. The G × E (linear) interaction was highly significant for grain Zn content and days to 50% flower when tested against pooled error, which revealed that there were genetic differences among entries in the broad spectrum of environments and also indicated the linear sensitivity of different entries. For grain Fe content the G × E (linear) interaction was non-significant, but the deviations from regression (pooled deviation over all the entries) was significant, indicating performance of some of the entries may not be predicted.

Analysis of variance according to AMMI model showed larger portion of the total sum of squares attributable to effect of entries for grain Fe and Zn and days to 50% flower, followed by G × E interaction in case of grain Fe and Zn, and environments in case of days to

50% flower. For 1000-grain mass nearly 83% of sum of squares was attributable to effect of entries, leaving very less proportion of variation attributable to other two components, of which, environment component at 3 df was significant (P <0.05) and G × E was not significant. Of the G × E interaction sum of squares, the first principal component axis (PCA 1) was highly significant ($P < 0.01$) and captured highest proportion of interaction variance at 30 df, for grain Fe (56%) and Zn (69%) content, and days to 50% flowering (80%), which has confirmed the adequacy to the AMMI model. This made it possible to construct the biplot and calculate entries and environment effects (Gauch and Zobel, 1996; Ebdon and Gauch, 2002; Kaya *et al.*, 2002).

The environments were diverse and caused significant variation in grain Fe and Zn content, and days to 50% flower as indicated by varying environmental indices (Eberhart and Russell model, 1966) and environmental PCA scores of AMMI model (Zobel *et al.*, 1988). The average grain Fe content in E_2, E_3 and E_4 was high, resulting into positive environmental indices, hence, they were suitable environments. On the contrary, E_1 was negative environment with lesser average grain Fe. This could be attributable to the soil Fe content of the fields where the experiments was conducted. The soil Fe content in E_2, E_3 and E_4 experiments were higher than E_1. However, E_2 and E_3 had PCA scores of near-zero indicated small interaction effects in these environments, and E_1 and E_4 had PCA scores deviating from zero in opposite direction suggesting large contrasting interaction effects. For grain Zn, E_2 alone was rich environment with positive environmental index whereas E_1, E_3 and E_4 had negative environmental indices. The soil Zn content of the respective experimental sites did not really reflected the same as E_2, E_3 and E_4 had higher soil Zn content than E_1. The environments, E_2 and E_4 had small interaction effect as indicated by PCA score near to zero and the remaining two had larger interaction effects as reflected by PCA score deviating from zero. For days to 50% flower, the summer season environments (E_1 and E_2) were favorable, where flowering in general was early (negative environment indices) compared to rainy

season environments (E_3 and E_4), which showed delayed flowering. The summer season environments had negative PCA score and the rainy season environments had positive PCA scores both deviating from zero suggesting larger interaction effects.

Among the 14 high Fe and Zn entries, 7 entries for both Fe and Zn had regression coefficient around unity and non-significant mean squared deviation suggesting that these entries were stable across environments with linear response to the environment and no deviation from linearity. Three entries for both Fe and Zn content had positive regression slope significantly deviating from unity suggesting the above or below average response of these to the changing environments, indicating their better adaptation to the favorable environments. Whereas, among the 14 entries with high grain Fe and Zn content, significant mean squared deviation was observed only in 3 entries, AIMP 92901 S1 183-2-2-B, SDMV 90031 S1-84-1-1-2-B and ICMB 00888 for both Fe and Zn content and, in an advance population progeny AIMP 92901 S1-15-1-2-B for Fe content, and in a seed parent 843B for Zn content, suggesting that the performance of these entries were highly fluctuating or unpredictability of its performance to the changing environments indicating their better adaptation to the favorable environments.

Among the top entries for grain Fe and Zn content, a seed parent 863B (75.1 mg kg^{-1} Fe and 53.9 mg kg^{-1} Zn) had non-significant deviation from regression (9.9 to 14.9) indicating its consistent response to the varying environment conditions and regression coefficient around unity (b_i = 0.68 to 0.69) indicating that fairly dynamic as the mean Fe and Zn content identically increase or decrease with changed environment and hence the response is predictable. For Zn content, an advance population progeny AIMP 92901 S1-15-1-2-B (62.6 mg kg^{-1}) had regression coefficient around unity (b_i = 0.82) with non-significant mean squared deviation (5.0) indicating their better stability over the varying environmental conditions.

The regression coefficient around unity and non-significant mean squared deviation were observed in all the medium and low entries (except 7 entries with significant regression

co-efficient for Zn content) for both Fe and Zn content suggesting that by and large these as stable across environments.

All the 14 high Fe and Zn content entries were early flowering (except 1), of which 7 had regression coefficient around unity with all of them having non-significant mean squared deviation (except ICMB 98222), indicating their average response to the environmental changes. Of these, 6 entries had positive regression slope significantly deviating from unity (b_i = 0.56 to 1.80; P <0.01), indicating its above or below average responses to changing environments. Among the medium and low Fe and Zn entries, regression coefficient around unity and non-significant mean squared deviation were observed for days to 50% flower (except 7 entries with significant regression co-efficient for Zn content) suggesting that by and large these were stable across environments.

Interpretation of G × E interaction in the AMMI analysis was done using biplot graph. The biplot shows not only average performance of the entries but also how it is achieved. Among 29 entries, 18 entries for grain Fe and 14 entries for grain Zn had PCA scores nearly zero irrespective of their main effects, indicating higher stability of these entries across environments with smaller interactions for both the traits. Of the 14 entries with high grain Fe and Zn, three, that consisted one each of seed parent (863B), population progeny (AIMP 92901 S1-15-1-2-B) and pollinator IPC 843 had higher main effects and smaller interaction effects as revealed by their PCA scores (near to zero) suggesting that the rank orders of these were stable for both grain Fe and Zn across environments. In addition to that, an early-maturing seed parent 843B and a population progeny ICMS 7704-S1-51-4-1-1-B were stable for grain Fe alone and four entries (EEBC, GB 8735, AIMP 92901 S1-183-2-2-B and ICMB 98222) were stable for grain Zn content alone. However, all the latter four had positive PCA scores deviating from zero indicating their probable adaptation to rainy season environments (E_4 and E_2).

For days to 50% flower, the entries had varying main effects but most of them (except 7) had smaller interaction effects indicating stability of entries across the environments. The summer season environments had negative PCA score and rainy season environments had positive PCA scores, both deviating from zero suggesting larger interaction effects. Most of the entries with high Fe and Zn content also showed stability in flowering behavior with their PCA scores nearly zero.

Based on both Eberhart and Russell and AMMI models, 863B was stable across the environments, as it had high grain Fe and Zn content, unit regression and non-significant deviation from regression as well as less interaction PCA scores. Apart from this, based on AMMI model, ICMB 88004 and 843B were also identified as stable genotypes across the environments with smaller interaction effects for both grain Fe and Zn content. The interpretation of stability of entries was more effective through AMMI model as the PCA scores were calculated based on the principal component analysis (a multiplicative analysis), which is executed on the covariance matrix of entries and environments, in addition to the calculation of main effects through additive ANOVA model.

5.3. GENE ACTION FOR GRAIN IRON AND ZINC CONTENT

An understanding of the inheritance of grain Fe and Zn content is essential for systematic and efficient genetic enhancement of grain Fe and Zn content in pearl millet. Genetic analyses were conducted in this study using a set of 10 × 10 diallel crosses according to Griffing's (1956), and Hayman's (1954) numerical and graphical approaches. This provides genetically valuable information within the framework of assumptions of diallel analysis (Christie and Shattuck, 1992).

Before discussing and interpreting the results on the estimates of genetic components of variation and derived genetic ratios, it is necessary to fulfill the assumptions of diallel analysis such as i) homozygous parents, ii) segregation is of diploid nature, iii) reciprocal crosses do not differ significantly, iv) multiple allelism does not exist, v) non-allelic

interaction is absent, and vi) genes are independently distributed among parents. Since, pearl millet is a cross-pollinated diploid species and the parents used in this study are homozygous lines, two of the six assumptions i.e., requirement of diploid segregation and homozygous parents are met. The assumption that the reciprocal crosses do not differ significantly was met in this study, especially for grain Fe and Zn as there was non-significant reciprocal effect. The assumption of independent distribution of genes among parents and the assumption of no multiple-allelism are made to simplify the model. Non-allelic interaction can always be tested as a null hypothesis. The multiple allelism can be detected in absence of epistasis and the basic requirement of Hayman's approach is to test presence or absence of non-allelic interaction (epistasis). The non-significant uniformity test and the unit regression of parent-offspring co-variance (Wr) on parental variance (Vr) and scatter of array points along the regression line for grain Fe and Zn, days to 50% flower and 1000-grain mass revealed the absence of epistasis, hence, confirming the validity of the additive-dominance model. When epistasis was ruled out, to know whether additive or dominance gene action is operative for the all the traits, dominance relationship was studied.

The overall difference between the hybrids and parental means averaged over all loci $((ML_1\text{-}ML_0)^2)$ and the regression line in the Wr-Vr graph intercepting above the origin on the covariance axis for grain Fe and Zn, and 1000-grain mass indicated partial dominance type of gene action operating for these traits. Further, the general combining ability (*gca*) of parents and specific combining ability (*sca*) effects of hybrids were also significantly different for all traits, indicating that both additive and non-additive effects were important in controlling the expression of these traits. However, the predictability ratio measured by $2\sigma^2gca/(2\sigma^2gca + \sigma^2sca)$ was around unity for grain Fe and Zn content, and 1000-grain mass, which implied the preponderance of additive gene action. Further, highly significant positive correlation between mid-parent value and hybrid performance, and no correlation between mid-parent value and mid-parent heterosis for grain Fe and Zn, and 1000-grain mass revealed

predominant role of additive gene action for these traits. Some of the earlier studies have also reported the greater importance of additive gene action (*gca* effects) for grain Fe and Zn content in crops like maize (Gorsline *et al.*, 1964; Arnold and Bauman, 1976 and Long *et al.*, 2004) and rice (Gregorio, 2002). The greater importance of additive gene action for 1000-grain mass in pearl millet was reported by Tyagi *et al.* (1975; 1982).

In case of days to 50% flower, the higher difference between the hybrids and the parental means averaged over all loci, and the regression line intercepting the Wr axis just below the origin was indicative of the overdominance type of gene action, which could result also from non-independent distribution of genes in the parents (in repulsion phase). Further, the predictability ratio $[2\sigma^2gca/(2\sigma^2gca + \sigma^2sca)]$ less than unity indicated importance of non-additive gene action. The predominance of non-additive gene action was also evident from the poor correlation between mid-parent value and hybrid performance, and highly significant correlation between mid-parent value and mid-parent heterosis. The non-additive gene effects for days to 50% flower were earlier reported by Khangura (1975), Kapoor *et al.* (1979) and Choi *et al.* (1989).

Under the additive-dominance genetic model, the total sum of squares partitioned into additive (D) and non-additive (H_1) genetic components. Analysis of variance showed significant additive and non-additive effects for all the traits studied. Further, the proportion of additive component was higher than non-additive component for grain Fe and Zn content, and for 1000-grain mass reflecting partial dominance. However, the additive component of variance for days to 50% flower was slightly lower than non-additive component suggesting prevalence of over-dominance in lower magnitude. This again confirms the observation made earlier using Wr-Vr graph and differences of hybrid and parental means averaged over all loci.

The overall dominance effect (h^2) was significant for grain Fe, days to 50% flower and 1000-grain mass, but it was not significant for grain Zn content, this affirms the observation

made based on the differences between hybrid and parental means over all loci, where the difference in case of Zn was almost negligible. The proportion of positive and negative genes (H_2) was significant for all the four traits and near-equality of H_1 and H_2 for grain Zn and days to 50% flower indicated positive and negative genes are in equal frequencies at all the loci exhibiting dominance. This was also supported by non-significant F statistic, mean co-variance of additive and non-additive components for these traits. For grain Fe, the size of H_1 and H_2 differed ($H_1 > H_2$) suggesting asymmetric distribution of dominant and recessive alleles, and the positive significant F statistic indicating the dominant alleles were more frequent than recessive alleles. In case of 1000-grain mass, $H_2 > H_1$, revealing asymmetric distribution of dominant and recessive alleles, whereas negative F statistic suggested the more prevalence of recessive alleles compared to dominant alleles. The ratio of $\{[\sqrt{(4DH_1)}+F)/[\sqrt{(4DH_1)}-F)\}$ also established the same facts on the distribution of dominant and recessive alleles in the parents as revealed by the relative sizes of H_1 and H_2.

The narrow-sense heritability (h^2_n) obtained from the ratio of additive variance to total phenotypic variation, was higher for grain Fe (80%) and Zn content (77%) and 1000-grain mass (67%), indicating additive genetic variance is a major component of the total genetic variance arising from the loci controlling for these traits. Whereas, relatively lower level of narrow-sense heritability was observed for days to 50% flower (56%), suggested both additive and dominance gene action playing significant role in inheritance of time to flower.

The larger *gca* effects are the general indicators that additive gene action is more important for the inheritance of these traits. The significant contribution of GCA in explaining genotypic variation indicated that, in general, *per se* line performance would be a good indicator of hybrid performance for grain Fe and Zn content among these inbreds. This fact was also confirmed as the correlation between *per se* performance with *gca* effects was positive and highly significant for grain Fe and Zn, and 1000-grain mass. The high Fe and Zn content parents were the best general combiners having positive significant *gca* effects and

the parents with low grain Fe and Zn content had significant negative *gca* effects. Considering the mean values and *gca* effects together for grain Fe and Zn content, the parents AIMP 92901 S1-15-1-2-B and 863B were identified as the best combiners for further breeding programs.

The distribution of array points in the Wr-Vr graph indicates the parental order of dominance; the parents near to the origin are supposed to have maximum dominant alleles and those on the other end having maximum number of recessive alleles. The scattered array points on regression line indicated diversity among the parents for all the four traits studied. Based on distribution of array points on Wr-Vr graph, the parent ICMS 8511-S1-17-2-1-1-B was found to possess excess of dominant alleles for both grain Fe and Zn content, as the array points were nearby the origin. However, the parents with high Fe and Zn content, ICMB 94111 (for Fe content), and AIMP 92901 S1-15-1-2-B (for Zn content), possessed excess of recessive alleles as they were far away from the origin. Examining the size of (Wr+Vr) values, which is inversely proportional to the parental order of dominance, drew similar conclusion.

For 1000-grain mass, the parent 863B positioned near the origin, possessing excess of dominant alleles, whereas the parent 81B was clustered away from the origin hence possessing excess of recessive alleles. For days to 50% flower the parents, 81B and MC 94 C2-S1-46-1-1-B possessed excess of dominant alleles and the parent ICMV 93074 S1-9-1-1-1-B possessed excess of recessive alleles since the array points were nearby origin and away from the origin, respectively. The remaining array points, for all the traits occupied intermediate positions, indicating nearly equal distribution of dominant and recessive alleles.

The correlation ($r_{Yr.\ (Wr+Vr)}$) between parental measurement, Yr, and the parental order of dominance (Wr+Vr), was significant and positive for grain Fe and Zn content, days to 50% flower (positive) and 1000-grain mass (negative), indicating most dominant alleles act in one direction and recessive alleles in the other. Further, association pattern also confirmed the

results of Wr-Vr graph with respect to the distribution of dominant and recessive genes among the parents. In general, the less Fe and Zn content in parents was due to higher frequency of dominant alleles with negative effects, and higher Fe and Zn content in parents was due to recessive alleles with positive effects. The high grain Zn parent 863B and low Zn parent ICMV 91059 S1-14-2-4-2-2-B were exceptions, where the former had higher frequency of dominant alleles with positive effects and the latter had recessive alleles with negative effects. The late-flowering in parents was mostly due to higher frequency of recessive alleles with positive effects, except for 81B in which it was due to higher frequency of dominant alleles with positive effects. The early-flowering in the parents was due to dominant alleles with negative effects except for AIMP 92901 S1-15-1-2-B in which it was due to recessive alleles with negative effects. In general, higher 1000-grain mass in parents was due to dominant alleles with positive effects except for ICMB 94111, in which it was due to recessive alleles with positive effects. The low 1000-grain mass in parents was due to recessive alleles with negative effects. The standardized deviation graph showed that the less grain Fe and Zn content in ICMS 8511 S-17-2-1-1-B was governed by dominant alleles with negative effects and the high grain Fe in the parent ICMB 94111 and high grain Zn content in the parent in AIMP 92901 S1-15-1-2-B was governed by recessive alleles. Similarly, for days to 50% flower, the earliness in ICMB 00888 was governed by dominant alleles and lateness in 81B was governed by recessive alleles. For 1000-grain mass, high grain mass in 863B is governed by recessive alleles and the low grain mass in 81B is governed by dominant alleles.

Predominance of fixable i.e., additive genetic variance coupled with comparatively higher narrow sense heritability with almost equal frequency of genes exhibiting increasing and decreasing effects for grain Fe and Zn content and 1000-grain mass suggested high effectiveness of progeny selection, pedigree selection or backcross breeding to develop lines and populations with increased levels of grain Fe and Zn and 1000-grain mass. The higher additive component of variance for these traits with higher magnitude of heritability also

prompts for recurrent selection method to improve the levels of grain Fe and Zn content. The rate of population improvement is directly proportional to the magnitude of additive variance.

5.4. HETEROSIS

Heterosis is the superiority in performance of hybrids with respect to their parents. The magnitude of heterosis usually depends on genetic divergence of parental lines. The hybrid with either significant positive or negative heterosis over the mid-parent indicates dominance of positive or negative genes. The hybrids with significant positive or negative heterosis over better parent indicates over-dominance of positive or negative genes and the hybrids where the F_1 value do not deviate significantly from their mid-parent values is indicative of additive gene effects.

There were significant differences between parents *vs* hybrids for grain Fe, days to 50% flower and 1000-grain mass indicated that there is possibility of selecting hybrids with significant difference over parents, whereas it was non-significant for grain Zn content. Frequency distribution of hybrids for mid-parent heterosis for grain Fe content indicated lesser frequency of hybrids (24%) with positive mid-parent heterosis compared to those having negative mid-parent heterosis and it was similar with better-parent heterosis. This result suggested that there is less likely of producing hybrids better than parents for grain Fe content. This could be attributed to the presence of more frequent dominant (negative effect on Fe content) alleles than the recessive alleles (positive effect on Fe content) among the parents. The parents with high Fe content produced hybrids with lower level of positive heterosis in lower frequency. None of the hybrids with high Fe content showed positive significant heterosis over its better-parent.

For grain Zn content, 53% of hybrids had positive mid-parent heterosis indicating almost equal frequency of hybrids with positive and negative mid-parent heterosis, whereas only 16% of hybrids had positive better-parent heterosis. This could be attributed to the distribution of equal frequency of dominant (negative effect on Zn) and recessive (positive

effect on Zn) alleles among the parents. Out of these positive mid-parent heterotic hybrids, around 60% of hybrids based on at least one high parent, indicating the parents with high Zn content produced hybrids with higher level of positive heterosis. The parents performing as good as hybrids for grain Fe and Zn was due to the high additive genetic variance as revealed by high predictability ratio suggesting predominant additive gene action operating for these traits. Further, the favorable alleles came from high Fe and Zn content lines, which had more frequency of recessive alleles with increasing effects on grain Fe and Zn content. This showed that there would be little opportunity, if any, to exploit heterosis for these micronutrients, and that high Fe and Zn levels in both parental lines will be required to breed high Fe and Zn hybrids.

Frequency distribution of mid-parent heterosis for days to 50% flower showed 97% hybrids possessing negative heterosis (up to -20%). About 75% hybrids had negative significant better-parent heterosis, i.e., flowering earlier than the early flowering parent. This could be either due to over-dominance or due to repulsion phase distribution of dominant genes. Among the negative mid-parent and better-parent heterotic hybrids, 67% of hybrids were based on at least one parent that had high Fe and Zn, indicating that the parents with high Fe and Zn content would produce early-flowering hybrids. Frequency distribution of hybrids for 1000-grain mass showed about 50% of hybrids had positive better-parent heterosis. About 40% of these hybrids had at least one parent with high Fe and Zn had positive better-parent heterosis for 1000-grain mass, indicating that the parents with high Fe and Zn content would produce hybrids with higher level of positive heterosis for seed size.

5.5. SCREENING EFFICIENCY FOR GRAIN IRON AND ZINC CONTENT

Selection for cryptic quality characters especially for trace elements, such as grain Fe and Zn content, demands costly and time consuming laboratory analysis. The magnitude of genetic variability can be comprehensively assessed and utilized in breeding, if the larger numbers of genotypes are studied. But, it would not be effective when the chemical analysis is

time consuming and costly. In such a case, it would not be possible to screen large number of genotypes or progenies limiting the effectiveness and progress of selection. The cost of the chemical analysis also varies for different methods of estimation and for different laboratories. The estimation cost of micronutrient also involves the cost of production and preparation of grain sample, which many a times escalate the total cost of micronutrient estimation.

In this study, three-pronged approach was followed to examine if the cost of grain Fe and Zn analysis could be reduced: (i) the reduction of the cost involved in the grain sample production, (ii) the management of analytical cost of Fe and Zn through estimation of inter-laboratory correlations and (iii) the use of staining technique for rapid and cheaper analysis of grain Fe content, as a discarding procedure when analyzing a large number of samples so that only a selected set of it only goes for laboratory analysis.

Production of selfed or open-pollinated (OP) seeds for micronutrient analysis is more cost-effective than producing sib-mated grain. It was observed that correlation between the selfed and sib-mated seed for both Fe and Zn was highly significant and positive, suggesting that the grain samples obtained through selfing could be as good as the sib-mated seeds for the grain Fe and Zn analysis. The use of open-pollinated grain for the grain Fe and Zn analysis was not found reliable due to possible contamination with the dust particles, which is a major sources of error in the estimation of Fe content.

A randomly selected 12 entries with a wide range of grain Fe and Zn levels based on the results from the laboratory analysis at NIN, Hyderabad were also analyzed at ICRISAT-Patancheru, India and at the Waite Analytical laboratory, Adelaide University, Australia as the cost of estimation of grain Fe and Zn in these laboratories is less than at NIN. The correlations for grain Fe and Zn content among the three laboratories were highly significant, indicating reliability of the results from any of these three laboratories. Since, the cost of grain Fe and Zn analysis at ICRISAT laboratory is only US$ 2 compared to NIN (US$ 20) and

Waite (US$ 12), it could be possible to screen large number of samples initially at ICRISAT and the selected few samples would be analyzed in either NIN or Waite laboratory for confirmation of the results from ICRISAT laboratory.

Grain Fe content of pearl millet will be quantitatively measured by chemical analysis using Atomic Absorption Spectrophotometry method (AAS) or ICP, which is costly and time consuming, hence not suitable to analyze large number of breeding lines or germplasm accessions. Further, the time taken to receive the analyzed results will also be more. The application of the Prussian blue stain has made possible the rapid estimation of grain Fe. This technique has recently been used to estimate the presence of Fe in the aleurone layer of rice grain (Prom-u-thai *et al.*, 2003) using stereomicroscope. However, that study only indicated the location of Fe in the aleurone layer. There was no information on Fe in other parts of the grain. In this study, Perls' Prussian blue was used to stain grain flour, and intensity of staining was used as a qualitative trait to distinguish entries with contrasting grain Fe content (high and low). The flour samples, when submerged with 2% Prussian blue solution (Prom-u-thai *et al.* 2003), in the Petri dishes, produced blue in entries having medium (less intense) to high Fe (more intense). No color developed in the entries with low iron content (31.2–40.6 mg kg^{-1}). The Prussian blue staining was effective in differentiating entries with high and low Fe content. The rank correlation of estimated grain Fe content and the color score was highly significant and positive (r=0.92; P <0.01) indicating that the higher Fe content in the grain, the more intense blue color. The intensity of blue color did not differ with varying concentrations (1% to 5%) of Prussian blue solution in high, medium and low entries. However, the color development was slow when treated with 1% solution. So the Prussian blue solution of 2% concentration was found to be effective in distinguishing the high and low iron entries. This standardized protocol using 2% Prussian blue solution was validated using 20 diverse B-lines with wide range of Fe content (32.4–69.0 mg kg^{-1}), where the similar results were observed indicating that the method is highly efficient for selecting B-lines with high grain Fe content.

