POTENTIAL OF SELENIUM (Se) ACCUMULATION AMONG PEARL MILLET [*Pennisetum glaucum* (L.) R. Br.] GENOTYPES

BY

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DECLARATION

I, Irene Shilulu, hereby declare that the work presented in this dissertation is my own and has never been submitted for a degree at this or any other University.

Signature---------- Date----------

APPROVAL

This dissertation of Irene Shilulu was approved as fulfilling part of the requirements of the award of the degree on Masters of Science in Plant Breeding and Seed Systems by the University of Zambia

Examiner's name	**Signature**	**Date**
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DEDICATION

To my lovely parents, husband (Kosmas), daughter (Angela) and sister (Krispina)

ABSTRACT

Beneficial effects of selenium (Se) can be delivered to humans through enriched plant foods. However, breeding plants to be more efficient at Se accumulation can complement enrichment efforts. This study was aimed at determining the inheritance of Se accumulation for the purpose of developing varieties of pearl millet (*Pennisetum glaucum (L.) R. Br*.) which accumulate high Se. In doing this the first step involved the determination of the magnitude of genetic variation in the Se content as a proxy for Se accumulation ability of the selected pearl millet genotypes followed by the determination of the gene action and heritability conditioning the Se accumulation trait. Thirty seven genotypes were screened for Se accumulation in a field experiment during 2011/2012 cropping season at University of Zambia (UNZA) Field Station. These genotypes were sprayed with Se in the form of sodium selenate (Na_2SeO_4) at a rate of 2 mg L^{-1} (288 m^3 ha^{-1}) through foliar application during vegetative stage, 30 days after emergence. The crop was grown to maturity and grains harvested. The grain samples were analysed for Se accumulation using the Atomic Absorption Spectrophotometer fitted with Graphite furnace (AAS-GF). The screened genotypes were further crossed in a North Carolina Design (NCD) II mating scheme, using 12 male and 25 female parents during 2012 offseason at National Irrigation Research Stations (NIRS), Nanga, Southern province of Zambia. The progenies were evaluated for Se accumulation and important agronomic traits in three sets in a Randomised Complete Block Design (RCBD). These progenies were also sprayed with Se and grain samples were analysed for accumulated Se following the same procedure as for parents. The Se analyses for the parental genotypes showed high coefficient of variation (CV) values of 135% for non-sprayed and 156% for sprayed genotypes, indicating the wide inherent genetic variability for grain Se accumulation among pearl millet genotypes. Out of 37 genotypes screened, before spraying, 29 were identified as Se accumulators while 8 were non-accumulators, however after spraying, 35 were designated as Se accumulators and 2 as non-accumulators. The amount of Se accumulated among the non-sprayed parental genotypes ranged from the lowest 0.00 µg g^{-1} to highest 0.09 µg g^{-1} for 570028 R1w and SDMV 59009 respectively while among the sprayed genotypes, the lowest was 0.01µg g^{-1} for 570028 R1w and highest was 0.63µg g^{-1} for NLC-C_3. Significant ($P<0.05$) differences in Se accumulation were also observed among crosses and the range across sets was 0.08 – 0.62 µg g^{-1}. The overall highest accumulator among all the crosses was ZPMDC x NEC-C_3 (0.62 µg g^{-1}) from set I while the overall lowest accumulator was NLBC-C_3 x TARAM (0.08 µg g^{-1}) from set III. This result indicated that pearl millet has the potential to accumulate Se and a wide genetic variation in grain Se accumulation exists in pearl millet genotypes. Significant ($p<0.05$) strong positive correlation ($r=0.652$) was noted between days to 50% flowering and Se concentration while days to maturity was significantly and negatively correlated ($r= -0.926$) to Se concentration indicating that selection for high Se content may be possible without significant compromise on grain yield.