This Prussian blue method could be used at room temperature and no costly and specific equipment is necessary other than Pestle and Mortar or cyclone mill (grinder) and simple glasswares. Using Pestle and Mortar for grinding, approximately 60-80 samples per day could be analyzed with two technicians and using cyclone mill approximately 120-140 samples could be analyzed. The estimation of grain Fe content with AAS or ICP involves higher cost, which ranges from US$ 1 to US$ 10 in various laboratories (excluding the shipping cost of the material). The chemical cost per sample in Perls' Prussian blue protocol used in this study, is about US$ 0.4 (when 500 g of potassium ferrocyanide approximately costs US$ 10 and 500 ml concentrated HCl approximately costs US$ 11) and cost of the technicians per sample would add another US$ 0.1 (when salary of each technician is approximately US$ 225 per month), hence making the total cost to US$ 0.6 per sample. Thus, this is simple, rapid and inexpensive method compared to the chemical analysis. This could be effectively used as the initial method of screening, and the genotypes identified for high grain Fe from this protocol could be subjected to actual laboratory analysis using AAS or ICP. This would save the cost involved in quantitative estimation of grain Fe. The highly significant positive correlation ($r = 0.84$; $P<0.01$) between grain Fe and Zn observed in this crop implies that this method would also be useful for indirect selection of genotypes in the high grain Zn group. Overall, the protocol was very efficient in distinguishing the entries with high and low Fe content and intensity of blue color served as qualitative selection criteria for grain Fe in pearl millet. When the large number of germplasm accessions or large number of progenies have to be screened for Fe content, this method will be useful in selecting pearl millet accessions or progenies with high Fe content or reject those with low Fe content.

5.6. FUTURE PROSPECTS

- Adequate genetic variation was found for grain Fe and Zn content in the improved populations and their progenies and also in the parental lines of hybrids. Initial results showed that selection for grain yield had no drastic effect on the grain Fe and Zn content of populations. Significant intra-population variability was observed for grain

Fe and Zn. Further studies are essential to assess the effect of selection for higher grain Fe and Zn on grain yield. The identified material for higher grain Fe and Zn, especially population progenies will need to be screened for downy mildew resistance.

- Since environment, especially soil type, has a strong influence on mineral content of grains, there is a need to validate these identified materials for stability of grain Fe and Zn across diverse soil types and soil fertility levels typical of the areas for which these cultivars are targeted. This is because pearl millet is grown in different soil types with varying levels of native soil fertility with/without farmer's managed fertility in India.
- Since the positive high correlation was observed between grain Fe and Zn content, and 1000-grain mass, selection for increased grain Fe and Zn content can be effectively made along with large seed size, which could lead to higher yields as there is positive correlation between grain yield and grain mass (Singh and Govila, 1989; Bidinger *et al.*, 1993). Hence, the traditional breeding component seeks not only to elevate the overall Fe and Zn content of elite breeding lines but also to combine the high Fe and Zn levels with other distinguishing features that are attractive to farmers and consumers.
- The ability of the plant to successfully acquire, distribute, utilize and store micronutrients in edible parts involves number of physiochemical mechanisms. It is interesting to note that wide differences exist in whole plant partitioning of micronutrients between vegetative and reproductive tissues in wheat (Grusak, 1994). Investigating the mechanisms of Fe and Zn uptake in the plants and their translocation to grains is an interesting area of research. Understanding the mechanism of micronutrient partitioning will help in modification of source-sink relationship in the plants to enhance the micronutrient mobilization to grains.
- According to Garnett and Graham (2005) increased Fe availability in the soil does not increase its content in the grain. This implies that simply increasing Fe levels by increased root uptake capacity or foliar fertilization would not be an effective way of

increasing grain levels of Fe and Zn. The research on phloem transport pathway indicates that breeding for better expression of the phloem pathway would be the best approach to improve the nutritional value of cereal grains. The characterization of the phloem transport pathways of Fe and Zn in pearl millet could be done.

- In the current study, majority of the increased grain Fe and Zn content lines used for genetic study were based on *iniadi* germplasm. The lines from this background might possess a valuable uptake transport and/or deposition strategy for moving Fe and Zn into the grain, whereas some other background might have increased reproductive remobilization. These hypotheses are worth investigation.
- The present study successfully identified contrasting parents for grain Fe and Zn content in pearl millet that could be used in the development of mapping populations for molecular markers identification. This has opened up an area of identification of quantitative trait loci (QTL) for grain Fe and Zn. Molecular marker approach might play a vital role in enhancing the efficiency of breeding efforts for increasing the Fe and Zn in pearl millet.
- Just increasing the concentration of micronutrients in a plant food and supplying that to the people suffering from micronutrient deficiencies does not guarantee their improved nutritional status. This is because not all the micronutrient in a plant food is bioavailable to persons consuming the food. Pearl millet grains contain phytates and other anti-nutritional factors as they form complexes with Fe and Zn, thus interfering with their bioavailability. Determining the bioavailability of Fe and Zn in the genetically enhanced new lines will provide the useful information on the available Fe and Zn to the target human population.

SUMMARY

CHAPTER 6

SUMMARY

The present study was undertaken to determine the magnitude of genetic variability for grain iron (Fe) and zinc (Zn) among diverse range of breeding lines, improved populations (among and within populations) and germplasm accessions; to assess the stability across different environments; to examine the relationships between them and with days to 50% flower and 1000-grain mass; and to develop an understanding of the nature of inheritance.

- A wide range of genetic variability, over two-fold was found for both grain Fe (30.1–75.7 mg kg^{-1}) and Zn (24.5–64.8 mg kg^{-1}) content in set I trial consisting of breeding lines, improved populations and germplasm accessions; and approximately two-fold variation (42.0–79.9 mg kg^{-1} Fe and 24.2–51.7 mg kg^{-1} Zn) in set II trial consisting of 69 improved populations originated from India and Africa with medium to high broad sense heritability, indicating good scope for effective selection for increased grain Fe and Zn content.

- In set I trial, largest variability and the highest values for both Fe and Zn were found in elite genetic background such as seed parents (863B and ICMB 88004) of released hybrids and the inbred lines derived from improved open-pollinated varieties (AIMP 92901 S1-15-1-2-B), besides in released open-pollinated varieties AIMP 92901 and GB 8735, cultivated in India and Africa, respectively. In set II trial, ICTP 8203, a high yielding, early-maturing and bold seeded commercial variety developed from *iniadi* land race had highest grain Fe and also high Zn content.

- The lines and populations with high Fe and Zn contents were often those largely based on *iniadi* germplasm (known for early-maturity and large seed), which has made the greatest impact on varietal and hybrid development in India and parts of Africa. Exploitation of this germplasm base for identifying sources with still higher Fe and Zn content would generate new set of breeding lines and genetic stocks for both varietal and

hybrid development. Breeding materials in elite genetic backgrounds that include released varieties and hybrid parents with high Fe and Zn contents, identified in this study, could be exploited for quick development of cultivars with high Fe and Zn and the consequent impact on nutritional security.

- Evaluation of about 65 progenies (S_{2-3}) derived from AIMP 92901 and GB 8735 showed two-to-three-fold within-population variability for grain Fe (40.9–118.9 mg kg^{-1} in AIMP progenies and 45.5–108.3 mg kg^{-1} in GB 8735 progenies) and for grain Zn (31.8–82.7 mg kg^{-1} in AIMP 92901 progenies and 33.8–70.5 mg kg^{-1} in GB 8735 progenies), suggesting good prospects of selection within-population for improvement of grain Fe and Zn.
- Large genetic variability detected within the populations (intra-population variability) indicated that there are prospects of enhancing the grain Fe and Zn levels by recurrent selection and also the progeny selection for development of inbred lines with higher Fe and Zn content. If such inbred lines selected for high grain Fe and Zn are good combiners for grain yield, they could be directly used in hybrid breeding. Otherwise, they could be used as source material and backcross breeding could be employed to transfer the high Fe and Zn content in good general combining lines using molecular marker-assisted selection. Since, most of the population progenies with high Fe and Zn content were of medium height, these lines could also be used for the development of potential pollinators, as most of the pollinators of the released hybrids are likely to have lower levels of grain Fe and Zn content.
- The study of initial and advanced cycle bulks of five composites improved for grain yield indicated that the selection for grain yield during recurrent selection cycles might not cause significant changes in the grain Fe and Zn content.
- Highly significant positive correlation between Fe and Zn showed good prospects of simultaneous genetic improvement for both micronutrients. Also, positive significant association with 1000-grain mass and negative association with days to 50% flower

suggested that it is possible to select for the high grain Fe and Zn genotypes in early-maturity and large-seed background as these are increasingly becoming farmer-preferred traits in most of the pearl millet growing areas.

- Highly significant positive correlations between sib-mated and selfed grain sources suggested to use selfed grain as suitable grain sample for grain Fe and Zn content analysis, as sib-mating is costly and open-pollinated grains are likely to carry dust contamination leading to over estimation of Fe and Zn values.
- The highly significant positive correlations of laboratory estimates of Fe and Zn among National Institute of Nutrition (NIN), Hyderabad, ICRISAT-Patancheru and Waite Analytical laboratory, Adelaide University, Australia showed the repeatability and consistency of grain Fe and Zn estimates across the laboratories.
- Based on both Eberhart and Russell and AMMI models, significant differences existed among environments and the entries. Highly significant G × E interaction was observed for Fe and Zn, and days to 50% flower, and such statistical interaction was of non-cross over type, as the correlation between seasons were highly significant and positive for Fe and Zn and days to 50% flower. A major portion of variance was attributable to genotypic effects (main effects) rather than environmental and genotype × environment interaction effects for both grain Fe and Zn content, indicating that the G × E interaction might not be serious.
- Further, the AMMI model of G × E analysis also showed that the rank orders of most of the entries for grain Fe and Zn were stable across environment as they had small interaction effects. This could be mainly attributed to the fact that the study was conducted in Alfisols at only one location with varying levels of soil Fe and Zn content. Hence, the experiments in diverse soil types and at diverse conditions would be essential to judge the nature of G × E interaction and stability of the entries. However, the seed parents 863B, 843B and ICMB 88004 with high grain Fe and Zn and stable across environments could be used as sources for further genetic improvement.

- Genetic component analysis using Hayman's graphical and numerical approach in a set of 10 × 10 diallel crosses revealed absence of epistasis for all the traits, due to non-significant uniformity test and unit regression of parent-offspring co-variance (Wr) on parental variance (Vr) confirming the adequacy of additive-dominance model.
- The general combining ability (*gca*) of parents and specific combining ability (*sca*) effects of hybrids were significantly different for all traits, indicating that both additive and non-additive effects were important in controlling the expression of these traits. However, the predictability ratio measured by $2\sigma^2gca/(2\sigma^2gca + \sigma^2sca)$ was around unity for grain Fe and Zn content, and 1000-grain mass implying the preponderance of additive gene action. Further, highly significant positive correlation between mid-parent value and hybrid performance, and no correlation between mid-parent value and mid-parent heterosis for grain Fe and Zn, and 1000-grain mass revealed predominant role of additive gene action for these traits. The regression line in Wr-Vr graph intercepting above the origin on the covariance axis for grain Fe and Zn, and 1000-grain mass again confirmed the partial dominance type of gene action operating for these traits.
- The parents with low Fe and Zn content, such as ICMS 8511-S1-17-2-1-B had excess of dominant alleles with decreasing effect on Fe and Zn content and the parents with high Fe and Zn content, such as ICMB 94111 (for Fe) and AIMP 92901 S1-15-1-2-B (for Zn) had excess of recessive alleles with increasing effects.
- Higher magnitude of narrow-sense heritability for grain Fe and Zn content and 1000-grain mass, indicated additive genetic variance is a major component of the total genetic variance arising from the loci controlling for these traits. Low level of heterosis over mid-parent for grain Fe and Zn content, with less frequency of hybrids with higher performance suggested that there would be little opportunity, if any, to exploit heterosis for these traits.
- The combining ability analysis showed strong positive correlation between *per se* performance and *gca* effects for Fe and Zn suggesting that the *per se* line performance

would be a good indicator of its general combining ability. Hence, the lines could be selected based on performance *per se*. Involvement of high additive genetic factors with partial dominance, low level of heterosis and no crosses showing higher than the better-parent for Fe and Zn prompt for breeding both seed parents and restorer parents with high Fe and Zn content to produce hybrids rich with these micronutrients. This is because the favourable alleles come from high Fe and Zn content lines, which have higher frequency of recessive alleles with increasing effects on the grain Fe and Zn content.

- Predominance of fixable i.e., additive genetic variance coupled with comparatively higher narrow sense heritability for grain Fe and Zn content suggested that effectiveness of progeny/pedigree/recurrent selection or backcross breeding to develop lines and populations with increased levels of grain Fe and Zn. While employing these breeding strategies, the simple and rapid method of selection using Prussian blue technique could substantially reduces the cost and increases the speed of improvement. This screening technique could be used for initial screening of large number of breeding lines or germplasm accessions before subjecting the selected stocks to the chemical analysis with AAS.

Based on the above results, it could be concluded that there are good prospects of genetic enhancement for grain Fe and Zn content in pearl millet in the early-maturity and bold-seed backgrounds.

REFERENCES

REFERENCES

Abdalla, A.A., A.H. Tinay, B.E. Mohamed and A.H. Abdalla. 1998. Proximate composition, starch, phytate and mineral contents of 10 pearl millet genotypes. *Food Chem.*, **63**: 243–246.

Abilgos-Ramos, R.G., R.V. Manaois, S.P. Escubio, D.G. Gemmabelle. G.C. Arocena and L.S. Sebastian. 2004. Grain quality and iron density of Philippine rice cultivars. Fourth International Crop Science Congress, Brisbane, Australia.

Aggarwal, A.J. 1992. Processing of pearl millet for its more effective utilization. Ph.D. thesis, CCS HAU, Hisar, India.

Ahmadi, M., W.J. Wiebold and J.E. Beuerlein. 1993. Grain yield and mineral composition of corn as influenced by endosperm type and nitrogen. *Commun. Soil Sci. Plant Anal.*, **24**: 2409–2426.

Aksel, R. and L.P.V. Johnson. 1963. Analysis of diallel cross: A worked example. *Adv. Frontiers Plant Sci.*, **2**: 37–53.

Al-Jibouri, H.R., P.A. Miller and H.F. Robinson. 1958. Genotypic and environmental variances and co-variances in an upland cotton cross of inter-specific origin. *Agron. J.*, **50**: 633–636.

Alpana, A. 1989. A study on nutritional value and utilization of sprouted pearl millet. M.Sc. thesis. CCS HAU, Hisar, Haryana, India.

Andrews, D.J. and K. Anand Kumar. 1996. Use of the West African pearl millet landrace *iniari* in cultivar development. *Plant Genet. Res. Newsl.*, **105**: 15–22.

Annapurani, S. and N.K. Murthy. 1985. Bioavailability of iron by *in vitro* method II from selected foods/diets and effect of processings. *Indian J. Nutr. Dietel.*, **22**: 95–105.

Archana, A. 1997. Utilization of processed pearl millet for the development of weaning and supplementary foods. M.Sc. thesis, CCS HAU, Hisar, India.

Arnold, J.M. and L.F. Bauman. 1976. Inheritance and interrelationships among maize kernel traits and elemental contents. *Crop Sci.*, **16**: 439–440.

Arnold, J.M., L.F. Bauman and H.S. Aycock. 1977. Interrelations among protein, lysine, oil, certain mineral element concentrations, and physical kernel characteristics in two maize populations. *Crop Sci.*, **17**: 421–425.

Baker, R.J. 1978. Issues in diallel analysis. *Crop Sci.*, **18**: 533–536.

Banziger, M and J. Long. 2000. The potential for increasing the iron and zinc density of maize through plant breeding. *Food Nutr. Bull.*, **21**: 397–400.

Barrow, N.J. 1987. The effects of phosphate on zinc adsorption by soils. *J. Soil Sci.*, **38**: 453.

Beard J.L., H. Dawson and D.J. Pinero. 1996. Iron metabolism: a comprehensive review. *Nutr. Rev.* **54**: 295–317.

Beebe, S., A. Gonzalez and J. Rengifo. 2000. Research on trace minerals in the common bean. *Food Nutr. Bull.*, **21**: 387–391.

Bhatnagar, S.K. 2003. Coordinator's review of All India Coordinated Pearl Millet Improvement Project 2002–2003. *In*: Indian Council of Agricultural Research, Mandor, Jodhpur, India.

Borade, V.P., S.S. Kadam and D.K. Salunkhe. 1984. Changes in phytate phosphorus and minerals during germination and cooking of horse gram and moth bean. *Plant Foods Hum. Nutr.*, **32**: 151.

Bothwell, T.H., R.D. Baynes, B.J. MacFarlane and A.P. MacPhail. 1989. Nutritional iron requirements and food iron absorption. *J. Int. Med.*, **226**: 357–365.

Bouis, H.E. 2003. Micronutrient fortification of plants through plant breeding: can it improve nutrition in man at low cost?. *Proc. Nutr. Soc.*, **62**: 403–411.

Bouis, H.E., R.D. Graham and R.M. Welch. 2000. The consultative group on international agricultural research (CGIAR) micronutrients project: Justification and objectives. *Food Nutr. Bull.*, **21**: 374–381.

Branca, F. and M. Ferrari. 2002. Impact of micronutrient deficiencies on growth: the stunting syndrome. *Ann. Nutr. Metabol.*, **46**: 8–17.

Briat J.F., I. Fobis-Loisy, N. Grignon, S. Lobreaux and N. Pascal. 1995. Cellular and molecular aspects of iron metabolism in plants. *Biol. Cell*, **84**: 69–81.

Brown J.C., H.M. Cathey, J.H. Bennett and R.W. Thimijan. 1979. Effect of light quality and temperature on Fe (III) reduction, and chlorophyll concentration in plants. *Agron. J.*, **71**: 1015–1021.

Brown, K. and S. Wuehler. 2000. Zinc and human health. *In*: Micronutrient initiative: Iron fortification of soy sauce in China. Food, Nutrition and Agriculture, **32**, Ottawa, Canada. Pp. 76–82.

Bruggemann, W., K. Maas-Kantel and P.R. Moog. 1993. Iron uptake by leaf mesophyll cells: the role of the plasma membrane bound ferric-chelate reductase. *Planta*, **190**: 151–155.

Brummer, G., K.G. Tiller, U. Herms and P.M. Clayton. 1983. Adsorption-desorption and/or precipitation-dissolution processes of Zn in soils. *Geoderma*, **31**: 337–354.

Brune, A., W. Urbach and K.J. Dietz. 1994. Compartmentation and transport of zinc in barley primary leaves as basic mechanisms involved in zinc tolerance. *Plant Cell Environ.*, **17**: 153–162.

Burton, G.W., A.T. Wallace and K.O. Rachie. 1972. Chemical composition and nutritive value of pearl millet (*Pennisetum typhoides*) grain. *Crop Sci.*, **12**: 187–188.

Caballero, B. 2002. Global patterns of child health: the role of nutrition. *Ann. Nutr. Metabol.*, **46**: 3–7.

Carpenter, C.E. and A.W. Mahoney. 1992. Contributions of heme and non-heme iron to human nutrition. *Crit. Rev. Food Sci. Nutr.*, **31**: 333–367.

Chakravarty, I. and K. Ghosh. 2000. Micronutrient malnutrition-present status and future remedies. *J. Indian Med Assoc.*, **98**: 539–542.

Chauhan, B.M., N. Suneja and C.M. Bhat. 1986. Nutritional value and fatty acid composition of some high yielding varieties of bajra. *Bull. Grain Technol.*, **21**: 41–42.

Choi, B.H., K.Y. Park and R.K. Park. 1989. Combining ability estimates for major traits in crosses between male sterile and fertile inbred lines of pearl millet. *Korean J. Breed.*, **21**: 40-46.

Christie, B.R. and V.I. Shattuck, 1992. The diallel cross: Design, analysis and use for plant breeders. *Plant Breed Rev.*, **9**: 9-36.

Cichy, K.A., S. Forster, K.F. Grafton and G.L. Hosfield. 2005. Inheritance of seed zinc accumulation in navy bean. *Crop Sci.*, **45**: 864–870.

Cisse, D., A.T. Guiro, B. Diaham, M. Souane, N.T.S. Doumbouga and S. Wade. 1998. Effect of food processing on iron availability of African pearl millet weaning foods. *Int. J. Food Sci. Nutr.*, **49**: 375–381.

Cook, J.D., M.B. Reddy and R.F. Hurrell. 1995. The effect of red and white wines on non-heme iron absorption in humans. *Am. J. Clin. Nutr.*, **61**: 800–804.

Coughtrey, A. and K. Mather. 1970. Interaction and gene association and dispersion in diallel crosses where gene frequencies are unequal. *Hered.*, **25**: 79–88.

Crossa, J., R.W. Zobel and H.G. Gauch, 1990. Additive and multiplicative interaction analysis of two international maize cultivar trials. *Crop Sci.*, **30**: 493–500.

Davidsson, L., A. Almgren, B. Sandstrom and R.F. Hurrell. 1995. Zinc absorption in adult humans: the effect of iron fortification. *Br. J. Nutri.*, **74**: 417–425.

Davidsson, L., J. Mackenzie, P. Kastenmayer, P.J. Aggett and R.F.Hurrell. 1996. Zinc and calcium apparent absorption from an infant cereal: a stable isotope study in healthy infants. *Br. J. Nutr.*, **75**: 291–300.

Deosthale, Y.G., K. Visweswar Rao, V. Nagarajan and K.C. Pant. 1971. Varietal differences in protein and amino acids of grain bajra (*Pennisetum typhoides*). *Indian J. Nutr. Diet.*, **8**: 301–308.

Desai, B.B. and G.K. Zender. 1972. Nutrient composition of hybrid and local bajra crops grown in medium black soil for assessing their nutritional requirements. *J. Indian Soc. Soil Sci.*, **20**: 175–182.

Donangelo, C.M., L.R. Woodhouse, S.M. King, G. Toffolo, D.M. Shames, F.E. Viteri, Z. Cheng, R.M. Welch and J.C. King. 2003. Iron and zinc absorption from two bean (*Phaseolus vulgaris* L.) genotypes in young women. *J. Agric. Food. Chem.*, **51**: 5137–5143.

Ebdon, J.S. and H.G. Gauch. 2002. Additive main effect and multiplicative interaction analysis of national turfgrass performance trials: I. Interpretation of Genotype × Environment interaction. *Crop Sci.*, **42**: 489-496.

Eberhart, S.A. and W.A. Russell. 1966. Stability parameters for comparing varieties. *Crop Sci.*, **6**: 36–40.

Ejeta, G., M.M. Hansen and E.T. Mertz. 1987. *In vitro* digestibility and amino acid composition of pearl millet (*Pennisetum typhoides*) and other cereals. *Proc. Nat. Acad. Sci.*, **84**: 6016–6019.

Epstein, E. 1972. Mineral Nutrition of Plants: Principles and Perspectives. Wiley, New York.

Fairweather-Tait, S. and R.F. Hurrell. 1996. Bioavailability of minerals and trace elements. *Nutr. Res. Rev.*, **9**: 295–324.

FAO and ICRISAT. 1996. The world sorghum and millet economies: Facts, trends and outlook. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT): Patancheru, Andhra Pradesh, India; and Food and Agricultural Organization of the United Nations: Rome, Italy. P.72.

FAO. 1997. Human nutrition in the developing world, by M. Latham. Food and Agricultural Organization of the United Nations, Rome. Italy.

FAO. 2000. Bulletin of Statistics. **1**, Food and Agricultural Organization of the United Nations, Rome, Italy. Pp. 16–36.