General combining ability (GCA) effects indicated that parent ZPMV 28402, a superior parent can be used in breeding programmes to introgress the Se accumulation trait, without compromising on grain yield. On the other hand, specific combining ability (SCA) revealed that crosses SOSANK x NL_0C-$C_{4,}$ SEPO x OKASHANA 1 and ZPMV 24801 x KUOMBOKA could be employed for effective utilisation in hybrid breeding programmes for Se accumulation trait. The variance components using Baker's ratio (0.36) indicated that non-additive effects were more important in conditioning the Se accumulation trait. Narrow sense heritability (h^2) of 0.28 for the Se accumulation trait was found to be very low and, therefore, suggesting a recurrent selection method to be employed in the improvement of this trait. This study has shown that it is feasible to develop pearl millet varieties that contain high Se in the grains. The positive implications for ensuring adequate Se intake for improved nutrition through a pearl millet diet are obvious.

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CHAPTER ONE

1.0 INTRODUCTION

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is the fifth most important cereal crop in the world after rice, wheat, maize and sorghum (Marthur, 2012; Sumathi *et al*., 2010). It is a widely grown rain-fed cereal crop in the arid and semi-arid regions of Africa and Southern Asia and can be grown in areas where rainfall is not sufficient for the cultivation of maize and sorghum. In other countries it is grown under intensive cultivation as a forage crop (Andrews and Kumar, 1992). Today, pearl millet is a staple food of more than 90 million people (The Syngenta Foundation for Sustainable Agriculture, 2002). It contributes to both rural food security and livelihood systems, as it provides good nutritional supplies and income sources to smallholder farmers. Areas planted to pearl millet are estimated at 17 million hectares annually in Africa and 10 million hectares in Asia (Rai *et al*., 2012) and the global production exceeds 10 million tons a year (National Research Council, 1996).

In India pearl millet occupies fourth position among cereal crops next to rice, wheat and sorghum while in sub-Saharan Africa; pearl millet is the third major crop with major producing countries being Nigeria, Niger, Burkina Faso, Chad, Mali, Mauritania and Senegal in the west, Sudan in the north, Uganda in the east and Namibia in the south.

In Namibia, pearl millet is the most important traditional cereal and staple crop followed by sorghum. The crop is widely grown for food and beverages in seven northern regions of the country such as Kavango, Oshikoto, Ohangwena, Oshana, Omusati, Kunene and Zambezi by more than 60% of subsistence farmers who reside in the rural areas covering about 355,200 ha of land (Ipinge, 2002). Subsistence farmers in Namibia depend on rain-fed smallholder agriculture for their livelihood and pearl millet plays critical social, economic and cultural roles in the Namibian societies (Ipinge, 2002). It accounts for 92% of cereal grain harvested in North Central Regions of Namibia (Keyler, 1995).

Pearl millet is preferred over other cereals because it is relatively drought tolerant, tolerant to high temperatures and can produce reasonable grain yields in soils of marginal fertility where other crops would fail. It is the only cereal adapted to low rainfall and high temperatures of most of Namibia (Keyler, 1995). It is highly nutritious as comparable to other cereals like maize, wheat or rice with high levels of metabolisable energy and protein, and a more balanced amino acid profile (Andrews and Kumar, 1992). Pearl millet products have been shown to have lower glycaemic index than similar products produced from wheat (Sehgal *et al*., 2004), thus increasing the food value of pearl millet for those prone to diabetics. In addition, pearl millet grains lack gluten, unlike most of the major cereals, thus enhancing its health value for those allergic to gluten (Dahlberg *et al*., 2004) and it is less prone to aflatoxin contamination than sorghum and maize.

Despite the importance of pearl millet, breeding programme in Africa have seldomly focused on improvement of this crop, especially its nutritional quality with respect to micronutrients; rather improvement has focused on yield and other components such as drought and disease tolerance. Micronutrients are commonly referred to as "vitamins and minerals" and they include minerals such as Se, fluoride (F), iron (Fe), iodine (I) and zinc (Zn). They also include vitamins such as vitamin A, C, D, E and K, as well as the B-complex vitamins. Micronutrients are vital to the proper functioning of all the body's systems.

One micronutrient that has recently gained importance due to its positive effects on human health is Se. Although the element is not considered an essential nutrient for plants, its concentration in edible tissues of cultivated plants presents an opportunity for inclusion in the human diets (Anderson and Scarf, 1983; Ip, 1998). The essential trace mineral, Se is of fundamental importance and has been shown to produce positive effects on human health by boosting the immune system. As a constituent of seleno-proteins called Glutathione peroxidise (GSHP x), Se has structural and enzymatic roles, of being best-known as an antioxidant and catalyst for the production of active thyroid hormone (Cartes *et al*., 2005).