FAO. 2002. State of food insecurity in the world. Food and Agricultural Organization of the United Nations, Rome. Italy.

FAO. 2003. FAOSTAT food balance sheet. Food and Agricultural Organization of the United Nations (http://apps.fao.org/faostat).

FAO/WHO. 2000. Preliminary report on recommended nutrient intakes. Joint FAO/WHO Expert Consultation on Human Vitamin and Mineral Requirements, FAO, Bangkok, Thailand, September 21–30, 1998, revised July 13, 2000. Food and

Agricultural Organization of the United Nations Rome, Italy and World Health Organization, Geneva, Switzerland.

FAO/WHO. 2002. Human vitamin and mineral requirements. Report of a joint FAO/WHO expert consultation. Rome. Food and Agricultural Organization of the United Nations Rome, Italy and World Health Organization, Geneva, Switzerland.

Fehr, W.R. 1982. Control of iron deficiency chlorosis in soybeans by plant breeding. *J. Plant Nutr.* **5**: 611–621.

Feil, B. and D. Fossati. 1995. Mineral composition of Triticale grains as related to grain protein. *Crop Sci.*, **35**: 1426–1431.

Feil, B., S.B. Moser, S. Jampatong and P. Stamp. 2005. Mineral composition of the grains of tropical maize varieties as affected by pre-anthesis drought and rate of nitrogen fertilization. *Crop Sci.*, **45**: 516–523.

Fonseca, S. and F.L. Patterson. 1968. Yield components, heritabilities and interrelationships in winter wheat (*Triticum aestivum* L.) *Crop Sci.*, **8**: 614–617.

Ganry, F. and J. Bideau. 1974. Effect of nitrogenous fertilization on the yields and nutritional value of millet, Souna III. *Agron. Trop. Nogent.*, **29**: 1006–1015.

Garnett, T.P. and R.D. Graham. 2005. Distribution and remobilization of iron and copper in wheat. *Ann. Bot.*, **95**: 817–826.

Gauch, H.G., and R.W. Zobel. 1996. AMMI analysis of yield trials. *In*: M.S. Kang and H.G. Gauch, Jr. (ed.) Genotype by environment interaction. CRC Press, New York. Pp. 85–112.

GENESTAT 8th Edition. 2002. The guide to Genstat ® release 6.1. Payne, R.W. Rothamsted Agricultural Experimental Station, UK.

Gibson, R.S. 1994. Zinc nutrition in developing countries. *Nutr. Res. Rev.*, **7**: 151–173.

Godsey, C.B., J.P. Schmidt, A.J. Schlegel, R.K. Taylor, C.R. Thompson and R.J. Gehl. 2003. Correcting iron deficiency in corn with seed row-applied iron sulfate *Agron. J.*, **95**: 160–166.

Golden, M.H.N. 1991. The nature of nutritional deficiency in relation to growth failure and poverty. *Acta Paediatrica Scandinavica*, **374**: 95–110.

Gomez, K.A. and A.A. Gomez. 1984. Statistical procedures for agricultural research. International Rice Research Institute Press, Philippines.

Gorsline, G.W., W.I. Thomas and D.E. Baker. 1964. Inheritance of P, K, Mg, Cu, B, Zn, Mn, Al, and Fe concentrations by corn (*Zea mays* L.) leaves and grain. *Crop Sci.*, **4**: 207–210.

Goswami, A.K., K.L. Sehgal and K.P. Sharma. 1969a. Chemical composition of bajra grains. 1. African entries. *J. Nutr. Diet.*, **6**: 287–290.

Goswami, A.K., K.P. Sharma and B.K. Gupta. 1969b. Chemical composition of bajra grains. 2. American entries. *J. Nutr. Diet.*, **6**: 291–294.

Goswami, A.K., K.L. Sehgal and B.K. Gupta. 1970a. Chemical composition of bajra grains. 3. Indian inbreds. *J. Nutr. Diet.*, **7**: 5–9.

Goswami, A.K., K.P. Sharma and K.L. Sehgal. 1970b. Chemical composition of bajra grains. 4. Indian varieties. *J. Nutr. Diet.*, **7**: 67–70.

Graham R.D., D. Senadhira, S. Beebe, C. Iglesias and I. Monasterio. 1999. Breeding for micronutrient density in edible portions of stable food crops: conventional approaches. *Field Crops Res.*, **60**: 57–80.

Graham, R.D. 1984. Breeding for nutritional characteristics in cereals. *Adv. Plant Nutr.*, **1**: 57–102.

Graham, R.D. and R. M. Welch. 1996. Breeding for staple-food crops with high micronutrient density. Agricultural Strategies for Micronutrients Working Paper No. 3. Washington, DC: International Food Policy Research Institute, Pp.1–72.

Graham, R.D., D. Senadhira, and I. Monasterio. 1997. A strategy for breeding staple-food crops with high micronutrient density. *Soil Sci. Plant Nutr.*, **43**: 1153–1157.

Graham, R.D., J.S. Ascher, and S.C. Hynes. 1992. Selecting zinc-efficient cereal genotypes for soils of low zinc status. *Plant Soil*, **146**: 241–250.

Graham, R.D., R.M. Welch and H.E. Bouis. 2001. Addressing micronutrient malnutrition through enhancing the nutritional quality of staple foods: principles, perspectives and knowledge gaps. *Adv. Agron.*, **70**: 77–142.

Grantham-McGregor, S.M. and C.C. Ani. 1999. The role of micronutrients in psychomotor sad cognitive development. *British Medical Bull.*, **55**: 511–527.

Gregorio, G.B. 2002. Progress in breeding for trace minerals in staple crops. *J. Nutr.*, **132**: 500-502.

Gregorio, G.B., D. Senadhira, H. Htut and R.D. Graham. 2000. Breeding for trace mineral density in rice. *Food Nutr. Bull.*, **21**: 382–386.

Griffing, B. 1956. Concept of general and specific combining ability in relation to diallel crossing systems. *Aust. J. Biol. Sci.*, **9**: 463–493.

Grusak M.A. 1994. Iron transport to developing ovules of *Pisum sativum*. I. Seed import characteristics and phloem iron-loading capacity of source regions. *Plant Physiol.* **104**: 649–655.

Hacisalihoglu, G., J.J. Hart and L.V. Kochian. 2001. High and low affinity zinc transport systems and their possible role in zinc deficiency in bread wheat. *Plant Physiol.*, **125**: 456–463.

Hadimani, N.A. and N.G. Malleshi. 1993. Studies on milling, physio-chemical properties, nutrient composition and dietary fibre content of millets. *J. Food Sci. Technol.*, **30**: 17–20.

Haltberg, L., M. Brune and L. Rossander. 1989. Iron absorption in man: Ascorbic acid and dose-dependent inhibition of phytate. *Am. J. Clin. Nutr.*, **49**: 140–144.

Hambidge, K.M., C.E. Casey and N.F. Krebs. 1986. Zinc. *In*: Trace Elements in Human and Animal Nutrition. Vol. 2, W. Mertz (ed.), Pp. 1–137. Academic Press, Inc., New York.

Hartwig, E.E., W.F. Jones and T.C. Kilen. 1991. Identification and inheritance of inefficient zinc absorption in soybean. *Crop Sci.*, **31**: 61–63.

Haslett, B.S., R.J. Reid and Z. Rengel. 2001. Zinc mobility in wheat: uptake and distribution of zinc applied to leaves and roots. *Ann. Bot.*, **87**: 379–386.

Hayman, B.I. 1954. The theory and analysis of diallel crosses. *Genet.*, **39**: 789–809.

Hercberg, S., P. Galan and H. Dupin. 1987. Iron deficiency in Africa. *World Rev. Nutr. Diet.*, **54**: 201–236.

Hoan, N. T., U.P. Rao and E.A. Siddiq. 1992. Genetics of tolerance to iron chlorosis in rice. *Plant soil*, **146**: 233–239.

Hoseney, R.C., D.J. Andrews, and H. Clark. 1987. Sorghum and pearl millet. *In*: Nutritional quality of Cereal Grains: Genetic and Agronomic Improvement. ASA, Monograph. **28**: 397–456.

House, W. A., D. R. Van Campen, and R. M. Welch. 1996. Influence of dietary sulfur containing amino acids on the bioavailability to rats of zinc in corn kernels. *Nutr. Res.*, **16**: 225–235.

House, W.A., R.M. Welch, S. Beebe and Z. Cheng. 2002. Potential for increasing the amounts of bioavailable zinc in dry beans (*Phaseolus vulgaris* L.) through plant breeding. *J. Sci. Food Agric.*, **82**: 1452–1457.

Hulse, J.H., E.M. Laing and O.E. Pearson. 1980. Sorghum and millets: their composition and nutritive value. Academic press, London, UK.

Imitiaz, M., B.J. Alloway, K.H. Shah, S.H. Siddiqui, M.Y. Memon, M. Aslam and P. Khan. 2003. Zinc nutrition of wheat: II: Interaction of zinc with other trace elements. *Asian J. plant Sci.*, **2**: 156–160.

International Zinc Association. 2000. Zinc and Human health. International Zinc Association. Brussels, Belgium.

Jambunathan, R. and V. Subramanian. 1988. Grain quality and utilization in sorghum and pearl millet. *In*: Proc. Workshop on Biotechnology for Tropical Crop Improvement, ICRISAT, Patancheru, India. Pp. 1330–1339.

Jinks, J.L. and B.I. Hayman. 1953. The analysis of diallel crosses. *Maize Genet. Coop. Newsl.*, **27**: 48–54.

Jorhem L. 1993. Determination of metals in foodstuffs by atomic absorption Spectrophotometry after dry ashing: NMKL Inter-laboratory study of lead, cadmium, zinc, copper, iron, chromium and nickel. *J. AOAC Int.*, **76**: 798–813.

Kapoor, R.L., S. Dass and S.R. Batra. 1979. Combining ability of some newly developed lines of pearl millet. *Indian J. Agric. Sci.*, 49: 253-256.

Kaya, Y., C. Palta and S. Taner. 2002. Additive main effects and multiplicative interactions analysis of yield performances in bread wheat genotypes across environments. *Turk. J. Agric. Forest.*, **26**: 275-279.

Khairwal, I.S. and S. Singh. 1999. Quantitative and qualitative traits. *In*: Pearl millet breeding (Khairwal, Rai, Andrews and Harinarayana, Eds.). Oxford and IBH Publishing Co. Pvt. Ltd. Pp. 119–155.

Khangura, B.S. 1975. Genetic analysis of some grain quality and yield attributes in pearl millet. Ph.D. thesis. Punjab Agric. Univ., Ludhiana, India.

Khetarpaul, N. and B.M. Chauhan. 1991. Effect of pure sequential culture fermentation by yeasts and lactobacilli on HCl-extractability of minerals from pearl millet. *Food Chem.*, **39**: 347–355.

Kim, M., D.T. Lee and Y.S. Lee. 1995. Iron absorption and intestinal solubility in rats are influenced by dietary proteins. *Nutr. Res.*, **15**: 1705–1716.

Kochian, L.V. 1991. Mechanisms of micronutrient uptake and translocation in plants. *In*: Micronutrients in Agriculture, ed. J.J. Mordvedt, F.R. Cox, L.M. Shuman, R.M. Welch, Madison, WI: *Soil Sci. Soc. Am.* 2nd ed. Pp. 229–296.

Kochian, L.V. 1993. Zinc absorption from hydroponic solution by plant roots. *In*: Zinc in soils and plants. Kluwer publishers, The Netherlands. Pp: 45–57.

Kumar, A. and B.M. Chauhan. 1993. Effects of phytic acid on protein digestibility (*In vitro*) and HCl-extractability of minerals in pearl millet sprouts. *Cereal Chem.*, **70**: 504–506.

Kumar, S. 1994. Food consumption and nutrient intakes. Adoption of hybrid maize in Zambia: Effects on gender roles, food consumption, and nutrition. International Food Policy Research Institute: Bull.**126**, Washington, D.C.

Kumar, V. and A.C. Kapoor. 1984. Trace mineral composition of different varieties of cereals and legumes. *Indian J. Nutr. Dietel.*, **21**: 137–143.

Kurien, P.P., M. Swaminathan and V. Subramanyan. 1961. The chemical composition and nutritive value of bajra (*Pennisetum typhoides*) and bajra diets. *Food Sci.*, **10**: 3–6.

Laulhere, J.P. and J.F. Briat. 1993. Iron release and uptake by plant ferritin: effects of pH, reduction and chelation. *Biochem. J.*, **290**: 693–699.

Lawan, M., F.L. Barnett, B. Kaleeq and R.L. Vanderlip. 1985. Seed density and seed size of pearl millet as related to field emergence and several seed and seedling traits. *Agron. J.*, **77**: 567–571

Layrisse, M., M.N. Garcia-Casal, L. Solano, M.A. Baron, F. Arguello, D. Llovera, J. Ramirez, I. Leets and E. Tropper. 1997. The role of vitamin A on the inhibitors of non-heme iron absorption: Preliminary results. *J. Nutr. Biochem.*, **8**: 61–67.

Lins, D.A.I. and F.R. Cox. 1988. Effect of soil pH and clay content on the zinc soil test interpretation for corn. *Soil Sci. Soc. Am. J.*, **52**: 1681–1685.

Long, J.K., M. Banziger and M. E. Smith. 2004. Diallel analysis of grain iron and zinc density in southern African-adapted maize inbreds. *Crop sci.*, **44**: 2019–2026.

Lush, J.L. 1940. Intra-site correlation and regression of offspring in rams as a method of estimating heritability of characters. *Proc. Amer. Soc. Animal Prod.*, **33**: 292–301.

Macintosh, M.S., 1983. Analysis of combined experiments. *Agron. J.*, **75**: 153–155.

Maiti, R.K. and F.R. Bidinger. 1981. Growth and development of pearl millet. *Res. Bull.*, **6**. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India.

Majumder, N.D., S.C. Rakshit and D.N. Borthakur. 1990. Genetic effects on uptake of selected nutrients in some rice (*Oryza sativa* L.) varieties in phosphorus deficient soil. *Plant Soil*, **123**: 117–120.

Malik, M. 1999. Functional and nutritional properties of newly developed hybrids and varieties of pearl millet. M.Sc. thesis, CCS HAU, Hisar, Haryana, India.

Mannar, V. and E. B. Gallego. 2002. Iron Fortification: Country level experiences and lessons learned. *J. Nutr.*, **132**: 856–858.

Marschner, H. and V. Romheld. 1994. Strategies of plants for acquisition of iron. *Plant Soil,* **165**: 261–274.

Mason, J.B. and M. Garcia. 1993. Micronutrient deficiency - the global situation. *SCN News,* **9**: 11–16.

Mason, J.B., M Lotfi, N. Dalmiya, K. Sethuraman, and M. Deitchler. 2001. The micronutrient report: current progress in the control of vitamin A, iodine, and iron deficiencies. *In*: Micronutrient Initiative: International development research center, Ottawa, Canada. (http://www.micronutrient.org/frame_html).

Mather, K. and J.L. Jinks. 1974. Biometrical Genetics. 2nd edn. Cornell University Press, Ithaca, New York.

Maziya-Dixon B., J.G. Kling, A. Menaki and A. Dixon. 2000. Genetic variation in total carotene, iron and zinc contents of maize and cassava genotypes. *Food Nutr. Bull.*, **21**: 419–422.

McGuire, J. 1993. Addressing micronutrient malnutrition. *SCN News,* **9**: 1–10.

Miller, R.O., J.S. Jacobsen and E.O. Skogley. 1993. Aerial accumulations and partitioning of nutrients by hard red spring wheat. *Commun. Soil Sci. Plant Anal.*, **24**: 2389–2407.

Mills, C.F. 1985. Dietary interactions involving trace elements. *Ann. Rev. Nutr.*, **5**: 173–193.

Monasterio, I. 1998. "CGIAR Micronutrient Project," Update No 3. International Food Policy Research Institute, Washington, D.C.

Monasterio, I. and R.D. Graham. 2000. Breeding for trace minerals in wheat. *Food Nutr. Bull.*, **21**: 392–396.

Ologhobo, A.D. 1989. Improving the nutritive value of soybean (*Glycine max* L.) through processing: biochemical and protein quality studies. *Trop. Agric.*, **66**: 290–296.

Parthasarathy Rao, P., P.S. Birthal, B.V.S. Reddy, K.N. Rai and S. Ramesh. 2006. Diagnostics of sorghum and pearl millet grains-based nutrition in India. *Int. Sorghum and Millets Newsl.* **47**: 93–96.

Ponnamperuma, F.N. 1976. Screening rice for tolerance to mineral stresses. *In*: Plant Adaptation to Mineral Stress in Problem Soils. M.J. Wright. (ed.). Cornell Univ. Agric. Exp. Stn., Ithaca, New York. Pp. 341–353.

Poonam, M. 2002. Effect of acid and heat treatment on nutrient composition and shelf life of pearl millet flour. M.Sc. thesis, CCS HAU, Hisar, Haryana, India.

Prom-u-thai, C., D. Bernie, G. Thomson and R. Benjavan. 2003. Easy and rapid detection of iron in rice grain. *ScienceAsia*, **29**: 203–207.

Raboy, V. 1996. Cereal low phytic mutants: A "global" approach to improving mineral nutritional quality. *Micronutri. Agric.*, **2**: 15–16.

Raboy, V., S.J. Hudson, and D.B. Dickinson. 1985. Reduced phytic acid content does not have an adverse effect on germination of soybean seeds. *Plant Physiol.*, **79**: 323–325.

Raboy, V., K.A. Young, J.A. Dorsch and A. Cook. 2001. Genetics and breeding of seed phosphorus and phytic acid. *J. Plant Physiol.*, **158**: 489–497.

Rahman, S., G.F. Vance and L.C. Munn. 1993. Salinity induced effects on the nutrient status of soil, corn leaves and kernels. *Commun. Soil Sci. Plant. Anal.*, **24**: 2251–2269.

Rai, K.N. and Anand Kumar, K., 1994. Pearl Millet improvement at ICRISAT- an update. *Int. Sorghum and Millets Newsl.*, **35**: 1–29.

Rai, K.N., D.S. Murty, D.J. Andrews and P.J. Bramel-Cox. 1999. Genetic enhancement of pearl millet and sorghum for the semi-arid tropics of Asia and Africa. *Genome*, **42**: 617–628.

Rai, K.N., F.R. Bidinger, K. Hussain Sahib and A.S. Rao. 1998. Registration of ICMP 94001 Pearl millet germplasm. *Crop Sci.*, **38**: 1411.

Rai, K.N., K. Anand Kumar, D.J. Andrews, A.S. Rao, A.G.B. Raj and J.R. Witcombe.1990. Registration of ICTP 8203 Pearl millet. *Crop Sci.*, **30**: 959.

Ramakrishnan, U., R. Manjrekar, J. Rivera, T. Gonzales-Cossio and R. Martorell. 1999. Micronutrients and pregnancy outcome: a review of the literature. *Nutr. Res.*, **19**: 103–159.

Rao, R.G. and M. Swaminathan. 1953. Chemical composition of different varieties of bajra. *Bull. Cent. Food Technol. Res. Inst.*, **3**: 68.

Rashid, A. and R.L. Fox. 1992. Evaluating internal zinc requirements of grain crops by seed analysis. *Agron. J.*, **84**: 469–474.

Reddy, B.V.S., S. Ramesh and T. Longvah. 2005. Prospects of breeding for micronutrients and β-carotene-dense Sorghums. *Int. Sorghum Millets Newsl.*, **46**: 10–14.

Reddy, M.B., R.F. Hurrell, M.A. Juillerat and J.D. Cook. 1996. The influence of different protein sources on phytate inhibition of nonheme-iron absorption in humans. *Am. J Clin. Nutr.*, **63**: 203 –207.

Rekha, N. 1997. Efficacy of processing techniques in the utilization of pearl millet for value added products. M.Sc. thesis, CCS HAU, Hisar, Haryana, India.

Rengel, Z. 1995. Carbonic anhydrase activity in leaves of wheat genotypes differing in Zn efficiency. *J. Plant Physiol.*, **147**: 251–256.

Rengel, Z., and R. D. Graham. 1995. Importance of seed zinc content for wheat growth on zinc-deficient soil. I. Vegetative growth. *Plant Soil,* **173**: 259–266.

Richard, L.A. 1954. Diagnosis and improvement of saline and alkali soils. USDA Handbook No. 60. Department of Agriculture, Washington, D.C.

Ripperger, H. and K Schreiber. 1982. Nicotianamine and analogous amino acids, endogenous iron carriers in higher plants. *Heterocycles,* **17**: 447–461.

Rooney, L.W. and C.M. McDonough. 1987. Food quality and consumer acceptance of pearl millet. *In*: Proceedings of the International Pearl Millet Workshop, ICRISAT Center, India. 7–11 April 1986. ICRISAT, Patancheru, A.P. 502 324, India. Pp. 43–61.

Saha, P.R., C.M. Weaver and A.C. Mason. 1994. Mineral bioavailability in rats from intrinsically labeled whole-wheat flour of various phytate levels. *J. Agric. Food Chem.*, **42**: 2531–2535.

Sahrawat, K.L., G. Ravi Kumar and J.K. Rao. 2002. Evaluation of triacid and dry ashing procedures for determining potassium, calcium, magnesium, iron, zinc, manganese and copper in plant materials. *Commun. Soil Sci. Plant Anal.*, **33**: 95–102.

Sandstead, H.H. 1991. Zinc deficiency: a public health problem. *Am. J. Dis. Child.,* **145**: 853–859.

Sandstead, H.H. 1994. Understanding zinc: Recent observations and interpretations. *J. Lab. Clin. Med.*, **124**: 322–327.

Sawhney, S.K. and M.S. Naik. 1969. Amino acid composition of protein fractions of pearl millet and the effect of Nitrogen fertilizations on its proteins. *Indian J. Genet.*, **29**: 395–406.

Schneeman, B.O. 2001. Linking agricultural production and human nutrition. *J. Sci. Food Agric.*, **81**: 3–9.

Sharma, A.N. 2003. Food security in India. Institute for human development, New Delhi. Pp 1-27.

Shukla, U.C. and K.N. Bhatia. 1971. Effect of nitrogen levels on the Fe and Mn concentration and uptake in hybrid and local varieties of pearl millet (*Pennisetum typhoides*). *Indian J. Agric. Sci.*, **41**: 790–794.

Sims, J.T. 1986. Soil pH effects on the distribution of plant availability of Mn, Cu and Zn. *Soil Sci. Soc. Am. J.*, **50**: 367–373.

Singh, P., U. Singh, K.A. Kumar and D.J. Andrews. 1987. Nutritional quality improvement in pearl millet. *J. Food Sci. Agri.*, **38**:41–48.

Snedeker, S. M., and J. L. Greger. 1983. Metabolism of zinc, copper, and iron as affected by dietary protein, cysteine, and histidine. *J. Nutr.*, **113**: 644–652.

Soliman, M.F. 1988. Effect of salinity on growth and micronutrient composition of corn plants. *Agrochemica*, **32**: 337–348.

Subbulakshmi, G. and M. Naik. 1999. Food fortification in developing countries - current status and strategies. *J. Food Sci. Technol.*, **36**: 371–395.

Theil, E.C., J.W. Burton and J.L. Beard. 1997. A sustainable solution for dietary iron deficiency through plant biotechnology and breeding to increase seed ferritin control. *Euro. J. Biochem.*, **51**: 28–31.

Timmer, C. P. 2003. Biotechnology and Food Systems in Developing Countries. *J. Nutr.*, **133**: 3319–3322.

Tukey, J.W. 1953. The problem of multiple comparisons, Ditto, Princeton University, Princeton, N.J.

Turner, J.H. 1953. A study of heterosis in upland cotton. I. Yields of hybrids compared with varieties. II. Combining ability and inbreeding effects. *Agron. J.*, **45**: 484-490.

Turnlund, J. R., J. C. King, W. R. Keyes, B. Gong, and M. C. Michel. 1984. A stable isotope study of zinc absorption in young men: Effects of phytate and cellulose. *Amer. J. Clini. Nutr.*, **40**: 1071–1077.