Selenium is also needed for the proper functioning of the immune system and appears to be a key nutrient in counteracting the development of virulence and inhibiting Human Immunodeficiency Virus (HIV) progression to Acquired Immuno Deficiency Syndrome (AIDS) by increasing the CD4 and T cell count (Melse – Boonstra *et al.*, 2007**)**. An elevated Se intake may be associated with reduced cancer risk and other conditions involving oxidative stress and inflammation have shown benefits of a higher Se status (Combs, 2001).

Though Se is such an essential nutrient in human health, its consumption remains low in sub-Saharan Africa population. Combs (2001) reported that it has been postulated that the vast majority of the world's population has suboptimal Se intakes, and, hence, is at increased risk of several diseases such as cancer, heart diseases, viral diseases and other conditions that involve increased levels of oxidative stress.

Enhancing Se intake via supplementation is costly and not practical because it carries a high risk of not reaching the whole population. Moreover, intake of Se through food is more equitable than through tablets. Therefore, genetic bio-fortification using both conventional breeding and modern biotechnology is considered to be the most promising and cost-effective approach to addressing micronutrient malnutrition, especially in the developing world (Stein *et al*., 2007; Bouis *et al*., 2011). In comparison to other strategies (agronomic biofortification, supplementation or dietary diversification), genetic biofortification provides a truly feasible means of reaching out to remote and rural areas to deliver naturally-fortified foods to population groups with limited access to diverse diets, supplements and commercially fortified foods (Bouis *et al.,* 2011).

Several studies suggest that it is possible to breed cultivars with enhanced mineral accumulation (Rai *et al.,* 2012). Studies on genetic enhancement of grain iron (Fe) and zinc (Zn) in pearl millet had shown high levels and large variability for both Fe and Zn contents in pearl millet grains (Rai *et al.,* 2012). This, therefore, indicates the potential of genetic enhancement for grain Se content in pearl millet as well. Progress in any crop improvement venture depends mainly on the magnitude of genetic variability and heritability of the trait of interest in the source material.

With respect to pearl millet, there appears to be a serious paucity of information on Se accumulation including the genetics of this trait in this crop. Currently, there is a study on mapping Se and Zn contents in some soils and food crops in Namibia. The study is being conducted by the Golden Valley Agricultural Research Trust (GART), Zambia under the Swedish International Development Agency (SIDA) supported project "Strengthening HIV/AIDS and Food Security Mitigating Mechanisms Amongst Smallholder Farmers in Botswana, Lesotho, Namibia and Zambia". However, this study will only be meaningful if the genetics and potential of Se accumulation of the food crops, especially pearl millet grown in the country are determined and evaluated. Hence, this study sought to contribute to better understanding of Se intake by pearl millet, an important staple grain in Namibia.

Namibia is among the countries with low Se intake. The situation is exacerbated by the absence of pearl millet varieties which accumulate Se in the Namibian pearl millet growing areas and the inherently low soil Se content. This has not only a negative spill-over effect in the diets of the subsistence pearl millet growers but places the rural population at a high risk of susceptibility to health conditions related to low Se intake.

Therefore, exploiting the genetic variability in the pearl millet crop for micronutrient density may be an effective method to improve Se intake in the Namibian population or breeding crop varieties with enhanced Se accumulation characteristics, may be plausible approaches to increase the Se concentration in human diet. This will, therefore, complement Se enrichment efforts by improving the diets of most people in need, increase agricultural production and ensure household nutrition security in Namibia and the entire sub-Saharan Africa.

Considering the high nutritional value of pearl millet and the potential contribution it can make in improving the nutrition of those heavily dependent on it as a major food crop, this study was, therefore, conducted with the following objectives:-

1.1 Main objective

The main goal of this study was to establish the basis for developing high Se accumulating pearl millet genotypes to be used in breeding programme through assessment of the magnitude of variability in Se accumulation among selected pearl millet varieties.