Tyagi, C.S., R.S. Paroda, N.D. Arora and K.P. Singh. 1975. Heterosis and combining ability in pearl millet. *Indian J. Genet.*, **35**: 403–408.

Tyagi, C.S., S. Lal and R.R. Karwasra. 1982. Genetic analysis of some quantitative characters in pearl millet. *Indian J. Agric. Sci.*, **52**: 215–219.

UNICEF. 2002. Vitamin A global initiative (www.unicef.org/vitamina). United Nations International Children's Emergency Fund, New York, USA.

Uprety, D.C. and A. Austin. 1972. Varietal differences in the nutrient composition of improved bajra (pearl millet) hybrids. *Bull. Grain Technol.*, **10**: 249–255.

Weiss, M.G. 1943. Inheritance and physiology of efficiency in iron utilization in soybeans. *Genet.*, **28**: 253–268.

Welch, R.M. 1986. Effects of nutrient deficiencies on seed production and quality. *Adv. Plant Nutr.*, **2**: 205–247.

Welch, R.M. 1993. Zinc concentrations and forms in plants for humans and animals. *In*: Zinc in Soils and Plants. A.D. Robson, (ed.) Kluwer Academic Publishers, Dordrecht. Pp. 183–195.

Welch, R.M. 1995. Micronutrient nutrition of plants. *Critical Rev. Plant Sci.*, **14**: 49–82.

Welch, R.M. 1999. Importance of seed mineral nutrient reserves in crop growth and development. *In*: Z. Rengel, ed. Mineral nutrition of crops: Fundamental mechanisms and implications. New York: Food Products Press, Pp. 205–226.

Welch, R.M. 2001. Micronutrients, agriculture and nutrition; linkages for improved health and well being. *In*: K. Singh, S. Mori, R.M. Welch eds. Perspectives on the micronutrient nutrition of crops. Jodhpur, India. Scientific Publ., Pp. 247–289.

Welch, R.M. 2002. Breeding strategies for biofortified staple plant foods to reduce micronutrient malnutrition globally. *J. Nutr.*, **132**: 495-499.

Welch, R.M. and R.D. Graham. 1999. A new paradigm for world agriculture: meeting human needs -productive, sustainable, and nutritious. *Field Crops Res.*, **60**: 1–10.

Welch, R.M. and R.D. Graham. 2002. Breeding crops for enhanced micronutrient content. *Plant Soil*, **245**: 205-214.

Welch, R.M and R. D. Graham. 2004. Breeding for micronutrients in staple food crops from a human nutrition perspective. *J. Exp. Bot.*, **55**: 353–364.

Welch, R.M., G.F. Combs Jr and J.M. Duxbury. 1997. Toward a `Greener' revolution. *Issues in Sci. Technol.*, **14**: 50–58.

Welch, R.M., W.A. House, S. Beebe and Z. Cheng. 2000. Genetic selection for enhanced bioavailable levels of iron in bean (*Phaseolus vulgaris* L.) seeds. *J. Agric. Food Chem.*, **48**: 3576–3580.

White, J.G. and R.J. Zasoski. 1999. Mapping soil micronutrients. *Field Crops Res.*, **60**: 11–26.

WHO. 1996. Trace elements in human nutrition and health. World Health Organization, Geneva, Switzerland. Pp. 1–343.

WHO. 1999. Malnutrition worldwide. World Health Organization. Geneva, Switzerland. (http://www.who.int/nut/malnutrition_worldwide.htm). Pp. 1–13.

WHO. 2001. Iron deficiency anemia: Assessment, prevention and control - a guide for programme managers. Geneva, M.G.

WHO. 2002. The world health report. 2002. Reducing risks, promoting healthy life. World Health Organization, Geneva, Switzerland. Pp. 1–168.

WHO/FAO. 1996. Preparation and use of food-based dietary guidelines. World Health Organization, Geneva, Switzerland and Food and Agricultural Organization of the United Nations Rome, Italy.

Witcombe, J.R., M.N.V.R. Rao and C.T. Hash. 1997. Registration of ICMV 88904 pearl millet. *Crop Sci.*, **37**: 1022–1023.

World Bank. 1994. The challenge of dietary deficiencies of vitamins and minerals. *In*: Enriching lives: overcoming vitamin and mineral malnutrition in developing countries. Washington DC: World Bank. Pp. 6–13.

Yip, R. 1997. The challenge of improving iron nutrition: limitations and potentials of major intervention approaches. *European J. Clinical Nutr.*, **51**: 16–24.

Zebarath, B.J., C.J. Warren and R.W. Sheard. 1992. Influence of the rate of nitrogen fertilization on the mineral concentration of winter wheat in Ontario. *J. Agric. Food Chem.*, **40**: 1528–1530.

Zhang, F.U., V. Romheld and H. Marschner. 1991. Diurnal rhythm of release of phytosiderophores and uptake rate of zinc in iron-deficient wheat. *Soil Sci. Plant Nutr.*, **37**: 671–678.

Zhang, M.W., B.J. Goa and Z.M. Peng. 2004. Genetic effects on Fe, Zn, Mn and P contents in Indica black pericarp rice and their genetic correlations with grain characteristics. *Euphytica,* **135**: 315–323.

Zobel, R.W., M.S. Wright and H.G. Gauch. 1988. Statistical analysis of a yield trial. *Agron. J.*, **80**: 388–393.

APPENDICES

Appendix I. Available soil Fe and Zn content (mg kg^{-1}) (DTPA extractable) in the experimental fields, ICRISAT - Patancheru.

		Summer 2004		Rainy 2004		Summer 2005		Rainy 2005		Summer 2006	
Sl. No.	Depth (cm)	Fe	Zn	Fe	Zn	Fe	Zn	Fe	Zn	Fe	Zn
1	0-15	6.1	2.1	18.2	2.4	10.7	3.1	17.9	3.9	8.0	2.5
	16-30	5.9	1.3	15.7	1.1	9.7	2.1	14.9	3.7	5.9	1.2
2	0-15	6.8	2.3	18.5	3.0	9.8	3.1	13.8	4.7	5.9	2.0
	16-30	5.9	1.0	18.1	1.6	8.4	1.6	11.1	2.6	5.8	0.9
3	0-15	6.8	1.4	12.8	3.0	8.4	2.8	12.5	5.1	5.7	2.2
	16-30	6.0	1.3	11.2	1.8	8.3	1.1	10.9	2.6	4.9	0.8
4	0-15	-	-	-	-	10.8	3.0	14.8	4.8	6.0	1.6
	16-30	-	-	-	-	9.6	1.2	10.0	2.0	5.1	1.1
5	0-15	-	-	-	-	11.5	3.1	15.4	5.1	5.9	1.9
	16-30	-	-	-	-	7.6	1.0	10.9	2.1	5.8	0.9
6	0-15	-	-	-	-	9.3	2.6	16.0	4.6	5.5	1.8
	16-30	-	-	-	-	8.5	1.1	12.7	2.5	5.5	1.0
	Mean	6.2	1.6	15.8	2.2	9.4	2.2	13.4	3.6	5.8	1.5
	Minimum	5.9	1.0	11.2	1.1	7.6	1.0	10.0	2.0	4.9	0.8
	Maximum	6.8	2.3	18.5	3.0	11.5	3.1	17.9	5.1	8.0	2.5
	SD (±)	0.45	0.52	3.11	0.63	2.77	0.86	2.44	1.21	0.76	0.78

Appendix II. Mean performance of 120 entries for grain Fe and Zn content, days to 50% flower and 1000-grain mass in set I trial, 2004 summer and rainy season, ICRISAT-Patancheru.

Entry	Fe content (mg kg^{-1})			Zn content (mg kg^{-1})			Days to 50% flower			1000-grain mass (g)		
	Summer	Rainy	Mean	Summer	Rainy	Mean	Summer	Rainy	Mean	Summer	Rainy	Mean
Inbred lines												
81 B	32.4	36.4	34.4	31.3	32.2	31.7	55	58	57	7.2	7.5	7.4
843 B	46.0	70.9	58.4	47.4	71.9	59.6	43	42	43	8.2	8.0	8.1
863 B	69.0	76.5	72.7	54.0	57.7	55.8	45	48	47	11.8	12.6	12.2
ICMB 88004	48.9	71.8	60.4	43.6	62.8	53.2	45	47	46	8.2	8.8	8.5
ICMB 89111	36.5	46.6	41.5	35.0	47.4	41.2	48	49	48	9.0	8.8	8.9
ICMB 91777	43.7	51.9	47.8	44.7	53.9	49.3	46	47	47	9.1	8.6	8.9
ICMB 93333	37.3	57.2	47.2	36.0	57.8	46.9	49	51	50	8.9	9.2	9.1
ICMB 94111	51.7	75.4	63.6	43.7	69.9	56.8	45	47	46	10.0	9.6	9.8
ICMB 94555	47.5	59.9	53.7	44.0	57.1	50.5	44	45	45	7.0	7.5	7.3
ICMB 95222	35.2	48.5	41.8	39.2	55.1	47.1	49	50	49	8.8	8.6	8.7
ICMB 97111	38.2	53.9	46.0	32.0	40.7	36.3	45	46	45	7.1	6.5	6.8
ICMB 98222	51.3	74.6	62.9	46.6	59.1	52.9	45	47	46	10.9	11.2	11.1
ICMB 00888	56.7	64.1	60.4	49.3	64.1	56.7	44	44	44	10.9	10.7	10.8
ICMB 91444	42.9	47.2	45.0	45.3	52.1	48.7	45	45	45	8.8	8.9	8.9
ICMB 01555	42.1	56.3	49.2	41.0	56.9	49.0	52	51	51	9.2	8.5	8.9
ICMB 01888	39.1	48.6	43.8	38.1	44.4	41.2	50	49	50	8.5	8.1	8.3
ICMB 00999	44.3	72.7	58.5	49.5	68.9	59.2	46	46	46	7.4	7.8	7.6
ICMB 90111	34.2	41.3	37.7	26.5	28.0	27.3	48	49	49	6.8	6.1	6.5
ICMB 98777	35.9	35.0	35.5	34.1	37.3	35.7	44	45	44	9.2	8.8	9.0
ICMB 97333	34.9	57.5	46.2	35.4	59.7	47.6	48	50	49	7.0	6.3	6.7
Partial inbreds												
AIMP 92901 S1-15-1-2-B	74.7	76.6	75.7	61.6	68.0	64.8	46	45	46	12.8	11.5	12.2
AIMP 92901 S1-183-2-2-B	49.2	102.0	75.6	46.5	80.8	63.7	45	45	45	10.8	10.0	10.4
AIMP 92901 S1-421-2-3-B	46.0	63.1	54.6	45.6	56.8	51.2	46	45	46	10.0	10.1	10.0
ICMR 312 S1-22-1-2-1-B	38.2	60.6	49.4	40.8	61.8	51.3	52	54	53	10.8	11.3	11.1
ICMR 312 S1-25-1-1-1-3-B	47.8	62.1	55.0	43.8	52.8	48.3	47	48	48	8.5	8.0	8.2
ICMR 312 S1-39-1-6-1-B	44.7	54.8	49.7	41.3	54.5	47.9	45	46	45	7.8	7.3	7.5
ICMS 7704 S1-13-3-3-1-3-B	35.0	43.1	39.0	40.9	44.8	42.9	49	50	49	8.1	8.0	8.1
ICMS 7704-S1-51-4-1-1-B	45.1	62.9	54.0	45.6	69.8	57.7	47	47	47	10.9	10.6	10.8
ICMS 7704-S1-80-1-3-B	41.6	35.6	38.6	31.7	36.7	34.2	45	46	45	7.8	7.4	7.6
ICMS 8274 S1-1-3-3-1-B	42.0	48.7	45.4	38.3	48.4	43.4	48	47	48	9.2	9.0	9.1
ICMS 8282 S1-8-5-6-1-B	47.0	49.6	48.3	40.0	49.9	44.9	44	45	44	8.5	8.1	8.3
ICMS 8511 S1-14-3-1-B	30.7	40.6	35.6	35.8	39.3	37.5	50	51	50	6.5	7.0	6.8
ICMS 8511 S1-17-2-1-1-B	30.8	29.4	30.1	25.0	24.0	24.5	52	54	53	7.9	7.6	7.8
ICMV 91059 S1-11-3-2-2-B-B	38.3	45.8	42.0	38.2	35.0	36.6	47	46	46	7.8	8.3	8.0
ICMV 91059 S1-14-2-4-2-2-B	31.2	41.5	36.3	29.1	38.0	33.5	55	57	56	7.9	8.5	8.2
ICMV 91059 S1-58-2-2-2-B	40.6	44.1	42.3	35.5	44.2	39.8	54	53	54	9.2	9.0	9.1
ICMV 93074 S1-9-1-1-1-B	40.3	46.7	43.5	43.4	47.8	45.6	49	50	50	8.8	9.0	8.9
ICMV 93074 S1-9-2-1-1-1-B	45.2	45.4	45.3	44.2	50.7	47.4	53	57	55	9.5	9.7	9.6
ICMV 93074 S1-9-2-2-1-B	36.7	49.0	42.9	38.2	39.3	38.7	51	54	53	9.0	8.4	8.7
MC 94 C2-S1-3-1-1-2-B	34.2	51.9	43.0	38.2	58.9	48.5	50	51	51	8.5	8.0	8.3
MC 94 C2-S1-46-1-1-B	40.8	48.9	44.8	40.8	40.9	40.9	47	49	48	8.0	7.8	7.9
MC 94 C2-S1-47-1-1-B	45.3	57.4	51.3	41.8	53.3	47.6	40	42	41	8.8	8.4	8.6
RCB-2 S1-33-1-3-3-2-B	45.7	65.7	55.7	41.1	57.1	49.1	49	47	48	9.2	10.0	9.6
RCB-2-S1-51-3-2-1-B	53.0	39.3	46.1	49.5	47.2	48.3	50	51	51	9.0	9.5	9.3
RCB-2-S1-121-1-2-1-B	46.2	49.5	47.9	44.2	51.0	47.6	42	44	43	8.5	8.3	8.4
RCB-2-S1-138-2-2-1-B	40.7	45.6	43.1	45.6	48.8	47.2	45	46	46	7.5	7.2	7.4
SDMV 90031-S1-84-1-1-2-B	53.2	84.8	69.0	50.8	45.4	48.1	43	47	45	9.9	10.8	10.4
SDMV 90031-S1-93-3-1-1-B	29.3	39.0	34.1	28.7	34.7	31.7	49	50	49	9.2	9.5	9.4
SDMV 93032-S1-5-2-1-1-B	30.9	41.4	36.2	39.4	46.1	42.7	50	51	50	9.0	9.5	9.2
SDMV 93032-S1-93-3-2-2	41.0	43.8	42.4	38.9	46.6	42.7	43	42	42	9.2	10.3	9.7

pendix II. Contd.

Entry	Fe content (mg kg^{-1})			Zn content (mg kg^{-1})			Days to 50% flower			1000-grain mass (g)		
	Summer	Rainy	Mean	Summer	Rainy	Mean	Summer	Rainy	Mean	Summer	Rainy	Mean
Potential pollinators												
HTP 94/54 (HHB 146)	36.5	33.2	34.9	31.1	32.3	31.7	47	49	48	9.8	10.8	10.3
G 73/107 (HHB 94)	36.3	48.5	42.4	33.7	40.0	36.8	50	51	50	8.0	8.1	8.0
H 77/29-2 (HHB 117)	36.9	47.7	42.3	40.8	48.1	44.4	45	46	45	8.9	9.0	8.9
H 77/833-2 (HHB 67)	33.1	36.7	34.9	34.5	41.0	37.7	47	48	48	8.7	8.4	8.6
J 104 (Sel.)	42.9	45.2	44.0	44.6	53.5	49.0	43	44	43	7.0	7.5	7.3
ICMP 451	39.6	38.4	39.0	36.0	34.9	35.4	48	47	48	10.0	10.5	10.3
ICMR 356	34.6	42.3	38.4	39.6	50.6	45.1	47	48	47	10.0	10.1	10.1
RIB 335/74 (RHB 30)	33.8	42.6	38.2	37.2	44.9	41.0	45	46	45	9.5	10.0	9.8
RIB 3135-18 (RHB 121)	34.7	42.5	38.6	33.6	42.8	38.2	41	42	41	7.2	7.5	7.4
CZI 98-11	36.6	47.4	42.0	38.4	46.9	42.6	42	42	42	6.8	6.9	6.9
CZI 9621	36.2	50.2	43.2	34.8	46.7	40.7	42	41	42	7.0	6.6	6.8
IPC 577 (Dual restorer)	41.9	42.6	42.3	41.5	38.0	39.7	41	40	41	6.8	6.2	6.5
IPC 804-2 (Dual restorer)	35.1	38.3	36.7	38.0	51.6	44.8	43	44	44	9.8	10.4	10.1
IPC 827-1 (Dual restorer)	38.2	57.3	47.7	42.6	50.9	46.7	47	48	48	9.5	10.0	9.8
IPC 873 (Dual restorer)	44.2	37.2	40.7	46.2	41.3	43.8	43	44	43	9.2	10.3	9.7
IPC 1518 (Dual restorer)	44.3	42.2	43.2	38.9	39.2	39.0	48	47	48	8.5	8.0	8.3
IPC 94	34.0	49.0	41.5	37.3	41.4	39.3	45	46	46	7.2	7.1	7.1
IPC 802	39.7	38.0	38.9	31.7	32.4	32.0	52	53	53	7.5	7.4	7.4
IPC 843	52.7	49.7	51.2	52.8	57.5	55.2	51	53	52	7.0	6.8	6.9
MIR 97171	50.9	60.4	55.7	42.8	50.7	46.7	42	44	43	8.0	7.8	7.9
Open pollinated varieties												
NCD2 Bulk	38.3	43.9	41.1	39.6	45.3	42.4	39	40	40	9.0	8.8	8.9
ICMR 312	48.5	53.0	50.7	40.0	47.6	43.8	47	48	47	9.5	10.0	9.8
Raj 171	41.2	45.5	43.4	41.2	55.0	48.1	44	45	44	10.0	10.3	10.1
SRC II C4	43.5	39.9	41.7	39.9	41.4	40.6	46	45	46	8.9	9.4	9.2
SOSAT C88	32.9	32.0	32.4	32.8	34.6	33.7	42	43	42	9.8	10.4	10.1
ICMV 221	47.5	67.0	57.3	43.4	56.7	50.1	43	43	43	11.0	11.5	11.3
GB 8735	52.3	73.1	62.7	45.2	61.0	53.1	42	44	43	12.7	12.2	12.5
MC 94	38.5	59.8	49.2	41.6	50.2	45.9	40	40	40	10.0	9.8	9.9
ICMV 91059	37.8	40.4	39.1	34.0	41.3	37.6	42	41	42	10.0	10.4	10.2
ICMS 7703	45.4	53.6	49.5	42.4	52.1	47.2	42	42	42	9.5	10.0	9.8
ICMS 7704	41.7	42.8	42.3	45.2	46.4	45.8	44	45	44	9.0	9.2	9.1
RCB 2	37.2	46.3	41.7	36.2	42.5	39.3	42	43	42	10.0	10.3	10.2
AIMP 92901	42.9	58.8	50.8	37.8	46.1	42.0	49	49	49	9.5	10.0	9.8
ICMS 8511	34.7	39.2	36.9	35.5	49.3	42.4	42	43	42	8.5	8.5	8.5
ICMV 93074	36.1	43.8	39.9	37.7	43.2	40.5	39	40	40	10.1	10.4	10.2
SDMV 90031	45.0	50.7	47.8	42.8	51.8	47.3	45	46	45	9.0	9.7	9.4
SDMV 93032	37.9	46.8	42.3	35.0	49.4	42.2	42	44	43	9.0	9.1	9.1
Jakhrana pop	39.4	44.1	41.8	35.6	46.8	41.2	42	44	43	10.0	10.2	10.1
Barmer pop	45.5	46.2	45.8	43.5	48.3	45.9	44	45	44	10.5	11.8	11.2
EEBC	51.0	64.1	57.5	45.5	59.2	52.4	39	38	39	9.6	10.0	9.8
WC-C 75	42.0	41.9	41.9	38.2	40.9	39.5	47	48	48	9.0	8.7	8.9
JBV 2	41.5	44.8	43.1	41.8	48.9	45.3	37	38	38	9.8	10.4	10.1
JBV 3	41.6	38.9	40.2	37.3	40.4	38.8	44	45	45	9.0	9.6	9.3
CO 8	48.5	48.2	48.4	42.6	47.2	44.9	39	40	40	9.5	9.7	9.6
AFB 2	36.2	43.0	39.6	37.2	39.6	38.4	42	43	42	9.5	10.0	9.8
ICMV 88908	45.2	58.6	51.9	42.7	50.4	46.5	42	42	42	10.2	11.0	10.6
HHVDBC	48.7	61.2	55.0	43.2	51.9	47.6	49	48	49	9.2	9.6	9.4
E Raj pop	40.4	44.1	42.2	38.8	40.9	39.9	40	41	41	9.8	10.3	10.0
CZP 9802	36.1	42.0	39.0	37.7	42.7	40.2	48	49	48	7.0	6.4	6.7
MCNELC	36.8	48.0	42.4	35.2	42.7	38.9	39	40	40	8.0	7.8	7.9

ppendix II. Contd.

Entry	Fe content (mg kg^{-1})			Zn content (mg kg^{-1})			Days to 50% flower			1000-grain mass (g)		
	Summer	Rainy	Mean	Summer	Rainy	Mean	Summer	Rainy	Mean	Summer	Rainy	Mean
rmplasm accessions												
IP 3122	42.9	49.3	46.1	44.6	52.9	48.7	39	40	39	7.0	6.9	6.9
IP 3859	46.8	52.8	49.8	45.0	53.5	49.3	45	44	45	7.2	6.4	6.8
IP 5830	42.0	43.0	42.5	43.6	43.9	43.7	50	49	50	8.5	8.6	8.6
IP 6244	42.0	40.4	41.2	37.3	38.8	38.1	50	51	50	9.2	9.1	9.1
IP 6271	36.3	37.2	36.7	39.8	41.3	40.5	51	52	51	9.8	9.5	9.6
IP 6764	49.6	51.3	50.4	42.9	45.8	44.3	58	59	59	10.0	9.5	9.7
IP 9453	39.5	55.5	47.5	45.8	51.0	48.4	60	62	61	8.1	8.0	8.1
IP 7946	39.7	42.5	41.1	39.1	38.5	38.8	51	50	50	7.0	7.9	7.5
IP 8032	39.4	46.7	43.0	38.0	44.4	41.2	44	44	44	10.0	11.1	10.6
IP 8964	47.7	61.1	54.4	43.0	54.7	48.8	60	62	61	9.2	8.6	8.9
IP 10781	37.2	35.2	36.2	34.9	36.0	35.4	54	53	53	10.0	9.5	9.8
IP 12000	36.5	33.9	35.2	39.1	39.7	39.4	52	55	54	8.9	8.5	8.7
IP 12012	43.4	37.1	40.3	46.3	41.7	44.0	47	46	46	10.5	9.8	10.2
IP 12089	37.7	41.8	39.8	34.1	41.4	37.7	55	55	55	10.0	10.7	10.4
IP 12240	50.2	51.6	50.9	41.1	46.4	43.7	60	61	61	9.2	9.6	9.4
IP 12245	35.4	33.5	34.5	40.3	42.3	41.3	62	63	62	8.0	8.8	8.4
IP 12313	34.7	36.5	35.6	39.2	37.6	38.4	68	69	68	10.5	10.1	10.3
IP 12341	35.7	33.8	34.8	38.2	34.2	36.2	67	70	69	10.8	11.6	11.2
IP 12383	35.0	46.9	41.0	36.7	43.4	40.1	61	62	62	7.5	7.1	7.3
IP 12384	41.7	43.6	42.7	41.2	45.9	43.5	58	61	59	8.2	7.9	8.1
Mean	41.5	49.5	45.5	40.1	47.7	43.9	47	48	47	8.9	9.1	9.0
Minimum	29.3	29.4	30.1	25.0	24.0	24.5	37	38	38	6.5	6.2	6.5
Maximum	74.7	102.0	75.7	61.6	80.8	64.8	68	70	69	12.7	12.2	12.5
LSD ($P = 0.05$)	10.5	16.8		6.4	11.1		3.1	3.0		0.8	0.8	
CV (%)	12.8	17.2		8.0	11.8		2.7	3.1		4.8	4.7	

Appendix III. Relationship of days to 50% flower and 1000-grain mass with grain Fe and Zn content (mg kg^{-1}) in variability trials, ICRISAT-Patancheru.

Trial	Character	Classes	No. of lines	Range		Mean	
				Fe	Zn	Fe	Zn
Set I	**Days to 50% flower**	35-40	8	39.9-57.5	38.9-52.4	46.0	44.9
		41-45	43	32.4-75.6	33.7-63.7	46.4	44.7
		46-50	44	34.1-75.7	27.3-64.8	46.6	44.3
		51-55	14	30.1-51.2	24.5-55.2	41.9	42.0
		56-60	4	34.4-50.4	31.7-44.3	41.0	38.3
		61-65	5	34.5-54.4	40.1-48.8	45.7	44.5
		66-70	2	34.8-35.6	36.2-38.4	35.2	37.3
	1000-grain mass (g)	6.5-8.0	29	30.1-58.5	24.5-59.2	43.4	41.3
		8.1-9.5	44	34.1-60.4	31.7-59.6	44.1	43.9
		9.6-11.0	39	32.4-75.6	31.7-63.7	46.1	44.4
		11.1-12.5	8	34.8-75.7	36.2-64.8	57.7	51.3
Set II	**Days to 50% flower**	41-45	29	49.0-79.9	34.2-50.2	58.4	40.3
		46-50	32	42.0-73.8	27.2-47.2	51.6	35..7
		51-55	7	44.4-70.1	30.4-49.8	55.7	38.8
		56-60	1	-	-	48.4	36.9
	1000-grain mass (g)	6.5-8.0	8	43.5-59.7	29.0-42.5	50.0	35.4
		8.1-9.5	22	43.7-58.9	28.6-43.2	51.3	36.6
		9.6-11.0	30	42.0-72.9	27.2-49.8	55.1	37.8
		11.1-12.5	6	44.1-73.8	35.8-47.7	63.0	43.5
		12.6-14.0	3	65.3-79.9	39.4-50.2	72.1	45.6
AIMP 92901 progeny trial (set IV)	**Days to 50% flower**	41-45	24	44.2-104.0	36.1-80.3	66.5	51.8
		46-50	34	40.9-118.9	31.8-82.7	66.5	51.9
		51-55	6	51.9-93.7	38.8-68.2	69.9	52.2
	1000-grain mass (g)	8.1-9.5	5	43.3-62.3	31.8-56.8	55.8	45.2
		9.6-11.0	29	40.9-93.7	34.3-68.2	67.5	53.1
		11.1-12.5	22	44.2-118.9	36.1-73.0	67.9	50.4
		12.6-14.0	8	50.3-104.0	41.9-80.3	68.2	55.3
GB 8735 progeny trial (set IV)	**Days to 50% flower**	35-40	3	73.7-89.1	53.4-61.7	79.3	57.7
		41-45	43	45.9-108.3	38.1-70.5	70.1	52.3
		46-50	21	45.5-96.0	33.8-67.2	68.1	53.1
		51-55	1	-	-	61.6	50.1
	1000-grain mass (g)	8.1-9.5	5	57.9-80.6	47.9-59.6	70.9	53.0
		9.6-11.0	28	45.5-84.4	33.8-67.2	65.3	52.0
		11.1-12.5	30	49.9-108.3	41.6-70.5	73.8	53.6
		12.6-14.0	5	45.9-89.1	39.5-65.5	69.8	50.9

Appendix IV. Mean performance of 69 improved populations for grain Fe and Zn content, days to 50% flower and 1000-grain mass in set II trial, 2004 rainy season and 2005 summer season, ICRISAT-Patancheru.

Trt. No	Population	Fe content (mg kg^{-1})			Zn content (mg kg^{-1})			Days to 50% flower			1000-grain mass (g)		
		Rainy 2004	Summer 2005	Mean	Rainy 2004	Summer 2005	Mean	Rainy 2004	Summer 2005	Mean	Rainy 2004	Summer 2005	Mean
1	3/4 HK	46.6	42.9	44.7	33.2	33.3	33.3	47	50	48	7.9	8.0	8.0
2	Acid Tolerant Pop	50.3	65.2	57.7	28.1	44.2	36.2	46	52	49	9.8	10.0	9.9
3	Afpop 90	50.6	49.5	50.0	29.1	28.1	28.6	50	48	49	9.3	9.1	9.2
4	Ankoutess	48.4	46.1	47.3	26.0	43.0	34.5	51	48	49	10.0	9.5	9.8
5	Bmr CIVT-133	49.7	45.9	47.8	32.5	36.7	34.6	50	48	49	7.7	7.2	7.4
6	Bmr CIVT-153	55.1	51.7	53.4	36.5	33.3	34.9	48	47	47	9.6	10.0	9.8
7	Bmr HKP-163	49.7	48.1	48.9	30.5	39.2	34.9	51	48	50	8.8	8.5	8.6
8	CGP	83.7	63.8	73.8	41.1	50.1	45.6	45	46	46	11.9	11.0	11.5
9	CIVT GMS	53.1	51.9	52.5	31.1	38.1	34.6	49	45	47	10.5	10.8	10.6
10	EDC	53.8	65.6	59.7	33.5	51.5	42.5	42	45	43	7.5	8.0	7.7
11	EXB	61.0	46.1	53.5	47.9	38.6	43.2	46	45	46	8.5	8.8	8.7
12	Faringuero	41.1	50.9	46.0	27.6	40.8	34.2	54	52	53	9.1	9.2	9.2
13	GAM 73	48.0	40.3	44.1	35.9	35.7	35.8	48	47	48	12.6	12.1	12.4
14	GGP Bulk (C_0)	85.3	71.7	78.5	51.7	48.7	50.2	42	43	42	14.0	13.2	13.6
15	GGT Bulk (C_0)	68.3	62.3	65.3	38.1	40.6	39.4	45	42	43	13.2	13.0	13.1
16	Guerinian	43.5	44.0	43.7	29.0	32.7	30.8	51	48	49	9.0	9.1	9.0
17	HICP GMS	47.7	40.8	44.2	33.2	39.4	36.3	49	47	48	9.7	9.8	9.8
18	Higrop	64.4	69.5	66.9	40.6	48.0	44.3	49	52	51	9.6	9.8	9.7
19	HiTip	58.0	56.7	57.3	32.1	43.0	37.5	42	40	41	7.8	7.5	7.6
20	HKP GMS	45.0	42.1	43.5	32.5	40.6	36.5	50	47	48	10.4	9.5	10.0
21	HTBC	58.9	39.1	49.0	37.3	37.3	37.3	41	41	41	9.5	9.2	9.3
22	ICMV 96752	53.4	53.3	53.3	30.9	42.7	36.8	44	43	43	8.7	9.0	8.8
23	ICMV 98109	58.4	48.8	53.6	37.5	40.7	39.1	41	44	42	9.4	9.2	9.3
24	ICMV 99902	64.2	43.5	53.9	40.4	35.3	37.8	44	42	43	10.3	10.0	10.1
25	ICMV-IS 85333	47.9	43.0	45.4	29.4	39.5	34.4	48	45	46	10.8	10.5	10.6
26	ICMV-IS 86330	44.0	55.7	49.9	24.2	36.6	30.4	51	52	51	9.4	9.5	9.4
27	ICMV-IS 88305	48.0	39.0	43.5	25.0	33.0	29.0	51	50	50	7.9	8.0	8.0
28	ICMV-IS 90311	58.7	50.8	54.7	30.0	43.2	36.6	50	48	49	9.0	9.2	9.1
29	ICMV-IS 92326	47.7	43.9	45.8	29.4	37.1	33.2	56	55	55	7.9	8.0	7.9
30	ICMV-IS 94206	50.2	39.2	44.7	26.4	33.2	29.8	48	47	47	10.0	10.2	10.1
31	ICMV-IS 92222	54.6	47.0	50.8	32.5	39.0	35.7	50	49	49	9.4	9.5	9.4
32	ICMV-IS 95303	47.3	41.8	44.5	34.3	34.1	34.2	48	50	49	7.2	7.0	7.1
33	ICMV-IS 99001	48.3	40.5	44.4	35.3	35.3	35.3	51	52	51	8.1	8.0	8.1
34	ICMV-IS 99002	45.0	46.5	45.8	31.8	36.5	34.1	46	48	47	9.9	10.1	10.0
35	ICMV-IS 99003	46.7	50.1	48.4	34.4	39.4	36.9	57	58	57	9.4	9.8	9.6
36	ICMV-IS 99005	46.0	38.0	42.0	26.6	27.9	27.2	49	50	50	10.4	9.8	10.1
37	ICRC 11	58.9	48.1	53.5	35.8	38.2	37.0	42	40	41	9.2	9.0	9.1
38	ICTP 8203	77.6	82.2	79.9	47.7	46.5	47.1	43	42	43	13.0	13.5	13.3
39	IAC-ISC-TCP-4	74.7	71.1	72.9	47.1	47.4	47.2	48	51	50	10.6	10.9	10.8
40	IAC-ISC-TCP-6	65.6	57.2	61.4	38.2	42.7	40.4	47	45	46	10.8	11.0	10.9
41	Jakharana (ICMP 97880)	58.9	56.1	57.5	34.3	49.1	41.7	41	44	42	8.6	9.0	8.8
42	Lubasi	61.9	71.3	66.6	42.2	47.3	44.7	52	50	51	11.8	11.0	11.4
43	MC-C10	60.9	38.9	49.9	40.7	35.9	38.3	44	42	43	8.3	8.2	8.3

Appendix IV. Contd.

Trt. No.	Population	Fe content (mg kg^{-1})			Zn content (mg kg^{-1})			Days to 50% flower			1000-grain mass (g)		
		Rainy 2004	Summer 2005	Mean	Rainy 2004	Summer 2005	Mean	Rainy 2004	Summer 2005	Mean	Rainy 2004	Summer 2005	Mean
44	MRC	54.2	79.1	66.7	29.3	44.8	37.0	41	41	41	9.5	9.8	9.7
45	NELC II	52.3	60.9	56.6	37.2	40.0	38.6	43	42	42	7.9	8.0	8.0
46	PVGGP-1	68.3	60.0	64.1	38.6	47.2	42.9	43	42	43	10.0	11.0	10.5
47	PVGGP-6	79.3	47.1	63.2	51.4	40.1	45.7	45	43	44	12.1	10.9	11.5
48	PVGGT-4	84.2	59.1	71.6	41.3	42.3	41.8	46	48	47	10.2	9.8	10.0
49	PVGGT-5	76.2	66.6	71.4	45.4	49.9	47.7	44	45	44	12.2	12.5	12.4
50	SADC White grain	54.6	37.6	46.1	38.8	36.6	37.7	46	45	46	10.3	10.5	10.4
51	Sasank	46.4	47.9	47.2	26.3	35.4	30.8	51	50	50	10.3	10.4	10.3
52	SDMV 89004	52.7	47.4	50.1	33.6	39.4	36.5	45	45	45	8.2	8.0	8.1
53	SDMV 92037	56.8	46.5	51.6	30.7	37.7	34.2	45	44	45	9.0	9.0	9.0
54	SDMV 95022	63.7	57.6	60.7	41.6	40.9	41.2	42	45	43	10.2	10.0	10.1
55	SDMV 95045	64.1	53.8	58.9	43.5	40.2	41.8	45	42	43	8.5	8.4	8.5
56	SenPop	54.5	38.0	46.2	29.8	35.0	32.4	46	48	47	10.0	9.5	9.8
57	SRBC	57.6	42.8	50.2	39.5	32.1	35.8	43	45	44	9.0	8.5	8.7
58	SRC III-C2	55.6	54.3	54.9	38.1	45.4	41.7	44	45	44	9.6	10.0	9.8
59	SSC-C7	57.5	46.2	51.8	39.1	38.3	38.7	43	44	43	9.6	9.2	9.4
60	Ugandi	74.7	65.5	70.1	42.1	57.6	49.8	52	55	54	10.2	10.0	10.1
61	W Raj Pop	52.1	49.0	50.5	37.1	43.4	40.2	42	45	44	8.6	8.8	8.7
62	EC 87 C_0	70.7	47.6	59.1	44.2	39.2	41.7	41	41	41	11.4	11.0	11.2
63	ELC C_0	59.9	58.9	59.4	37.7	44.5	41.1	46	42	44	10.2	10.5	10.3
64	IKMV Bulk	56.9	50.1	53.5	36.4	37.2	36.8	48	47	47	10.0	9.5	9.7
65	LaGrap	70.2	60.3	65.2	45.9	45.7	45.8	48	52	50	10.2	11.0	10.6
66	SSC C1 Brist	61.7	63.9	62.8	37.9	42.9	40.4	43	44	43	10.4	10.5	10.5
67	ICMS 7704 (Check)	54.3	51.6	52.9	25.4	36.6	31.0	46	47	47	9.8	10.2	10.0
68	RCB 2 (Check)	57.7	42.8	50.2	40.0	36.3	38.1	43	43	43	10.2	9.2	9.7
69	WC-C 75 (Check)	56.3	58.9	57.6	34.4	43.2	38.8	46	45	46	9.2	9.5	9.4
	Mean	57.6	52.1	54.8	35.7	40.2	38.0	46	46	46	9.7	9.7	9.7
	Minimum	41.1	37.6	42.0	24.2	27.9	27.2	41	40	41	7.2	7.0	7.1
	Maximum	85.3	82.2	79.9	51.7	57.6	50.2	57	58	57	13.0	13.5	13.3
	LSD ($P = 0.05$)	14.6	12.8		10.3	10.1		5.8	6.7		2.8	3.2	
	CV (%)	12.7	12.3		14.5	12.6		13.4	10.5		3.1	3.0	

Appendix V. Mean performance of AIMP 92901 (S_3) progenies for grain Fe and Zn content, days to 50% flower and 1000-grain mass, 2005 rainy and 2006 summer seasons, ICRISAT-Patancheru.

Trt. No.	Progeny	Fe content (mg kg^{-1})			Zn content (mg kg^{-1})			Days to 50% flower			1000-grain mass (g)		
		Rainy 2005	Summer 2006	Mean	Rainy 2005	Summer 2006	Mean	Rainy 2005	Summer 2006	Mean	Rainy 2005	Summer 2006	Mean
1	AIMP 92901-S1-15-4-1	52.5	63.7	58.1	37.0	36.6	36.8	42	43	43	12.5	11.2	11.9
2	AIMP 92901-S1-15-4-3	81.0	74.0	77.5	59.0	55.8	57.4	44	45	45	14.0	13.0	13.5
3	AIMP 92901 S1-16-2-1	48.5	55.5	52.0	36.0	41.5	38.8	51	50	51	12.0	11.8	11.9
4	AIMP 92901 S1-16-2-3	55.5	55.5	55.5	41.0	61.5	51.3	44	46	45	13.2	12.0	12.6
5	AIMP 92901 S1-23-2-1	69.5	74.4	72.0	45.5	63.6	54.6	46	48	47	11.0	10.5	10.8
6	AIMP 92901 S1-27-3-1	59.5	80.5	70.0	47.0	65.4	56.2	41	44	43	10.2	9.5	9.9
7	AIMP 92901 S1-35-1-1	59.0	49.7	54.3	44.0	49.7	46.8	51	51	51	10.2	10.8	10.5
8	AIMP 92901 S1-41-2-1	58.0	80.2	69.1	52.0	65.6	58.8	45	47	46	11.0	10.5	10.8
9	AIMP 92901 S1-45-1-2	58.5	56.1	57.3	47.5	52.6	50.1	45	44	45	14.3	13.2	13.8
10	AIMP 92901 S1-45-1-4	45.5	58.4	51.9	42.5	55.1	48.8	42	43	43	10.5	9.5	10.0
11	AIMP 92901 S1-66-1-1	67.0	55.4	61.2	48.0	41.3	44.6	41	44	43	9.2	8.8	9.0
12	AIMP 92901 S1-75-1-1	93.5	59.7	76.6	51.0	58.7	54.9	42	44	43	9.8	10.0	9.9
13	AIMP 92901 S1-83-1-3	64.0	60.4	62.2	38.5	52.6	45.5	47	48	48	11.5	11.0	11.3
14	AIMP 92901 S1-83-4-2	70.5	84.8	77.6	46.0	58.9	52.5	50	49	50	10.0	9.8	9.9
15	AIMP 92901 S1-103-2-2	63.5	65.3	64.4	46.0	60.4	53.2	48	47	48	10.2	9.5	9.9
16	AIMP 92901 S1-105-1-1	61.5	61.3	61.4	38.5	53.4	46.0	42	42	42	12.2	11.8	12.0
17	AIMP 92901 S1-119-2-1	54.5	66.7	60.6	45.5	55.9	50.7	43	44	44	9.5	8.9	9.2
18	AIMP 92901 S1-119-2-2	64.5	60.0	62.3	49.5	64.2	56.8	47	48	48	9.2	8.8	9.0
19	AIMP 92901 S1-148-2-3	51.0	72.4	61.7	47.0	62.6	54.8	42	43	43	13.3	12.2	12.8
20	AIMP 92901 S1-183-1-1	82.0	94.0	88.0	46.5	51.0	48.8	45	46	46	9.6	9.8	9.7
21	AIMP 92901 S1-183-1-2	78.5	88.8	83.6	43.5	56.0	49.7	42	41	42	13.2	12.2	12.7
22	AIMP 92901 S1-183-2-2	87.5	96.4	91.9	54.5	57.8	56.1	50	52	51	11.0	10.8	10.9
23	AIMP 92901 S1-187-1-3	43.5	55.5	49.5	39.5	45.5	42.5	50	49	50	11.2	12.0	11.6
24	AIMP 92901 S1-194-3-2	58.5	55.2	56.9	41.5	43.2	42.4	48	47	48	10.2	10.5	10.4
25	AIMP 92901 S1-197-1-1	51.0	49.6	50.3	40.5	43.3	41.9	47	46	47	12.9	13.0	12.9
26	AIMP 92901 S1-211-2-3	84.0	115.5	99.8	57.0	70.1	63.5	47	45	46	11.4	11.5	11.5
27	AIMP 92901 S1-218-1-3	43.5	46.9	45.2	46.0	45.0	45.5	44	45	45	12.0	12.4	12.2
28	AIMP 92901 S1-239-1-1	36.5	45.4	40.9	29.0	39.6	34.3	46	47	47	10.2	10.5	10.4
29	AIMP 92901 S1-242-1-1	54.5	57.5	56.0	57.5	56.7	57.1	48	50	49	12.5	13.0	12.7
30	AIMP 92901 S1-242-2-1	74.5	85.7	80.1	51.0	68.4	59.7	47	48	48	12.4	11.4	11.9
31	AIMP 92901 S1-253-1-1	51.0	62.0	56.5	50.0	48.7	49.4	45	47	46	10.0	9.8	9.9
32	AIMP 92901 S1-261-3-1	68.5	67.0	67.8	43.5	57.5	50.5	45	46	46	12.3	12.5	12.4
33	AIMP 92901 S1-271-3-1	53.0	59.6	56.3	44.0	56.7	50.3	41	42	42	10.5	9.8	10.2
34	AIMP 92901 S1-271-3-2	58.5	69.1	63.8	63.0	66.2	64.6	46	47	47	9.5	9.6	9.6
35	AIMP 92901 S1-271-3-3	52.5	58.8	55.7	53.0	58.6	55.8	47	48	48	11.9	12.2	12.0
36	AIMP 92901 S1-272-2-1	47.0	52.8	49.9	37.5	47.4	42.4	45	46	46	11.8	12.4	12.1
37	AIMP 92901 S1-272-2-2	55.5	57.0	56.3	35.0	49.0	42.0	47	48	48	11.5	10.8	11.1

ppendix V. Contd.

Trt. No.	Progeny	Fe content (mkg^{-1})			Zn content mg kg^{-1})			Days to 50% flower			1000-grain mass (g)		
		Rainy 2005	Summer 2006	Mean	Rainy 2005	Summer 2006	Mean	Rainy 2005	Summer 2006	Mean	Rainy 2005	Summer 2006	Mean
38	AIMP 92901 S1-272-2-3	51.0	64.9	58.0	44.5	52.6	48.6	47	50	49	9.5	9.6	9.6
39	AIMP 92901 S1-296-2-1	80.5	90.4	85.4	51.5	63.9	57.7	45	45	45	10.2	9.5	9.9
40	AIMP 92901 S1-296-2-2	81.5	80.6	81.0	48.0	51.0	49.5	44	47	46	11.4	11.6	11.5
41	AIMP 92901 S1-310-2-2	45.0	57.0	51.0	46.0	58.0	52.0	44	43	44	12.0	12.3	12.2
42	AIMP 92901 S1-310-2-3	48.0	52.3	50.1	35.0	45.6	40.3	46	47	47	10.5	10.6	10.6
43	AIMP 92901 S1-343-2-1	54.5	52.5	53.5	38.5	48.5	43.5	43	42	43	11.9	12.2	12.1
44	AIMP 92901 S1-344-1-1	81.0	86.6	83.8	65.0	65.1	65.0	44	46	45	12.0	11.4	11.7
45	AIMP 92901 S1-348-1-1	64.0	87.3	75.6	60.5	75.9	68.2	51	53	52	11.4	10.5	10.9
46	AIMP 92901 S1-359-1-1	59.0	88.0	73.5	42.5	55.2	48.8	42	42	42	11.3	10.8	11.1
47	AIMP 92901 S1-359-2-2	61.0	94.6	77.8	45.5	60.0	52.8	45	47	46	11.2	10.0	10.6
48	AIMP 92901 S1-383-2-3	62.0	103.2	82.6	70.0	95.4	82.7	48	48	48	11.2	9.5	10.3
49	AIMP 92901 S1-403-1-2	34.5	53.8	44.2	28.5	43.7	36.1	44	45	45	12.0	11.4	11.7
50	AIMP 92901 S1-403-1-4	42.5	44.0	43.3	29.0	34.7	31.8	49	50	50	9.5	9.3	9.4
51	AIMP 92901 S1-406-1-1	97.5	110.5	104.0	68.0	92.7	80.3	41	42	42	8.8	8.9	8.9
52	AIMP 92901 S1-415-2-1	93.5	80.4	86.9	57.5	71.7	64.6	46	47	47	12.3	12.2	12.3
53	AIMP 92901 S1-421-2-3	56.0	56.6	56.3	51.0	48.5	49.7	45	46	46	10.8	9.8	10.3
54	AIMP 92901 S1-423-2-2	104.0	87.9	95.9	53.5	60.8	57.2	45	45	45	11.2	11.5	11.4
55	AIMP 92901 S1-438-3-2	88.0	99.5	93.7	50.5	72.8	61.6	50	51	51	10.9	10.7	10.8
56	AIMP 92901 S1-459-1-1	59.5	81.1	70.3	51.5	58.9	55.2	47	48	48	10.3	10.5	10.4
57	AIMP 92901 S1-460-2-1	56.0	64.0	60.0	40.5	57.7	49.1	45	46	46	10.2	9.5	9.8
58	AIMP 92901 S1-460-3-1	54.5	60.5	57.5	41.5	55.5	48.5	47	47	47	10.2	10.5	10.3
59	AIMP 92901 S1-460-3-3	62.0	60.5	61.2	43.5	50.9	47.2	48	49	49	10.0	10.7	10.3
60	AIMP 92901 S1-480-1-1	72.5	83.9	78.2	47.5	61.1	54.3	49	50	50	10.8	9.5	10.2
61	AIMP 92901 S1-480-1-3	103.5	134.3	118.9	65.5	80.4	73.0	49	50	50	11.2	11.7	11.5
62	AIMP 92901 S1-486-1-1	65.5	67.1	66.3	50.5	54.5	52.5	44	44	44	10.8	9.5	10.2
63	AIMP 92901 S1-486-1-2	59.5	62.8	61.2	45.5	59.0	52.3	42	45	44	10.8	9.8	10.3
64	AIMP 92901 S1-488-2-1	52.5	51.2	51.9	41.0	42.7	41.8	51	52	52	9.5	9.2	9.4
	Mean	63.2	70.4	66.8	47.0	56.8	51.9	46	47	46	11.1	10.8	10.9
	Minimum	34.5	44.0	40.9	28.5	34.7	31.8	41	41	42	8.8	8.8	8.9
	Maximum	104.0	134.3	118.9	70.0	95.4	82.7	51	53	52	14.3	13.2	13.8
	LSD (P=0.05)	11.8	15.0	13.5	8.3	13.8	11.3	3.2	4.2	3.1	3.4	2.7	3.1
	CV (%)	9.4	10.7	10.3	8.9	12.1	11.0	7.5	7.9	8.4	5.2	7.4	6.3

Appendix VI. Mean performance of GB 8735 (S_2) progenies for grain Fe and Zn content, days to 50% flower and 1000-grain mass, 2005 rainy and 2006 summer seasons, ICRISAT-Patancheru.