1.2 Specific objectives

1. To ascertain the magnitude of variation for Se accumulation among pearl millet genotypes
2. To evaluate the progenies of population crosses for Se accumulation and agronomic characteristics
3. To determine gene action and heritability conditioning the Se accumulation trait in pearl millet

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Pearl millet taxonomy and its uses

Pearl millet is an annual, allogamous, diploid cereal, which belongs to the family *Poaceae*, subfamily *Panicoideae*, tribe *Paniceae*, subtribe *Panicinae*, genus *Pennisetum*, section *Penicillaria* and it possesses 2n-2x-14 chromosomes (Joshi *et al*., 2000). It is a predominantly cross-pollinated crop with 75–80% out-crossing (Rai *et al.*, 1999). Pearl millet is a highly tillering diploid tropical C4 cereal crop. It bears grains on the surface of erect candle shaped terminal spikes. Grain size varies from 0.5 to over 2.0 g/100, and, depending on the size of the spike, grain number per spike ranges from 500 to 3,000 (Andrews, 1990).

In Africa and the developing countries of Asia, pearl millet grains are used mostly for human consumption. Traditionally, these grains are used in such diverse food types as leavened and unleavened flat breads, porridges, steamed foods, and rice-like boiled products and in alcoholic as well as non-alcoholic beverages (Murty and Kumar, 1995). Pearl millet flour can be substituted for up to 20% of the wheat flour in making leavened bread (Rai *et al.*, 1999). It is also grown as a fodder crop, e.g. in Brazil, the United States of America, South Africa and Australia. In addition to grain and forage uses, pearl millet crop residues and green plants also provide sources of animal feed, building material, and fuel for cooking, particularly in dry land areas (IFAD, 1999).

2.2 Pearl millet production

2.2.1 Sub-Sahara African region and Indian subcontinent

Estimates by the Food and Agriculture Organisation of the United Nations (FAO) statistics and relative importance of pearl millet in different countries indicate an annual grain production of about 18 million tons from a planted land area of 27

million ha mostly in the dry regions of Africa (60% of area and 58% of production) and the Indian subcontinent (38% of area and 41% of production) (Marthur, 2012). Production statistics over the past 10 years show a 20% increase in area planted in Africa, with a 12% increase in yield. Much of the increased area is in Burkina Faso, Chad, Mali, Niger and Nigeria, but yield levels increased only in the latter two countries (Marthur, 2012). During the same period, the area planted to pearl millet in India declined by 16%, but yield levels increased by 30%. The average crop yield was estimated by NRC (1997) to be around 0.6 tonnes per hectare (around 0.8 t ha^{-1} in India), but ranged between 0.3 t ha^{-1} in Southern Africa to over 0.8 t/ha in Nigeria and Uganda under wetter conditions.

2.2.2 Namibia

In Namibia, the average households cultivates an average field size of 4.2 hectares and over 90% of these fields are planted with pearl millet with an average pearl millet yield estimated at around 0.35 t ha^{-1} which is amongst the lowest in the world (CAC, 2010). According to the Namibian Agricultural Union (2004), about 96, 370 tons of pearl millet was produced in 2004 compared with only 55,597 tons of maize in the same year. This further indicated that, total cereal production in 2004 contributed 14 percent to gross agricultural production of which pearl millet accounted for 64 percent (Mendelsonh, 2006).

2.3 Adaptation and climatic requirements

Pearl millet is highly adapted to drought, representing an essential component of the food security and livelihood of many million poor farmers that inhabit dry lands and semi-arid ecosystems throughout sub-Saharan Africa. It is grown in areas with low rainfall of 200-600 mm per annum. Primarily a tropical plant, pearl millet is often referred to as the "Camel", because of its exceptional ability to tolerate drought (Ong, 1983a). Even with minimal rainfall pearl millet will typically still produce reasonable yields. Pearl millet is suitable to well drained light (sandy) and medium (loamy) soils and can grow in nutritionally poor soils. It does well in a range of soil reactions, acid, neutral and basic (alkaline) soils (Ong, 1983a).

Smallholder farmers conserve and cultivate innumerable cultivars of pearl millet, often adapted to local agro-ecological factors, livelihood needs, and cultural values.

2.4 Nutritional importance of pearl millet

Pearl millet is a principal source of energy, protein, vitamins, and minerals for millions of people in the regions where it is cultivated as the dominant crop (Hulse *et al*., 1980). Like sorghum, pearl millet is generally composed of 9-13% protein but large variations in protein content, ranging from 6-21%, have been reported (Baker, 2003). Pearl millet grains are usually made up of 70% carbohydrates and consist almost exclusively of starch. It accounts for 24% of total calorie intake as compared to 23% for maize and 13% for wheat (Hulse *et al*., 1980).

Apart from pearl millet being a source of energy and protein, no study or breeding programme in Africa has published information on the improvement of micronutrients like selenium (Se). Se is one of the important micronutrients needed in human health.

2.5 Selenium (Se) as an element

Selenium with atomic number 34, is the third member of the Group 6 (IV) of the Periodic Table (IPCS, 1987). It has an atomic mass of 78.96 and has six naturally occurring stable isotopes. Se is present in the earth's crust, often in association with sulphur containing minerals. It is normally found in concentrations of 50–90μg kg^{-1}, but higher concentrations can be associated with some volcanic, sedimentary and carbonate rocks (Keller, 2000).

Selenium concentrations in soils vary widely from 5 to 1 200 000 μg kg^{-1}, being higher in soils of more recent volcanic origin (IPCS, 1987). The element occurs in soils in several forms, according to its possible oxidation states: selenides (Se^{2-}), amorphous or polymeric elemental Se (Se_0), selenites (Se^{4+}) and selenates (Se^{6+}) (IPCS, 1987).

2.6 Selenium as a micronutrient and its importance to human health

Selenium is an essential micronutrient for animals and humans that is obtained from dietary sources including cereals, legumes and vegetables. Seleno-methionine (Se-met) in cereals is the major form of Se intake by humans and its concentration increases with increasing soil Se (Schrauzer, 2000). The element plays an essential role in boosting the immune system by affecting the antioxidant defence systems, thyroid hormone metabolism and redox control of enzymes and proteins (Gibson, 2005).

In combating cancer cells, Se acts as pro-oxidant rather than antioxidant, inducing apoptosis through the generation of oxidative stress. Epidemiological studies have pointed to inverse associations between nutritional Se status and cancer risks (Combs and Gray, 1998), cardiovascular diseases (Oldfield, 1999) and immune system functions (Baum *et al.*, 1997). The findings from recent human intervention trials (Clark *et al.*, 1996; Yu *et al.*, 1997) have stimulated interest in a cancer-preventive role for Se. In addition to its cancer preventive ability, Se also has an anti-viral effect (Baum *et al.*, 1997; Yu *et al.*, 1997).

Selenium is reported to be a crucial nutrient for HIV-infected individuals and is a potent inhibitor of HIV replication in vitro. Plasma Se is a strong predictor of HIV progression and it has been suggested that low plasma Se is a greater risk factor for mortality than other antioxidants or a low CD4+ count (Rayman, 2000 cited by Melse -Boonstra *et al*., 2007). Studies done so far on Se and HIV/AIDS showed a positive association between Se status and delayed disease progression or increased mortality (Baum *et al.*, 1997). Hence, a Se-rich diet is one way of ensuring a high Se in the serum.

2.7 Selenium accumulation by plants

Although plants have no known physiological requirement for Se, many plant species accumulate large amounts of it from soil and water. Se in the selenate form is readily taken up by plants, converted into organic forms (which are very suitable for humans and animals), and loaded into grains and other edible parts (Koljonen,

1974). Se uptake by plants varies according to available soil Se concentration, its bioavailability for uptake into plant roots (which depends heavily on redox equilibria in the soil, but also on several other factors including low pH, high contents of iron (Fe) and aluminium (Al) oxides, sulphur (S) and phosphate (P) levels and species of plants (Oldfield, 1999). More regions of the world, Africa inclusive are characterised by moderate to low Se bioavailability than rich or high soil levels (Combs, 2001). Se content in the soil varies from 0.01mg kg^{-1} in deficient areas to 1,200mg kg^{-1} in soils rich in organic matter in areas with high Se (Keller, 2000).