Trt. No.	Progeny	Fe content (mg kg^{-1})			Zn content (mg kg^{-1})			Days to 50% flower			1000-grain mass (g)		
		Rainy 2005	Summer 2006	Mean	Rainy 2005	Summer 2006	Mean	Rainy 2005	Summer 2006	Mean	Rainy 2005	Summer 2006	Mean
1	GB 8735 S1-3-1	59.5	69.1	64.3	41.0	52.0	46.5	42	44	43	10.5	11.0	10.8
2	GB 8735 S1-3-2	62.5	60.7	61.6	42.5	57.6	50.1	51	52	52	11.2	10.5	10.9
3	GB 8735 S1-11-1	66.5	84.9	75.7	48.0	65.3	56.6	42	44	43	12.5	11.8	12.2
4	GB 8735 S1-11-2	70.0	106.8	88.4	51.5	79.6	65.5	42	41	42	14.2	13.0	13.6
5	GB 8735 S1-13-1	66.5	94.8	80.6	47.5	71.7	59.6	44	45	45	8.9	9.0	9.0
6	GB 8735 S1-13-2	60.5	72.7	66.6	40.5	59.0	49.8	44	45	45	9.2	9.4	9.3
7	GB 8735 S1-15-1	55.0	62.8	58.9	41.0	44.1	42.5	45	46	46	12.0	11.2	11.6
8	GB 8735 S1-15-2	47.0	66.6	56.8	34.0	54.8	44.4	47	48	48	11.4	10.7	11.1
9	GB 8735 S1-16-1	62.5	87.2	74.8	45.5	75.3	60.4	42	44	43	12.2	11.2	11.7
10	GB 8735 S1-16-2	73.5	80.7	77.1	45.5	69.9	57.7	41	43	42	11.5	11.0	11.3
11	GB 8735 S1-20-1	87.0	91.1	89.1	47.0	59.7	53.4	40	40	40	14.0	13.2	13.6
12	GB 8735 S1-23-1	60.0	71.4	65.7	41.5	58.7	50.1	45	44	45	12.0	11.4	11.7
13	GB 8735 S1-23-2	66.0	70.3	68.2	50.0	59.4	54.7	39	42	41	10.8	11.2	11.0
14	GB 8735 S1-25-1	78.5	78.3	78.4	52.5	63.1	57.8	47	48	48	12.1	12.0	12.1
15	GB 8735 S1-25-2	66.0	61.1	63.5	37.5	48.8	43.1	42	44	43	10.9	11.0	11.0
16	GB 8735 S1-28-1	63.5	59.0	61.3	46.0	64.7	55.3	44	46	45	9.8	9.5	9.7
17	GB 8735 S1-36-1	47.0	52.7	49.9	37.0	46.3	41.6	44	46	45	11.4	11.5	11.5
18	GB 8735 S1-36-2	51.5	97.3	74.4	36.5	62.6	49.5	41	44	43	12.0	11.4	11.7
19	GB 8735 S1-37-1	61.5	65.4	63.4	49.5	67.4	58.4	45	45	45	11.0	10.8	10.9
20	GB 8735 S1-37-2	64.5	92.1	78.3	54.0	80.4	67.2	43	44	44	9.5	9.6	9.6
21	GB 8735 S1-38-1	81.0	68.0	74.5	51.0	53.5	52.3	44	46	45	9.8	9.7	9.8
22	GB 8735 S1-38-2	62.5	61.8	62.1	41.5	52.1	46.8	42	43	43	13.1	12.8	13.0
23	GB 8735 S1-47-1	47.0	68.9	57.9	33.5	62.4	47.9	46	47	47	8.8	9.2	9.0
24	GB 8735 S1-47-2	57.0	56.7	56.8	36.0	59.7	47.8	44	45	45	12.0	11.5	11.8
25	GB 8735 S1-53-1	89.0	79.8	84.4	57.0	71.6	64.3	45	46	46	11.0	10.4	10.7
26	GB 8735 S1-53-2	73.0	74.4	73.7	49.0	66.9	58.0	38	40	39	9.8	10.0	9.9
27	GB 8735 S1-54-1	70.5	67.8	69.1	48.5	63.9	56.2	45	44	45	10.2	9.5	9.9
28	GB 8735 S1-55-1	54.0	74.6	64.3	38.5	54.8	46.6	45	46	46	11.0	9.7	10.4
29	GB 8735 S1-55-2	76.5	68.5	72.5	45.0	63.4	54.2	47	48	48	12.5	11.4	11.9
30	GB 8735 S1-56-1	51.0	66.9	59.0	29.0	47.3	38.1	42	44	43	11.2	10.8	11.0
31	GB 8735 S1-56-2	54.5	64.1	59.3	36.5	53.3	44.9	43	44	44	12.4	11.5	11.9
32	GB 8735 S1-62-1	56.5	57.0	56.7	41.0	41.3	41.1	43	44	44	11.0	10.5	10.8
33	GB 8735 S1-62-2	53.5	59.7	56.6	37.0	42.5	39.8	41	42	42	9.8	9.5	9.7
34	GB 8735 S1-63-1	59.0	71.2	65.1	46.0	61.8	53.9	44	46	45	9.7	9.6	9.7
35	GB 8735 S1-63-2	61.5	78.5	70.0	57.0	73.4	65.2	45	46	46	9.7	9.4	9.6
36	GB 8735 S1-64-1	63.5	75.3	69.4	46.5	48.2	47.4	42	47	45	11.8	10.7	11.3

Appendix VI. Contd

Trt. No.	Progeny	Fe content (mg kg^{-1})			Zn content (mg kg^{-1})			Days to 50% flower			1000-grain mass (g)		
		Rainy 2005	Summer 2006	Mean	Rainy 2005	Summer 2006	Mean	Rainy 2005	Summer 2006	Mean	Rainy 2005	Summer 2006	Mean
37	GB 8735 S1-64-2	41.0	80.6	60.8	30.0	56.0	43.0	41	43	42	12.5	11.4	12.0
38	GB 8735 S1-69-1	76.0	98.1	87.1	49.5	73.4	61.5	45	46	46	11.4	10.8	11.1
39	GB 8735 S1-69-2	77.0	110.9	93.9	43.0	68.0	55.5	41	42	42	12.5	12.0	12.3
40	GB 8735 S1-71-1	60.0	71.2	65.6	45.0	60.8	52.9	43	42	43	12.0	11.5	11.8
41	GB 8735 S1-71-2	87.5	110.3	98.9	53.5	75.7	64.6	41	42	42	11.8	10.2	11.0
42	GB 8735 S1-72-1	104.5	112.0	108.3	58.0	83.0	70.5	44	46	45	11.4	10.4	10.9
43	GB 8735 S1-78-1	55.0	91.2	73.1	48.5	66.8	57.6	44	46	45	12.3	12.0	12.2
44	GB 8735 S1-80-1	66.0	80.6	73.3	45.5	67.3	56.4	47	49	48	8.9	8.0	8.5
45	GB 8735 S1-80-2	61.5	70.2	65.9	46.5	59.5	53.0	45	47	46	11.4	10.5	10.9
46	GB 8735 S1-85-1	64.5	71.6	68.1	53.0	68.2	60.6	41	42	42	11.3	11.2	11.3
47	GB 8735 S1-85-2	53.0	75.1	64.0	45.0	75.2	60.1	45	46	46	10.8	10.9	10.9
48	GB 8735 S1-88-1	73.0	77.1	75.0	58.5	64.8	61.7	40	40	40	11.2	10.7	10.9
49	GB 8735 S1-88-2	71.0	65.5	68.3	58.0	58.2	58.1	40	42	41	10.5	9.5	10.0
50	GB 8735 S1-89-1	71.0	55.8	63.4	50.0	48.6	49.3	46	47	47	12.5	12.1	12.3
51	GB 8735 S1-89-2	83.0	74.7	78.9	57.5	60.7	59.1	42	44	43	12.0	10.7	11.4
52	GB 8735 S1-91-1	94.0	101.3	97.6	52.5	63.2	57.9	44	46	45	10.9	11.3	11.1
53	GB 8735 S1-91-2	70.5	79.9	75.2	59.5	64.6	62.1	45	47	46	11.0	10.7	10.9
54	GB 8735 S1-99-1	69.0	62.9	66.0	44.5	61.5	53.0	45	46	46	10.5	10.3	10.4
55	GB 8735 S1-99-2	71.5	82.8	77.1	47.0	67.8	57.4	40	42	41	12.4	11.7	12.1
56	GB 8735 S1-101-1	55.5	66.7	61.1	46.5	56.6	51.5	42	44	43	10.3	10.2	10.3
57	GB 8735 S1-101-2	42.0	48.9	45.5	28.5	39.2	33.8	48	46	47	9.7	9.9	9.8
58	GB 8735 S1-112-1	54.5	57.3	55.9	37.5	49.4	43.4	47	46	47	10.2	10.8	10.5
59	GB 8735 S1-112-2	49.0	64.1	56.6	36.0	53.0	44.5	44	45	45	9.6	10.0	9.8
60	GB 8735 S1-113-1	53.0	71.4	62.2	38.5	51.2	44.9	43	45	44	10.5	9.5	10.0
61	GB 8735 S1-122-1	74.0	78.1	76.0	48.5	54.4	51.4	42	44	43	9.6	9.0	9.3
62	GB 8735 S1-125-1	86.5	105.6	96.0	57.0	77.5	67.2	46	45	46	12.5	11.4	12.0
63	GB 8735 S1-135-1	62.0	91.6	76.8	41.5	55.8	48.7	44	47	46	11.8	12.0	11.9
64	GB 8735 S1-135-2	58.0	77.3	67.6	37.5	52.1	44.8	45	45	45	12.0	11.2	11.6
65	GB 8735 S1-140-1	54.5	58.2	56.3	44.0	57.0	50.5	44	47	46	12.5	11.2	11.9
66	GB 8735 S1-140-2	60.5	63.0	61.8	50.5	55.1	52.8	47	46	47	11.5	10.7	11.1
67	GB 8735 S1-142-1	39.5	52.2	45.9	31.5	47.5	39.5	45	45	45	13.5	12.5	13.0
68	GB 8735 S1-142-2	70.0	80.4	75.2	40.0	59.6	49.8	43	44	44	12.4	11.9	12.2
	Mean	64.5	75.1	69.8	45.1	60.4	52.7	44	45	44	11.2	10.8	11.0
	Minimum	39.5	48.9	45.5	28.5	39.2	33.8	38	40	39	8.8	8.0	8.5
	Maximum	104.5	112.0	108.3	59.5	83.0	70.5	51	52	52	14.2	13.2	13.6
	LSD (P=0.05)	10.1	22.8	19.3	8.5	19.2	15.4	6.9	7.2	4.8	2.8	2.5	2.4
	CV (%)	10.8	15.3	14.0	9.1	15.9	14.8	4.2	7.6	6.9	5.2	6.5	6.3

Appendix VII. Environment wise mean performance and PCA scores of entries for grain Fe and Zn content in stability trials, summer and rainy seasons of 2004 and 2005, ICRISAT-Patancheru.

Trt. No.	Entry	Fe content (mg kg^{-1})						Zn content (mg kg^{-1})					
		Summer 2004 (E1)	Rainy 2004 (E2)	Summer 2005 (E3)	Rainy 2005 (E4)	Pooled Mean	PCA I	Summer 2004 (E1)	Rainy 2004 (E2)	Summer 2005 (E3)	Rainy 2005 (E4)	Pooled Mean	PCA I
1	81 B	32.4	36.4	40.6	36.5	36.5	-0.76	31.3	32.2	27.8	29.4	30.2	1.18
2	843 B	46.0	70.9	59.3	61.6	59.4	0.46	47.4	71.9	51.5	50.6	55.3	-0.98
3	863 B	69.0	76.5	78.5	76.4	75.1	-0.59	54.0	57.7	47.7	56.3	53.9	0.49
4	AIMP 92901 S1-15-1-2-B	74.7	76.6	94.5	92.1	84.5	-0.07	61.6	68.0	56.5	64.3	62.6	0.32
5	AIMP 92901 S1-183-2-2-B	49.2	102.0	59.7	69.4	70.1	1.40	46.5	80.8	40.0	38.1	51.4	-0.69
6	CZI 96-21	36.2	50.2	38.9	50.4	43.9	0.02	34.8	46.7	31.3	42.2	38.7	0.04
7	CZI-98-11	36.6	47.4	41.2	52.0	44.3	0.10	38.4	46.9	32.7	40.0	39.5	0.06
8	EEBC	51.0	64.1	66.5	83.2	66.2	1.99	45.5	59.2	46.5	53.2	51.1	0.10
9	GB 8735	52.3	73.1	67.9	75.7	67.2	1.24	45.2	61.0	41.7	51.4	49.8	-0.51
10	HTP 94/54 (HHB 146)	36.5	33.2	44.0	32.8	36.6	-1.75	31.1	32.3	31.5	30.7	31.4	1.39
11	ICMB 00888	56.7	64.1	49.6	46.4	54.2	-2.76	49.3	64.1	32.7	36.1	45.5	-2.47
12	ICMB 00999	44.3	72.7	57.1	80.9	63.7	2.78	49.5	68.9	45.0	57.1	55.1	-1.00
13	ICMB 88004	48.9	71.8	63.2	65.1	62.2	0.30	43.6	62.8	44.5	45.2	49.0	-0.98
14	ICMB 90111	34.2	41.3	38.8	45.5	39.9	-0.38	26.5	28.0	24.9	26.1	26.4	1.13
15	ICMB 94111	51.7	75.4	66.9	70.6	66.2	1.14	43.7	69.9	41.8	44.2	49.9	-1.90
16	ICMB 98222	51.3	74.6	69.4	77.2	68.1	1.97	46.6	59.1	42.4	49.3	49.3	-0.27
17	ICMS 7704-S1-51-4-1-1-B	45.1	62.9	59.0	57.0	56.0	0.03	45.6	69.8	44.1	49.1	52.1	-1.59
18	ICMS 8511 S1-17-2-1-1-B	30.8	29.4	31.8	32.8	31.2	-1.42	25.0	24.0	20.4	21.7	22.7	1.79
19	ICMV 91059 S1-58-2-2-2-B	40.6	44.1	48.4	57.3	47.6	0.23	35.5	44.2	35.9	43.5	39.8	0.37
20	ICMV 93074 S1-9-1-1-1-B	40.3	46.7	50.6	52.0	47.4	-0.14	43.4	47.8	38.8	37.8	41.9	0.34
21	IPC 843	52.7	49.7	66.7	68.9	59.5	0.30	52.8	57.5	51.9	53.1	53.8	0.61
22	Jakharana pop	39.4	44.1	49.0	56.8	47.3	0.16	35.6	46.8	38.6	45.4	41.6	0.83
23	MC 94 C2-S1-46-1-1-B	40.8	48.9	43.9	52.6	46.5	-0.32	40.8	40.9	32.0	38.0	37.9	1.14
24	NCD2 Bulk	38.3	43.9	40.7	47.3	42.5	-0.66	39.6	45.3	32.5	41.1	39.6	0.31
25	SDMV 90031-S1-84-1-1-2-B	53.2	84.8	54.1	48.2	60.1	-1.70	50.8	45.4	38.6	42.1	44.2	0.91
26	SDMV 90031-S1-93-3-1-1-B	29.3	39.0	36.5	49.0	38.4	0.61	28.7	34.7	24.8	32.0	30.0	1.00
27	SDMV 93032-S1-93-3-2-2	41.0	43.8	42.0	52.6	44.8	-0.26	38.9	46.6	30.8	38.5	38.7	-0.26
28	SOSAT C88	32.9	32.0	37.9	33.6	34.1	-1.35	32.8	34.6	28.3	30.4	31.5	0.98
29	WCC 75	42.0	41.9	43.1	52.8	44.9	-0.58	38.2	40.9	31.4	38.1	37.1	0.65
	Mean	44.7	56.6	53.1	57.8	53.1	-	41.4	51.3	37.5	42.2	43.1	-
	Environment PCA	-4.74	0.71	-0.16	4.20	-	-	-3.92	0.58	2.55	0.79	-	-

Appendix VIII. Mean performance, mid-parent and better-parent heterosis for grain Fe and Zn content in 10 × 10 diallel trial, rainy season-2005, ICRISAT- Patancheru.

Sl. No.	Hybrid	Fe content (mg kg^{-1})			Zn content (mg kg^{-1})		
		Mean	Mid heterosis	Better heterosis	Mean	Mid heterosis	Better heterosis
1	863B × ICMB 94111	70.6	1.70	1.00	43.6	7.08	5.06
2	863B × ICMB 00888	63.3	-6.63	-9.39	41.9	1.13	0.96
3	863B × AIMP 92901 -S1-15-1-2-B	70.7	2.07	1.14	47.5	15.26**	14.29*
4	863B × ICMB 95222	54.7	-7.13	-21.75**	37.3	-1.71	-10.19
5	863B × ICMV 93074 S1-9-1-1-1-B	69.3	11.42	-0.91	39.9	6.02	-3.85
6	863 B × MC 94 C2-S1-46-1-1-B	57.3	-3.67	-17.98**	33.8	-7.64	-18.54**
7	863B × 81B	53.7	5.23	-23.18**	36.1	7.77	-13.16*
8	863B × ICMS 8511 S1-17-2-1-1-B	48.1	-10.75	-31.14**	33.5	-3.46	-19.34**
9	863B × ICMV 91059 S1-14-2-4-2-2-B	56.6	0.38	-18.98**	40.5	26.43**	-2.49
10	ICMB 94111 × ICMB 00888	56.5	-16.11**	-18.04**	36.2	-11.10*	-12.64*
11	ICMB 94111 × AIMP 92901 -S1-15-1-2-B	58.4	-15.10**	-15.28*	34.8	-13.86*	-14.78*
12	ICMB 94111 × ICMB 95222	50.5	-13.55*	-26.74**	36.1	-2.87	-9.67
13	ICMB 94111 × ICMV 93074 S1-9-1-1-1-B	54.9	-10.94	-20.31**	37.9	2.67	-5.25
14	ICMB 94111 × MC 94 C2-S1-46-1-1-B	54.5	-7.68	-20.94**	33.7	-5.90	-15.60*
15	ICMB 94111 × 81B	44.3	-12.36	-35.74**	31.9	-2.40	-20.18**
16	ICMB 94111 × ICMS 8511 S1-17-2-1-1-B	37.1	-30.53**	-46.13**	26.7	-21.18**	-33.11**
17	ICMB 94111 × ICMV 91059 S1-14-2-4-2-2-B	51.8	-7.39	-24.85**	33.5	7.20	-16.18*
18	ICMB 00888 × AIMP 92901 -S1-15-1-2-B	61.4	-8.68	-10.59	46.2	12.28*	11.51
19	ICMB 00888 × ICMB 95222	47.2	-16.89*	-28.18**	34.6	-8.67	-16.43*
20	ICMB 00888 × ICMV 93074 S1-9-1-1-1-B	54.6	-9.15	-16.98*	38.1	1.42	-7.89
21	ICMB 00888 × MC 94 C2-S1-46-1-1-B	53.3	-7.22	-18.96**	35.6	-2.73	-14.09*
22	ICMB 00888 × 81B	45.4	-7.22	-30.92**	34.6	3.59	-16.43*
23	ICMB 00888 × ICMS 8511 S1-17-2-1-1-B	46.8	-9.70	-28.79**	34.3	-1.06	-17.23**
24	ICMB 00888 × ICMV 91059 S1-14-2-4-2-2-B	50.2	-7.57	-23.62**	32.4	1.46	-21.66**
25	AIMP 92901-S1-15-1-2-B × ICMB 95222	55.4	-4.86	-19.23**	39.7	5.59	-2.78
26	AIMP 92901-S1-15-1-2-B × ICMV 93074 S1-9-1-1-1-B	63.1	2.60	-8.01	38.3	2.64	-6.20
27	AIMP 92901 -S1-15-1-2-B × MC 94 C2-S1-46-1-1-B	52.4	-10.95	-23.60**	37.2	2.53	-8.90
28	AIMP 92901 S1-15-1-2-B × 81B	49.2	-2.31	-28.27**	33.8	2.16	-17.14**
29	AIMP 92901-S1-15-1-2-B × ICMS 8511 S1-17-2-1-1-B	47.4	-11.07	-30.94**	30.8	-10.43	-24.65**
30	AIMP 92901-S1-15-1-2-B × ICMV 91059 S1-14-2-4-2-2-B	51.2	-8.22	-25.40**	36.0	13.52	-11.92
31	ICMB 95222 × ICMV 93074 S1-9-1-1-1-B	40.7	-20.39**	-25.17**	33.9	-0.54	-1.36
32	ICMB 95222 × MC 94 C2-S1-46-1-1-B	44.7	-7.80	-8.96	34.0	2.77	-1.16
33	ICMB 95222 × 81 B	38.2	-4.58	-20.25*	27.5	-7.86	-19.88*
34	ICMB 95222 × ICMS 8511 S1-17-2-1-1-B	40.5	-5.67	-15.45	29.3	-5.84	-14.74
35	ICMB 95222 × ICMV 91059 S1-14-2-4-2-2-B	38.8	-14.50	-18.93*	28.4	-0.29	-17.46*
36	ICMV 93074 S1-9-1-1-1-B × MC 94 C2-S1-46-1-1-B	56.6	9.24	3.92	29.1	-11.09	-13.81
37	ICMV 93074 S1-9-1-1-1-B × 81B	47.7	10.08	-12.43	31.6	6.76	-6.51
38	ICMV 93074 S1-9-1-1-1-B × ICMS 8511-S1-17-2-1-1-B	42.2	-8.73	-22.54**	28.5	-7.46	-15.58*
39	ICMV 93074 S1-9-1-1-1-B × ICMV 91059 S1-14-2-4-2-2-B	45.8	-5.92	-15.86*	29.9	6.04	-11.64
40	MC 94 C2-S1-46-1-1-B × 81B	40.9	0.70	-16.69	25.4	-11.09	-19.96*
41	MC 94 C2-S1-46-1-1-B × ICMS 8511 S1-17-2-1-1-B	37.6	-13.59	-23.41**	26.1	-12.30	-17.65*
42	MC 94 C2-S1-46-1-1-B × ICMV 91059 S1 -14-2-4-2-2-B	55.4	20.28*	12.69	28.7	5.65	-9.66
43	81B × ICMS 8511 S1-17-2-1-1-B	41.0	17.02	8.08	29.3	10.14	5.26
44	81B × ICMV 91059 S1-14-2-4-2-2-B	37.4	-0.31	-12.81	27.8	15.86	9.32
45	ICMS 8511 S1-17-2-1-1-B × ICMV 91059 S1-14-2-4-2-2-B	37.0	-8.45	-13.74	29.2	15.74	4.67
	Mean	50.6	-5.66	-17.87	34.1	0.72	-10.19
	Minimum	37.0	-30.53	-46.13	25.4	-21.18	-33.11
	Maximum	70.7	20.28	12.69	47.5	26.43	14.29
	LSD ($P = 0.05$)	8.2			5.6		
	CV (%)	10.3			10.3		

Appendix IX. Mean performance, mid-parent and better-parent heterosis for days to 50% flower and 1000-grain mass in 10 × 10 diallel trial, rainy season 2005, ICRISAT- Patancheru.