Selenium enters the human food chain when plants accumulate it from the soil and incorporate it during synthesis of new molecules, typically as Se-substituted analogues of thio-molecules. Seleno-methionine is the major organic seleno-molecule in cereal grains, legumes and soybeans, as well as in Se-enriched yeast used in Se supplements, but a number of other organic and inorganic molecular species are also present (Schrauzer, 2000).

Plant species vary widely in Se uptake and accumulation. Some plants accumulate Se in direct relationship to the amount available from the soil, whereas others (Se accumulators) may accumulate Se in concentration orders of magnitude above that in the majority of species (Zayed *et al.*, 1998). Plants are divided into three groups based on their capacity for accumulating and tolerating Se (Rosenfeld and Beath, 1964; Zayed *et al.*, 1998). The following represent the three groups:

- Primary accumulators (hyper-accumulators), such as some *Astragalus*, *Stanleya*, and *Xylorhiza* species, are able to accumulate several thousand mg Se kg^{-1} of leaf tissue. These species preferentially grow on seleniferous soils and often contain levels of Se that are toxic to horses and cattle.
- Secondary accumulators (Se absorbers), such as some *Brassica* species (cabbage, cauliflower, broccoli, Indian mustard etc) which can accumulate up to 1,000mg Se kg^{-1} of leaf tissue. These species are not confined to seleniferous soils, but are able to accumulate Se if it is present in the soil.
- Non-accumulators which include most grains and grasses and these usually do not accumulate more than 50mg Se kg^{-1} (0.05mg Se g^{-1}) under field conditions. Most cultivated plants are Se non-accumulators and they are

sensitive to its presence in the growth medium (Zhang *et al.*, 2007). Cereals are inherently very poor in both concentration and bioavailability of microelements such as Zn, Fe, and Se in the seeds, particularly when grown in microelement-deficient soils (FAO/WHO, 2001).

Results from studies done on determining the distribution of Se in cereal grains revealed that Se, like sulphur (S), is more evenly distributed throughout the wheat grain, with a higher proportion stored in the endosperm (Lyons *et al*., 2004). The Se content and the chemical form of Se within plants may be altered by manipulation of plant genetics or by agricultural production conditions (Koljonen, 1974).

Zayed *et al.* (1998) stated that Se is taken up by plants as selenate, selenite, and organic Se. However, Sors *et al.* (2005) indicated that plants absorb Se from soil primarily as selenate and translocate it to foliage specifically to chloroplasts, where it follows the sulphur - assimilation pathway. They further stated that the presence of sulphate was a limiting factor which influences the uptake of Se by most plants. Lyons *et al*. (2004) also reported that Se concentrations in plants may be reduced following application of sulphate as a result of competition between sulphate and selenate for transporters in plant roots. Se and sulphur (S) compete with each other in the biochemical pathways, leading to synthesis of seleno-methionine (Se-met) and methionine in plant cells (Lyons *et al.,* 2004).

Hopper and Parker (1999) stated that phosphate concentration also influences selenite uptake, which may hint at a common uptake pathway while Zayed *et al.* (1998) reported that plants other than hyper-accumulators take in Se regardless of the presence of sulphate, because they possess an alternate pathway for uptake and retention of Se.

2.8 Selenium intake by humans

Most people obtain almost all of their Se from the foods they eat. In plant and animal tissues, Se is found mostly bound to proteins. Therefore, the most important food sources of Se are meats and seafood (0.3–0.5 mg kg^{-1}) because of their high protein contents and cereals (0.1–10 mg kg^{-1}) because they tend to be consumed in

large amounts (NRC, 1983). In contrast, foods with relatively low protein levels, such as vegetables and fruits, tend to have relatively low Se contents of <0.01 mg kg^{-1} (NRC, 1983). In all cases, the Se content of foods reflects the available Se content of the soils on which the crops are grown.