Hybrid	Days to 50% flower			1000-grain mass (g)		
	Mean	Mid heterosis	Better heterosis	Mean	Mid heterosis	Better heterosis
863B × ICMB 94111	47	-6.27*	-8.39**	12.9	2.63	1.44
863B × ICMB 00888	44	-8.01**	-14.84**	9.7	-22.54**	-23.43**
863B × AIMP 92901 -S1-15-1-2-B	47	-5.02	-8.39**	13.9	18.77**	11.48**
863B × ICMB 95222	48	-9.49**	-11.18**	13.4	20.62**	7.89*
863B × ICMV 91059-S1-58-2-2-2-B	46	-10.97**	-10.97**	13.5	22.35**	8.24*
863 B × MC 94 C2-S1-46-1-1-B	45	-8.11**	-12.26**	11.5	-0.90	-7.11
863B × 81B	48	-7.64**	-8.81**	13.0	26.89**	4.64
863B × ICMS 8511 S1-17-2-1-1-B	47	-11.95**	-14.11**	11.5	5.70	-7.16
863B × ICMV 91059 S1-14-2-4-2-2-B	47	-8.97**	-9.55**	13.7	29.24**	10.22**
ICMB 94111 × 863B	46	-8.25**	-10.32**	14.7	16.79**	15.44**
ICMB 94111 × ICMB 00888	45	-3.57	-8.78**	14.3	12.71**	12.71**
ICMB 94111 × AIMP 92901 -S1-15-1-2-B	45	-6.85*	-8.11*	11.6	-1.91	-8.91*
ICMB 94111 × ICMB 95222	47	-8.09**	-11.80**	11.8	5.17	-6.89
ICMB 94111 × ICMV 91059-S1-58-2-2-2-B	44	-12.87**	-14.84**	11.5	3.08	-9.72*
ICMB 94111 × MC 94 C2-S1-46-1-1-B	46	-5.19	-7.43*	12.4	5.16	-2.49
ICMB 94111 × 81B	49	-3.58	-6.92*	11.7	12.91**	-7.73*
ICMB 94111 × ICMS 8511 S1-17-2-1-1-B	48	-8.04**	-12.27**	12.8	15.60**	0.52
ICMB 94111 × ICMV 91059 S1-14-2-4-2-2-B	46	-8.85**	-11.46**	10.2	-5.32	-20.03**
ICMB 00888 × 81 B	44	-8.71**	-15.48**	12.8	1.70	0.52
ICMB 00888 × ICMB 94111	44	-5.71	-10.81**	13.7	7.86*	7.86*
ICMB 00888 × AIMP 92901 -S1-15-1-2-B	43	-6.52*	-10.42**	12.8	8.54*	0.79
ICMB 00888 × ICMB 95222	44	-10.58**	-18.63**	12.6	11.47**	-1.31
ICMB 00888 × ICMV 91059-S1-58-2-2-2-B	42	-12.89**	-19.35**	11.7	5.36	-7.73*
ICMB 00888 × MC 94 C2-S1-46-1-1-B	43	-6.23*	-9.22**	13.4	13.50**	5.24
ICMB 00888 × 81B	49	1.72	-6.92*	13.2	27.15**	3.91
ICMB 00888 × ICMS 8511 S1-17-2-1-1-B	46	-6.44*	-15.34**	13.1	18.31**	2.88
ICMB 00888 × ICMV 91059 S1-14-2-4-2-2-B	45	-5.88*	-13.38**	11.7	8.86*	-8.05*
AIMP 92901 S1-15-1-2-B × 863B	46	-7.69**	-10.97**	11.7	0.53	-5.63
AIMP 92901 S1-15-1-2-B × ICMB 00888	48	-2.05	-3.38	13.4	13.79**	5.66
AIMP 92901 S1-15-1-2-B × ICMB 94112	44	-5.07	-9.03**	12.6	6.65	-0.97
AIMP 92901-S1-15-1-2-B × ICMB 95222	46	-9.51**	-14.29**	11.6	11.88**	6.24
AIMP 92901-S1-15-1-2-B × ICMV 91059 S1-58-2-2-2-B	45	-9.70**	-12.90**	11.9	16.51**	9.33*
AIMP 92901 -S1-15-1-2-B × MC 94 C2-S1-46-1-1-B	47	-0.35	-1.39	12.8	17.73**	17.55**
AIMP 92901 S1-15-1-2-B × 81B	49	-2.97	-7.55*	12.1	27.77**	11.16*
AIMP 92901-S1-15-1-2-B × ICMS 8511 S1-17-2-1-1-B	52	1.63	-4.29	11.7	15.27**	7.34
AIMP 92901-S1-15-1-2-B × ICMV 91059 S1-14-2-4-2-2-B	45	-10.30**	-14.01**	10.1	2.88	-7.19
ICMB 95222 × 863B	48	-8.86**	-10.56**	10.9	-2.20	-12.53**
ICMB 95222 × ICMB 94111	48	-7.44**	-11.18**	11.7	3.63	-8.26*
ICMB 95222 × ICMB 00888	48	-2.39	-11.18**	11.6	3.42	-8.44*
ICMB 95222 × AIMP 92901-S1-15-1-2-B	52	2.95	-2.48	11.7	12.88**	7.19
ICMB 95222 × ICMV 91059 S1-58-2-2-2-B	49	-7.59**	-9.32**	10.1	4.36	3.06
ICMB 95222 × MC 94 C2-S1-46-1-1-B	46	-8.61**	-14.29**	11.3	9.68*	4.29
ICMB 95222 × 81 B	51	-5.00	-5.59	11.7	31.19**	19.59**
ICMB 95222 × ICMS 8511 S1-17-2-1-1-B	51	-6.17*	-6.75*	9.8	2.26	0.17
ICMB 95222 × ICMV 91059 S1-14-2-4-2-2-B	52	-1.26	-2.48	9.0	-3.55	-8.64
ICMV 91059 S1-58-2-2-2-B × 863B	45	-12.90**	-12.90**	13.1	19.05**	5.31
ICMV 91059 S1-58-2-2-2-B × ICMB 94111	47	-7.59**	-9.68**	10.7	-3.83	-15.78**
ICMV 91059 S1-58-2-2-2-B × ICMB 00888	44	-8.71**	-15.48**	9.6	-14.10**	-24.77**
ICMV 91059 S1-58-2-2-2-B × AIMP 92901-S1-15-1-2-B	47	-6.35*	-9.68**	11.8	15.17**	8.07

ppendix IX. Contd.

Sl. No.	Hybrids	Days to 50% flower			1000-grain mass (g)		
		Mean	Mid heterosis	Better heterosis	Mean	Mid heterosis	Better heterosis
30	ICMV 91059 S1-58-2-2-2-B × ICMB 95222	51	-3.16	-4.97	10.1	4.70	3.40
31	ICMV 91059 S1-58-2-2-2-B × MC 94 C2-S1-46-1-1-B	46	-7.43**	-11.61**	10.8	6.09	-0.31
32	ICMV 91059 S1-58-2-2-2-B × 81B	50	-5.10	-6.29*	8.5	-3.16	-10.71*
33	ICMV 91059 S1-58-2-2-2-B × ICMS 8511-S1-17-2-1-1-B	49	-7.55**	-9.82**	10.5	10.43*	9.52
34	ICMV 91059 S1-58-2-2-2-B × ICMV 91059 S1-14-2-4-2-2-B	48	-8.33**	-8.92**	8.5	-7.22	-11.06*
35	MC 94 C2-S1-46-1-1-B × 863B	46	-6.08*	-10.32**	13.1	12.78**	5.72
36	MC 94 C2-S1-46-1-1-B × ICMB 94111	44	-8.65**	-10.81**	11.4	-3.04	-10.09**
37	MC 94 C2-S1-46-1-1-B × ICMB 00888	44	-4.03	-7.09*	13.6	15.48**	7.08
38	MC 94 C2-S1-46-1-1-B × AIMP 92901 S1-15-1-2-B	43	-8.77**	-9.72**	13.5	23.74**	23.55**
39	MC 94 C2-S1-46-1-1-B × ICMB 95222	46	-9.27**	-14.91**	13.0	25.39**	19.23**
40	MC 94 C2-S1-46-1-1-B × ICMV 91059-S1-58-2-2-2-B	45	-8.11**	-12.26**	9.4	-7.95	-13.50**
41	MC 94 C2-S1-46-1-1-B × 81B	48	-4.67	-10.06**	11.0	16.37**	1.38
42	MC 94 C2-S1-46-1-1-B × ICMS 8511 S1-17-2-1-1-B	47	-7.89**	-14.11**	13.0	28.62**	19.94**
43	MC 94 C2-S1-46-1-1-B × ICMV 91059 S1 -14-2-4-2-2-B	45	-10.07**	-14.65**	9.5	-3.57	-12.88**
44	81 B × 863 B	48	-8.92**	-10.06**	13.6	32.98**	9.66*
45	81B × ICMB 94111	49	-4.89	-8.18**	10.4	0.08	-18.22**
46	81B × ICMB 00888	49	1.72	-6.92*	11.7	12.69**	-7.92*
47	81B × AIMP 92901 S1-15-1-2-B	50	-0.99	-5.66	10.6	11.95**	-2.60
48	81B × ICMB 95222	48	-10.63**	-11.18**	10.1	13.25**	3.23
49	81B × ICMV 91059 S1-58-2-2-2-B	50	-5.10	-6.29*	11.2	27.48**	17.54**
50	81B × MC 94 C2-S1-46-1-1-B	49	-2.67	-8.18**	10.5	11.27*	-3.07
51	81B × ICMS 8511 S1-17-2-1-1-B	50	-6.83**	-7.98**	9.8	11.83*	3.90
52	81B × ICMV 91059 S1-14-2-4-2-2-B	50	-4.43	-5.03	8.3	-0.99	-4.94
53	ICMS 8511 S1-17-2-1-1-B × 863B	50	-5.66*	-7.98**	12.2	11.68**	-1.91
54	ICMS 8511 S1-17-2-1-1-B × ICMB 94111	48	-8.04**	-12.27**	13.0	17.86**	2.49
55	ICMS 8511 S1-17-2-1-1-B × ICMB 00888	50	1.02	-8.59**	9.5	-13.79**	-25.03**
56	ICMS 8511 S1-17-2-1-1-B × AIMP 92901 S1-15-1-2-B	53	2.93	-3.07	12.2	20.53**	12.23**
57	ICMS 8511 S1-17-2-1-1-B × ICMB 95222	50	-7.41**	-7.98**	8.9	-7.29	-9.18
58	ICMS 8511 S1-17-2-1-1-B × ICMV 91059 S1-58-2-2-2-B	48	-8.81**	-11.04**	10.7	13.24**	12.31*
59	ICMS 8511 S1-17-2-1-1-B × MC 94 C2-S1-46-1-1-B	48	-4.61	-11.04**	9.8	-2.96	-9.51*
60	ICMS 8511 S1-17-2-1-1-B × 81B	51	-4.97	-6.13*	8.4	-4.20	-10.99*
61	ICMS 8511 S1-17-2-1-1-B × ICMV 91059 S1-14-2-4-2-2-B	50	-6.25*	-7.98**	9.6	6.06	2.48
62	ICMV 91059 S1-14-2-4-2-2-B × 863B	51	-2.56	-3.18	11.8	11.50**	-4.91
63	ICMV 91059 S1-14-2-4-2-2-B × ICMB 94111	49	-2.95	-5.73	9.2	-14.35**	-27.65**
64	ICMV 91059 S1-14-2-4-2-2-B × ICMB 00888	47	-2.42	-10.19**	12.9	20.50**	1.78
65	ICMV 91059 S1-14-2-4-2-2-B × AIMP 92901 S1-15-1-2-B	50	-0.33	-4.46	9.4	-4.75	-14.07**
66	ICMV 91059 S1-14-2-4-2-2-B × ICMB 95222	49	-8.18**	-9.32**	9.7	4.13	-1.36
67	ICMV 91059 S1-14-2-4-2-2-B × ICMV 91059 S1-58-2-2-2-B	52	-0.64	-1.27	9.4	2.97	-1.29
68	ICMV 91059 S1-14-2-4-2-2-B × MC 94 C2-S1-46-1-1-B	46	-8.05**	-12.74**	9.8	-0.51	-10.12*
69	ICMV 91059 S1-14-2-4-2-2-B × 81B	49	-6.96**	-7.55*	8.9	5.35	1.14
70	ICMV 91059 S1-14-2-4-2-2-B × ICMS 8511 S1-17-2-1-1-B	52	-1.88	-3.68	9.4	3.49	0.00
	Mean	47	-6.07	-9.54	11.4	8.55	-0.65
	Minimum	42	-12.9	-19.35	8.3	-22.54	-27.65
	Maximum	53	2.95	-1.27	14.7	32.98	23.55
	LSD ($P = 0.05$)	8.9			2.8		
	CV (%)	9.8			8.6		
	orrelation between mid-parent value and hybrid performance	r = 0.23			r=0.69**		

, ** Significant at 5% and 1% probability levels, respectively.

PUBLICATIONS

Plant Breeding
© 2007 ICRISAT
Journal compilation © 2007 Blackwell Verlag, Berlin

doi:10.1111/j.1439-0523.2007.01322.x

Prospects of breeding biofortified pearl millet with high grain iron and zinc content

G. VELU[1], K. N. RAI[2,4], V. MURALIDHARAN[1], V. N. KULKARNI[2], T. LONGVAH[3] and T. S. RAVEENDRAN[3]

[1]Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India; [2]International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India; [3]National Institute of Nutrition, Hyderabad 500 007, Andhra Pradesh, India; [4]Corresponding author, E-mail: k.rai@cgiar.org

With 1 figure and 1 table

Received November 20, 2005/Accepted August 7, 2006
Communicated by H. H. Geiger

Abstract

Development of crop cultivars with elevated levels of micronutrients is being increasingly recognized as one of the approaches to provide sustainable solutions to various health problems associated with micronutrient malnutrition, especially in developing countries. To assess the prospects of this approach in pearl millet (*Pennisetum glaucum*), a diverse range of genetic materials, consisting of 40 hybrid parents, 30 each of population progenies and improved populations, and 20 germplasm accessions, was analysed for grain iron (Fe) and zinc (Zn) content, deficiencies of which adversely affect human health. Based on the mean performance in two seasons at ICRISAT, Patancheru, India, large variability among the entries was found, both for Fe (30.1–75.7 mg kg^{-1} on dry weight basis) and Zn (24.5–64.8 mg kg^{-1}). The highest levels of grain Fe and Zn were observed in well-adapted commercial varieties and their progenies, and in the parental lines of hybrids, which were either entirely based on *iniari* germplasm, or had large components of it in their parentage. There were indications of large within-population genetic variability for both Fe and Zn. The correlation between Fe and Zn content was positive and highly significant ($r = 0.84$; $P < 0.01$). These results indicate that there are good prospects of simultaneous selection for both micronutrients, and that selection within populations, especially those with the predominantly *iniari* germplasm, is likely to provide good opportunities for developing pearl millet varieties and hybrid parents with significantly improved grain Fe and Zn content in pearl millet.

Key words: *Pennisetum glaucum* · grain iron and zinc · genetic resources · correlation · biofortification

Micronutrient malnutrition, resulting from the dietary deficiency of some of the important minerals such as iron (Fe) and zinc (Zn), and vitamin A, has been reported to be a widespread food-related health problem, affecting more than two billion people worldwide (WHO 2002). This problem is particularly serious in developing countries where most of the population has limited access to meat, fruits and vegetables, which are generally rich sources of these micronutrients. The HarvestPlus initiative of the Consultative Group on International Agricultural Research (CGIAR) has recently embarked on addressing this issue through the development of improved crop cultivars with elevated levels of these micronutrients. Pearl millet [*Pennisetum glaucum* (L.) R. Br.], grown on more than 26 million ha in the arid and semi-arid regions of Asia and Africa, and serving as a major source of dietary energy for a vast population in these regions, is one of the crops included in this initiative. A pearl millet breeding line with yellow grain colour derived from a germplasm accession originating from Burkina Faso, with *β*-carotene (precursor of vitamin A) levels as high as 137 μg 100 g^{-1} has been identified at ICRISAT, Patancheru, India (Hash et al. 1996). Attempts are underway to identify molecular markers of the gene(s) associated with this trait and use molecular marker technology to transfer it into commercial open-pollinated varieties and hybrid parents. Pearl millet has high grain Fe and Zn content. There are reports of up to 580 mg kg^{-1} Fe and 70 mg kg^{-1} Zn in pearl millet germplasm accessions (Jambunathan and Subramanian 1988), but neither the identity of these accessions is available, nor have the micronutrient analyses protocols been reported. The initial objective of the HarvestPlus project on pearl millet is to initiate an intensive search to identify sources of higher grain Fe and Zn content in this crop. This research reports on the extent of variability for Fe and Zn content in grains, the consistency of their expression across environments, and the relationship between Fe and Zn in a diverse range of materials, to assess the prospects of their genetic enhancement in cultivar development.

Materials and Methods

The experimental material consisted of 120 entries of pearl millet, which included 30 each of improved populations and population progenies (partial inbreds), and 20 each of germplasm accessions, seed parents and pollinators. This was inclusive of the three controls, consisting of the first ICRISAT-bred and the most widely cultivated open-pollinated variety 'WC C75', the seed parent (81B) and the pollen parent (ICMP 451) of the first ICRISAT-bred and the most widely cultivated hybrid 'ICMH 451'. The trial was laid out in a randomized complete block design with two replications during the 2004 summer and rainy seasons (hereafter referred to as environments) at ICRISAT, Patancheru, India. Each entry was grown in four rows, 4 m long with 75 cm spacing between the rows and 15 cm spacing between plants within the rows. The trials were conducted in Alfisols with applied fertilizer levels of 78 kg ha^{-1} N (50% basal and 50% top dressing) and 35 kg ha^{-1} P during both the summer and the rainy season. The trials were irrigated eight times during the [illegible] the rainy season to ensure ther[illegible]

micronutrient levels in soil measured in six samples (three each at 0–15 cm and 15–30 cm depth) at the time of planting varied from 6.2 ± 0.45 mg kg^{-1} Fe and 1.6 ± 0.52 mg kg^{-1} Zn for the field used during the summer season to 15.8 ± 3.11 mg kg^{-1} Fe and 2.2 ± 0.63 mg kg^{-1} Zn for the field used during the rainy season.

Sib-mated grains were produced for laboratory analysis of Fe and Zn content. Plants were selfed with parchment paper bags at the initiation of panicle emergence. Bulk pollen collected from 50–60 plants in a plot was used to cross 20 plants of the same plot. The sib-mated panicles were harvested at physiological maturity, machine-threshed (Wintersteiger ID7805114 – Single head thresher, Ried, Austria), and the grains cleaned of any glumes before being transferred

to paper envelopes with metal seals. Precautions were taken in each step to avoid any contamination of grains with dust particles and any other extraneous matter. The grain samples from the trials were analysed using an atomic absorption spectrophotometry (Thermo Electron Corporation, Cambridge, UK) fitted with a GFS97 autosampler, a system equivalent to the Inductively Coupled Plasma Atomic Absorption Spectrophotometry at the National Institute of Nutrition, Hyderabad, India. Pearl millet samples were powdered to pass through a 10-mesh sieve using a cyclone sample mill (Udy Corporation, Fort Collins, CO, USA). Aliquots of the powdered sample were taken as two subsamples from each entry and ashed. Dry ashing and mineral solution preparation were carried out according to the method described by Jorhem (1993). The analytical method used was validated using NIST standard certified reference material (1584A). Every day an in-house quality control sample was ashed and quantified, together with a blank, to expose any systematic errors. For blanks, no major interference was found. Discrepancies between in-house quality control samples and concentrations quantified were below 5% and the coefficient of variation for single measurements was below 10%. Grain Fe and Zn content data (expressed in mg kg on a dry weight basis) were analysed for individual environments, as well as across the two environments following a fixed model analysis of variance (Gomez and Gomez 1984).

Results

The average grain Fe content in the trial was comparable to the average Zn content both in the summer season (41.5 mg/kg Fe and 40.1 mg/kg Zn) and rainy season (49.5 mg/kg Fe and 47.7 mg/kg Zn) (Table 1). It was also observed that the average grain Fe and Zn contents in the rainy season were 19–20 % higher than those in the summer season. This may be due to the high Fe and Zn levels in the soil, as the field used during the rainy season had 155% more Fe and 38% more Zn than the field used during the summer season. Analysis of variance (data not presented) showed highly significant differences among the entries for grain Fe and Zn content in both seasons ($P < 0.001$). There were also significant entry × environment interactions for both micronutrients, but the correlation of micronutrient content of genotypes between the two seasons was highly significant, both for Fe ($r = 0.66$; $P < 0.01$) and Zn ($r = 0.69$; $P < 0.01$), indicating high levels of consistency of the rankings of entries across the two seasons for both Fe and Zn content.

Based on the mean performance across the two environments, a large variability among the entries was observed for both Fe (30.1–75.7 mg/kg) and Zn content (24.5–64.8 mg/kg) (Table 1). A majority of the entries had grain Fe content in the range of 35–55 mg/kg and Zn content in the range of 35–50 mg/kg (Fig. 1). Although the differences among the three controls (81B, 'ICMP 451' and 'WC-C75') were not statistically significant, the open-pollinated variety 'WC-C75' had the highest levels of both Fe (41.9 mg/kg) and Zn (39.5 mg/kg) content. Twenty-one entries had 28–81% higher grain Fe than

Table 1. Selected pearl millet inbred lines and populations with high grain Fe and Zn content, 2004 summer and rainy seasons, ICRISAT, Patancheru

	Fe content (mg/kg)			Zn content (mg/kg)		
Inbred line/population	Summer	Rainy	Mean	Summer	Rainy	Mean
Hybrid parent (n = 40)						
863 B	69.0	76.5	72.7	54.0	57.7	55.8
ICMB 94111	51.7	75.4	63.6	43.7	69.9	56.8
ICMB 98222	51.3	74.6	62.9	46.6	59.1	52.9
ICMB 00888	56.7	64.1	60.4	49.3	64.1	56.7
ICMB 88004	48.9	71.8	60.4	43.6	62.8	53.2
ICMB 00999	44.3	72.7	58.5	49.5	68.9	59.2
843 B	46.0	70.9	58.4	47.4	71.9	59.6
ICMB 94555	47.5	59.9	53.7	44.0	57.1	50.5
MIR 97171	50.9	60.4	55.7	42.8	50.7	46.7
Population progenies (n = 30)						
AIMP 92901 S1-15-1-2-B	74.7	76.6	75.7	61.6	68.0	64.8
AIMP 92901 S1-183-2-2-B	49.2	102.0	75.6	46.5	80.8	63.7
SDMV 90031-S1-84-1-1-2-B	53.2	84.8	69.0	50.8	45.4	48.1
RCB-2 S1-33-1-3-3-2-B	45.7	65.7	55.7	41.1	57.1	49.1
ICMR 312 S1-25-1-1-1-3-B	47.8	62.1	55.0	43.8	52.8	48.3
AIMP 92901 S1-421-2-3-B	46.0	63.1	54.6	45.6	56.8	51.2
ICMS 7704-S1-51-4-1-1-B	45.1	62.9	54.0	45.6	69.8	57.7
Improved population (n = 30)						
GB 8735	52.3	73.1	62.7	45.2	61.0	53.1
ICEBC	51.0	64.1	57.5	45.5	59.2	52.4
ICMV 221	47.5	67.0	57.3	43.4	56.7	50.1
HHVDBC	48.7	61.2	55.0	43.2	51.9	47.6
Germplasm accession (n = 20)						
IP 8964	47.7	61.1	54.4	43.0	54.7	48.8
Control						
81 B	32.4	36.4	34.4	31.3	32.2	31.7
ICMP 451	39.6	38.4	39.0	36.0	34.9	35.4
WC-C 75	42.0	41.9	41.9	38.2	40.9	39.5
Trial (n = 120)						
Minimum	29.3	29.4	30.1	25.0	24.0	24.5
Maximum	74.7	102.0	75.7	61.6	80.8	64.8
Mean	41.5	49.5	45.5	40.1	47.7	43.9
LSD (P = 0.05)	10.5	16.8		6.4	11.1	
CV (%)	12.8	17.2		8.0	11.8	

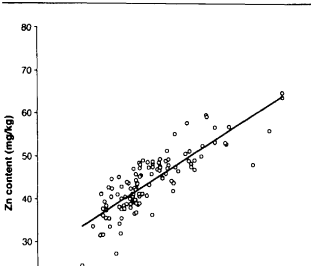


Fig. 1: Relationship between grain Fe and Zn content in pearl millet

that of 'WC-C75' (Table 1). Of these, 15 entries also had 25 - 62% higher Zn content than that of 'WC-C75'. Two S_4 progenies derived from an open-pollinated variety ('AIMP 92901') had the highest levels of both Fe (about 76 mg/kg) and Zn (about 65 mg/kg), which exceeded the grain Fe and Zn of 'AIMP 92901' (50.8 mg/kg Fe and 42.0 mg/kg Zn) by 46% and 52%, respectively. 'AIMP 92901' is a high-yielding open-pollinated variety, collaboratively developed by ICRISAT and the National Agricultural Research Project Centre of Marathwada Agricultural University, Aurangabad, Maharashtra, India. 'AIMP 92901' was released in 1998 as 'Samrudhi' for cultivation in pearl millet zone B (central and southern states) of India. This variety was developed from a Bold-seeded Early Composite that was constituted from open-pollinated varieties and several other progenies produced from the *iniari* germplasm. The *iniari* landrace has been observed as a promising germplasm with several positive attributes such as early maturity, large seed size and compact panicles (Andrews and Anand Kumar 1996). An S_5 progeny derived from another variety 'SDMV 90031' also had a high Fe content (69.0 mg/kg), which was 44% more than that in the original population (Fe 47.8 mg/kg). 'SDMV 90031' was developed largely based on the *iniari* germplasm by ICRISAT in partnership with the national research programmes in the southern African region. Evaluation of just two or three progenies from each of these two populations revealed large intra-population variability, with an Fe content in the range of 46-76 mg/kg in AIMP 92901 progenies and 43-69 mg/kg in the SDMV 90031 progenies, while the Zn content was in the range of 51-65 mg/kg in the AIMP 92901 progenies and 32-48 mg/kg in the SDMV 90031 progenies. This showed that evaluation of a larger number of progenies from these varieties might lead to identification of progenies with still higher grain Fe and Zn content.

Among the inbred lines, produced as parental lines of hybrids, the maintainer line 863B had the highest level of Fe (72.7 mg/kg), and it was amongst the top five hybrid parents for high Zn content (55.8 mg/kg) as well. It is interesting that, except for MIR 97171, all the other hybrid parents with high Fe and Zn content were either directly selected from *iniari* germplasm such as 863B, ICMB 98222 and ICMB 88004, or involved a large proportion of *iniari* germplasm in their parentage, such as ICMB 94111, ICMB 00888, ICMB 00999, 843B and ICMB 94555.

Among the open-pollinated varieties, 'GB 8735', also developed largely from the *iniari* germplasm, had the highest grain Fe (62.7 mg/kg) and Zn (53.1 mg/kg) content. This early-maturing and large-seeded variety, developed by ICRISAT in partnerships with the national programmes in the western and central African region, has been released in Chad, Mauritania, Nigeria and Benin. Amongst the other populations with high Fe and Zn level, they were either derived entirely from the *iniari* germplasm, such as EEBC and ICMV 221 (= ICMV 88904), or had a substantial proportion of the *iniari* germplasm in their parentage such as HHVDBC. ICMV 221, having performed better than the popular commercial variety ICTP 8203 (15% higher grain yield than ICTP 8203), was first released in India and subsequently in Kenya and Eritrea in eastern Africa. EEBC is a photoperiod-insensitive, extra-early maturing population (matures in < 65 days) with large seed size (13.14 g/1000) and a high level of resistance to downy mildew. High Head Volume Dwarf B-Composite (HHVDBC) is a dwarf population (110 cm plant height) of mid-late maturity (90 days to mature), large seed size (13.5 g/1000) and thick panicles (40 mm diameter). Both EEBC and HHVDBC have been extensively used at ICRISAT for breeding seed parents.

Discussion

These results showed that the high levels of grain Fe and Zn content in pearl millet are almost twice those in sorghum (Reddy et al. 2005) and 50% more than those in maize (Gregorio 2002). Furthermore, these entries with high Fe and Zn content are some of the released and widely-cultivated varieties and hybrid parents of popular high-yielding hybrids, which can now be used for bioavailability evaluation.

There was a highly significant and positive correlation between the Fe and Zn content in both the summer season ($r = 0.78$; $P < 0.01$) and the rainy season ($r = 0.82$; $P < 0.01$). Based on the mean performance across the two seasons, this correlation was still higher ($r = 0.84$; $P < 0.01$) (Fig. 1), indicating that simultaneous genetic improvement for the elevated levels of both micronutrients should be highly effective. Highly significant and positive correlation between grain Fe and Zn content has also been observed in maize (Maziya-Dixon et al. 2000), wheat (Graham et al. 1999) and sorghum (Reddy et al. 2005). Highly significant positive correlations of 1000-grain weight with Fe ($r = 0.80$; $P < 0.01$) and Zn ($r = 0.85$; $P < 0.01$) content per grain indicated that breeding for the higher levels of these micronutrients could be achieved without compromising the large grain size, which is a preferred grain trait in several African countries (Chintu et al. 1994, Ipinge et al. 1994) and in central and southern parts of India (especially in Maharashtra).

Thus, the results of this study indicate that prospects of genetic enhancement for increased levels of both Fe and Zn content in pearl millet are high, and that intensive exploitation of *iniari* germplasm is likely to lead to the identification of lines with still higher Fe and Zn content in relatively elite agronomic backgrounds. This may enable rapid development of high-yielding open-pollinated varieties and hybrid parents with

high grain Fe and Zn content. Much higher grain Fe and Zn levels have been reported in some of the earlier studies (Jambunathan and Subramanian 1988, Abdalla et al. 1998, Malik 1999). The higher grain Fe and Zn densities reported in these studies could be due either to environmental influence or to different germplasm, indicating the need to screen even non-*iniari* germplasm for identifying additional sources of high grain Fe and Zn content.

Acknowledgements

This research was done with funding support from the Harvest Plus Challenge Program of the Consultative Group on International Agricultural Research (CGIAR).

References

Abdalla, A. A., A. H. El Tinay, B. E. Mohamed, and A. H. Abdalla, 1998: Proximate composition, starch, phytate and mineral contents of 10 pearl millet genotypes. Food Chem. **63**, 243–246.

Andrews, D. J., and K. Anand Kumar, 1996: Use of the West African pearl millet landrace *iniadi* in cultivar development. Plant Genet. Res. Newsl. **105**, 15–22.

Chintu, E. M., E. S. Monyo, and S. C. Gupta, 1994: On-Farm evaluation of pearl millet varieties in Malawi for farmer preferences, grain yield and food quality traits. Drought tolerant crops for Southern Africa. In: Proceedings of the SADC/ICRISAT Regional Sorghum and Pearl Millet Workshop, 27–33. Gaborone, Botswana.

Gomez, K. A., and A. A. Gomez, 1984: Statistical Procedures for Agricultural Research. International Rice Research Institute Press, Philippines.

Graham, R. D., D. Senadhira, S. Beebe, C. Iglesias, and I. Monasterio, 1999: Breeding for micronutrient density in edible portions of stable food crops: conventional approaches. Field Crops Res. **60**, 57–80.

Gregorio, G. B., 2002: Progress in breeding for trace minerals in staple crops. J. Nutr. **132**, 500S–502S.

Hash, C. T., A. G. Bhaskar Raj, S. Appa Rao, and U. Singh, 1996: New sources of yellow endosperm and β-carotene in pearl millet. In: Proceedings of the International Conference on Genetic Improvement of Sorghum and Pearl Millet, 650–IX, USA.

Ipinge, S. A., W. R. Leebner, and E. S. Monyo, 1994: On-Farm evaluation of pearl millet varieties in Malawi for farmer preferences, grain yield and food quality traits. Drought tolerant crops for Southern Africa. In: Proceedings of the SADC/ICRISAT Regional Sorghum and Pearl Millet Workshop, 35–42. Gaborone, Botswana.

Jambunathan, R., and V. Subramanian, 1988: Grain quality and utilization in sorghum and pearl millet. In: Proceedings of Workshop on Biotechnology for Tropical Crop Improvement, 133–139. ICRISAT, Patancheru, India.

Jorhem, L., 1993: Determination of metals in foodstuffs by atomic absorption spectrophotometry after dry ashing: NMKL inter laboratory study of lead, cadmium, zinc, copper, iron, chromium and nickel. J. AOAC Int. **76**, 798–813.

Malik, M., 1999: Functional and Nutritional Properties of Newly Developed Hybrids and Varieties of Pearl Millet. MSc thesis, CCS Haryana Agricultural University, Hisar, Haryana, India.

Maziya-Dixon, B., J. G. Kling, A. Menkir, and A. Dixon, 2000: Genetic variation in total carotene, iron and zinc contents of maize and cassava genotypes. Food Nutr. Bull. **21**, 419–422.

Reddy, B. V. S., S. Ramesh, and T. Longvah, 2005: Prospects of breeding for micronutrients and β-carotene-dense sorghums. Int. Sorghum Millets Newsl. **46**, 10–14.

WHO, 2002: Reducing risks and promoting healthy life. Report 61–105. World Health Organization, Geneva, Switzerland.

A Rapid Screening Method for Grain Iron Content in Pearl Millet

G Velu[1], VN Kulkarni[2], V Muralidharan[1], KN Rai[1]*, T Longvah[3], KL Sahrawat[1] and TS Raveendran[1]

[1. Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India; 2. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India; 3. National Institute of Nutrition, Hyderabad 500 007, Andhra Pradesh, India]

*Corresponding author: k.rai@cgiar.org

Introduction

Enhancing grain iron (Fe) content is one of the effective ways of increasing the Fe intake and reducing the incidence of Fe-deficiency anaemia (Welch and Graham 2002). Large genetic variability for grain Fe content has been reported in many crops (Graham et al. 1999). Pearl millet [*Pennisetum glaucum* (L.) R.Br.] is a major source of dietary energy for millions of people living in the arid and semi-arid tropical regions of Africa and Asia. It has on an average 50 mg kg^{-1} of grain Fe, which is more than wheat, rice and maize. Studies with limited germplasm have shown large genetic variability for this trait, indicating good opportunities to select and/or breed millet genotypes with still higher grain Fe (Jambunathan and Subramanian 1988; Abdalla et al. 1998). The bottleneck in this process is the high cost of Fe estimation. At present Fe estimation is done with digests using Atomic Absorption Spectrophotometry (AAS) and Inductively Coupled Plasma-Atomic Absorption Spectrophotometry (ICP), which require relatively expensive instruments and are time-consuming. These chemical analyses would be prohibitively costly for evaluating a large number of progenies during the course of a breeding program. A procedure based on Perls' Prussian blue stain was proposed for rapid screening of grain Fe content in rice (Prom-u-thai et al. 2003; Krishnan et al. 2003) which involves scoring color intensity in the embryo of cut and treated seeds (with 2% Prussian blue) through a stereo-microscope. The objective of this research was to simplify the method and assess its effectiveness in screening for grain Fe content in pearl millet.

Materials and Methods

Experimental materials. During the dry season of 2004, 120 entries were grown at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India for estimation of grain Fe content by the AAS method (Jorhem 1993) at the National Institute of Nutrition (NIN), Hyderabad, India. Out of these, 12 pearl millet lines (6 B-lines and 6 partial inbreds) were chosen with a wide range of grain Fe content (30.8–74.7 mg kg^{-1}) and were classified based on the Fe content as high (51.7–74.7 mg kg^{-1}), medium (40.3–40.8 mg kg^{-1}) and low (30.8–35.7 mg kg^{-1}) to standardize the Perls' Prussian blue procedure. Subsequently, the procedure was validated using 20 B-lines (counterparts of the designated A-lines developed at ICRISAT-Patancheru) of pearl millet with a wide range of grain Fe content (32.4–69.0 mg kg^{-1}). The grain samples obtained from sib mating during the dry season of 2004 at ICRISAT-Patancheru, were used for the experiments. Precautions were taken to avoid external contamination of grain with dust in the field and during threshing and cleaning.

Reagents. In rice, the Perls' Prussian blue method with Prussian blue solution of 2% concentration was used in identifying high grain Fe genotypes (Prom-u-thai et al. 2003). The same concentration was initially used in our method and the reagents were prepared as follows.

1) Aqueous potassium ferrocyanide (2%): 10.0 g potassium ferrocyanide was mixed with distilled water, and the volume was made to 500 mL. The solution was transferred into an acid-cleaned brown bottle for storage, which remains stable for 6 months.

2) Aqueous hydrochloric acid (2%): 10.0 mL concentrated hydrochloric acid (HCl) was mixed with distilled water to make the volume 500 mL. This dilute HCl was transferred into a brown bottle for storage, which remains stable for 6 months.

3) Prussian blue solution: This solution was prepared by mixing equal volumes of 2% HCl and 2% ferrocyanide solutions. This solution was freshly prepared every time during testing. The unused solution was discarded.

In the Perls' Prussian blue protocols described for different histopathological reactions, solutions with 1% to 5% of equal volumes of HCl and potassium ferrocyanide solution have been used. Hence, we too tried 1%, 2%, 3% and 5% Prussian blue solutions for staining the lines with high, medium and low grain Fe content in one experiment.

Staining procedure. Dry pearl millet grain samples were ground into flour with a pestle and mortar and 0.5 g of the flour of each sample was transferred into one quarter of a partitioned petri dish. The pestle and mortar were cleaned adequately with distilled water after grinding each sample to avoid contamination with the flour from the previous sample. The Prussian blue solution (10 ml) was poured

onto the flour in each quarter of the petri dishes. The color development was recorded after 10 minutes and the color intensity was visually scored on a 1–4 scale, where 1 = no color; 2 = less intense blue color; 3 = medium blue color and 4 = more intense blue color. The blue color intensity in the flour was compared with the Fe density of genotypes measured through AAS at NIN, Hyderabad, India. Rank correlation between color score and measured Fe density ranks was estimated (Gomez and Gomez 1984).

Results and Discussion

Pearl millet grains flour when treated with 2% Prussian blue solution in the petri dishes produced varying intensity of blue color in genotypes having medium to high Fe content (Fig. 1). In genotypes having a high Fe content (51.7–74.7 mg kg^{-1}), the blue color was more intense than in those having medium Fe content (40.3–40.8 mg kg^{-1}) (Table 1). No color developed in genotypes with low Fe content (31.2–40.6 mg kg^{-1}). These results suggest that Prussian blue staining was effective in differentiating genotypes with high and low Fe content. The rank correlation between measured grain Fe content and the color intensity score was highly significant and positive ($r = 0.92$; $P < 0.01$), indicating that higher the Fe content in the grain, the more the intensity of blue.

The feasibility of fine-tuning the protocol was tested by varying the concentration (1%, 2%, 3% and 5%) of the Prussian blue solution. But the intensity of blue color did not vary with varying concentrations of Prussian blue solution (Fig. 2) in pearl millet lines with high, medium and low Fe content. However, color development was slow when treated with 1% solution, suggesting that Prussian blue of 2% concentration would be optimum for effective discrimination of pearl millet lines for grain Fe content.

The standardized protocol using 2% Prussian blue solution was validated using 20 diverse B-lines with a wide range of Fe content (32.4–69.0 mg kg^{-1}) (Table 2). Color development was more intense in B-lines with Fe content ranging from 48.9 mg kg^{-1} to 69.0 mg kg^{-1}. On the contrary, no color developed in B-lines having an Fe content from 32.4 mg kg^{-1} to 39.1 mg kg^{-1} with the exception of ICMB 93333, which in spite of having low Fe content (37.3 mg kg^{-1}), showed less intense blue color as against the expected no color. Medium color intensity was noticed in B-lines having an Fe content of 42.9–46.0 mg kg^{-1}. The seed parent ICMB 94555, in spite of having moderate levels of Fe (47.5 mg kg^{-1}), showed less intense blue staining, which could be considered as an exception. All the B-lines having Fe content above the average of 43.4 mg kg^{-1} showed either medium or high intensity blue color staining, indicating that lines with high grain Fe content cannot be missed by this method. There was highly significant and positive correlation ($r = 0.91$; $P < 0.01$) between the measured Fe content and the color intensity score, again indicating that higher content of grain Fe in the genotypes is associated with intense blue color development.

In general, the intensity of blue color served as a reliable qualitative selection criterion for grain Fe in pearl millet. The method was efficient in separating genotypes

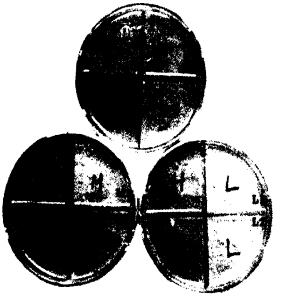


Figure 1. Differential Prussian blue staining of pearl millet grain flour with varying levels (H- high, M- medium and L-low) of grain Fe content. (H1- 863B, H2 - AIMP 92901 S1-15-1-2-B; M1 - MC 94 C2-S1-46-1-1-B; M2 - ICMV 93074 S1-9-1-1-1-B; L1 - 81B; and L2 - ICMB 90111).

Figure 2. The intensity of blue colour in high (H), medium (M) and low (L) Fe content genotypes with varying concentrations of Prussian blue staining solution.

with high grain Fe from those with low grain Fe. When a large number of germplasm accessions or progenies or breeding lines have to be screened for Fe content, this method will be highly efficient in discarding accessions or progenies with low Fe content or vice versa.

The Prussian blue method could be used at room temperature, and no costly and specific equipment is necessary other than a pestle and mortar or cyclone mill (grinder) and simple glassware. Using a pestle and mortar for grinding, approximately 60–80 samples per day could be analyzed with two technicians; using a cyclone mill, approximately 120–140 samples could be analyzed. Estimation of grain Fe content with AAS or ICP involves higher cost, which ranges from US$ 1 to US$ 10 in various laboratories (excluding the shipping cost of the material).

These chemical procedures are also time-consuming in terms of receiving the results of analyses. The chemical cost per sample in the Perls' Prussian blue protocol used in our study is about US$ 0.4 (when 500 g of potassium ferrocyanide approximately costs US$ 10 and 500 mL

Table 1. Prussian blue staining pattern and grain iron (Fe) content in pearl millet, ICRISAT-Patancheru, Andhra Pradesh, India, dry season, 2004.

Entry	Class	Fe content (mg kg^{-1})	Color class	Color score
ICMS 8511 S1-1/-2-1-1-B	Low	30.8	No color	1
ICMV 91059 S1-14-2-4-2-2-B	Low	31.2	No color	1
81 B	Low	32.4	No color	1
ICMB 90111	Low	34.2	No color	1
ICMB 95222	Low	35.2	No color	1
ICMV 91059 S1-58-2-2-2-B	Medium	40.6	Less intense blue color	2
ICMV 93074 S1-9-1-1-1-B	Medium	40.3	Medium blue color	3
MC 94 C2-S1-46-1-1-B	Medium	40.8	Medium blue color	3
ICMB 94111	High	51.7	More intense blue color	4
ICMB 00888	High	56.7	More intense blue color	4
863 B	High	69.0	More intense blue color	4
AIMP 92901 S1-15-1-2-B	High	74.7	More intense blue color	4

Table 2. Prussian blue staining reaction and grain iron (Fe) content in 20 pearl millet B-lines, ICRISAT-Patancheru, Andhra Pradesh, India, dry season, 2004.

B-line	Fe content[1] (mg kg^{-1})	Color class	Color score
81 B	32.4	No color	1
ICMB 90111	34.2	No color	1
ICMB 97333	34.9	No color	1
ICMB 95222	35.2	No color	1
ICMB 98777	35.9	No color	1
ICMB 89111	36.5	No color	1
ICMB 93333	37.3	Less intense blue color	2
ICMB 97111	38.2	No color	1
ICMB 01888	39.1	No color	1
ICMB 01555	42.1	Less intense blue color	2
ICMB 91444	42.9	Medium blue color	3
ICMB 91777	43.7	Medium blue color	3
ICMB 00999	44.3	Medium blue color	3
843 B	46.0	Medium blue color	3
ICMB 94555	47.5	Less intense blue color	2
ICMB 88004	48.9	More intense blue color	4
ICMB 98222	51.3	More intense blue color	4
ICMB 94111	51.7	More intense blue color	4
ICMB 00888	56.7	More intense blue color	4
863 B	69.0	More intense blue color	4

1. Mean = 43.4 mg kg^{-1}; LSD (P = 0.05); CV (%) = 12.3

concentrated HCl approximately costs US$ 11). The cost of the technicians per sample would add another US$ 0.1 (assuming each technician's salary is approximately US$ 225 per month), hence taking the total cost to US$ 0.5 per sample. Thus, this is a simple, rapid and inexpensive method compared to both chemical analyses and the method followed in rice. This could be effectively used as an initial method of screening, and genotypes identified for high grain Fe from this protocol could be subjected to actual laboratory analysis using AAS or ICP. This would save the cost involved in quantitative estimation of grain Fe. The highly significant positive correlation ($r = 0.84$; $P < 0.01$) between grain Fe and Zn observed in pearl millet (G. Velu, unpublished) implies that this method would also be useful for indirect selection of genotypes with high grain Zn. However, care should be taken while handling potassium ferrocyanide, which is low toxic under normal conditions, and releases highly toxic cyanide gas when heated.

References

Abdalla AA, El Tinay AH, Mohamed BE and **Abdalla AH.** 1998. Proximate composition, starch, phytate and mineral contents of 10 pearl millet genotypes. Food Chemistry 63:243–246.

Gomez KA and **Gomez AA.** 1984. Statistical procedures for agricultural research. Philippines: International Rice Research Institute Press.

Graham R, Senadhira S, Beebe S and **Iglesias C.** 1999. Breeding for micronutrient density in edible portions of food crops: conventional approaches. Field Crops Research 60:57–80.

Jambunathan R and **Subramanian V.** 1988. Grain quality and utilization in sorghum and pearl millet. Pages 133–139 *in* proceedings of the workshop on biotechnology for tropical crop improvement. Patancheru, Andhra Pradesh, India: ICRISAT.

Jorhem L. 1993. Determination of metals in foodstuffs by atomic absorption spectrophotometry after dry ashing. NMKL Interlaboratory study of lead, cadmium, zinc, copper, iron, chromium and nickel. Journal of AOAC International 76:798–813.

Krishnan S, Datta K, Baisakh N, Vasconcelos M and **Datta SK.** 2003. Tissue-specific localization of β-carotene and iron in transgenic rice (*Oryza sativa* L.). Current Science 84:1232–1234.

Prom-u-thai C, Bernie D, Thomson G and **Benjavan R.** 2003. Easy and rapid detection of iron in rice grain. ScienceAsia 29:203–207.

Welch RM and **Graham RD.** 2002. Breeding crops for enhanced micronutrient content. Plant and Soil 245: 205–214.