However, a review of the literature by Melse – Boonstra *et al.* (2007) revealed that worldwide, cereals were the most important dietary source of Se with a concentration ranging from 1–55 mg/100 g (550 mg kg^{-1}) fresh weight, followed by fruit and vegetables with concentration of 10 mg kg^{-1}. They further stated that Se intake from cereals was about 10 times higher than from meat.
Thus, increasing the levels of Se in staple crops may have health benefits for humans that extend beyond simply meeting the basic nutritional requirement for Se. The preeminent role of pearl millet in human diets makes it a logical candidate for biofortification efforts.

Increased human Se intake may be achieved through increased consumption of high Se foods, use of Se-rich fertilisers (agronomic biofortification), increased consumption of plants that naturally accumulate much Se, sprouting seeds in Se-enriched media, crop biofortification (plant breeding) for enhanced Se accumulation, plant production in most Se-rich areas, supplementation of livestock, food fortification and supplementation of individuals (Haug *et al.*, 2007).

However, supplementation of livestock with Se is unlikely to be an efficient strategy to increase Se level in the human population. In New Zealand, a small increase in the Se content of human foods was observed after the introduction of Se supplementation for farm animals in the 1960s (Thomson and Robinson, 1980). Furthermore, the addition of selenate to NPK fertilisers for use on crops and pastures in Finland since 1984 has been an effective and safe method to increase the status of the entire population (Aro *et al.*, 1995). However, this strategy is very costly. Therefore, a strategy of breeding staple crops with enhanced ability to fortify themselves with micronutrients offers a sustainable, cost-effective alternative, which is more likely to reach those most in need and has the added advantage of requiring no change in current consumer behaviour to be effective.

2.8.1 Recommended dietary allowance for selenium

Selenium is an important element for human nutrition and, hence, various national and international organisations have established recommended daily intakes. The joint WHO/FAO consultation on preparation and use of food-based dietary guidelines (FAO/WHO, 1998) listed recommended daily intakes of 6–21 μg of Se for infants and children, according to age, 26 and 30 μg of Se for adolescent females and males, respectively, and 26 and 35 μg of Se for adult females and males, respectively. In 2000, the United States National Academy of Sciences Panel on Dietary Oxidants and Related Compounds revised the recommended intake of Se to 55μg day^{-1} for both men and women and 70 μg day^{-1} for women during pregnancy and lactation. Recommended Se intakes for children are between 15 μg day^{-1} for infants 0–6 months of age and 30 μg day^{-1} for children 4–8 years old (NAS, 2000).

Table 1: Recommended Dietary Se Allowance per age group

Age group	**Recommended Dietary Se Allowance** (μg day^{-1})
Children 1-3 years	20
Children 4-8 years	30
Children 9-13 years	40
Adults and children 14 years and up	55
Pregnant women	60
Breastfeeding women	70

Source: WebMd medical reference 2012

According to Melse – Boonstra *et al.* (2007), the recommended daily intake of Se is 55 mg day^{-1} for adults. While McIntosh (2007) stated that the recommended daily intake (RDI) of Se for adult men is 70 μg day^{-1} and for women is 60 μg day^{-1}. However, Kamwesinga *et al.* (2012) are in line with FAO/WHO that Se intake differs with age and conditions (Table 1). However, it is clear that the position with regard to the dietary allowance of Se is complex.

Because of concern about the adverse effects of exposure to excessively high levels of Se, various national and international organizations have established upper limits of exposure to Se. The United States National Academy of Sciences Panel on Dietary Oxidants and Related Compounds set an upper tolerable limit for Se at 400 μg day^{-1} (NAS, 2000). This level was also recommended by FAO/WHO (1998) and the United Kingdom Expert Group on Vitamins and Minerals (UK EGVM, 2002). These levels are far beyond what an average person would be exposed to in sub-Saharan Africa where both the soils and food crops are deficient in Se.

2.8.2 Prevalence of deficiency in selenium on humans

It is postulated that many people do not take in adequate selenium as per recommended dietary intake and this is regarded as a major health problem for 0.5 to 1 billion people worldwide (Combs, 2001). Se deficiency is prevalent in many areas of the world, especially portions of sub-Saharan Africa, East Asia, and many areas of Europe have Se intakes below the US recommended daily allowances (Combs, 2001). Studies from South Africa and Malawi showed that Se intakes of women and children were mostly below the recommended intake levels. Inadequate plasma Se concentrations were found in various African countries such as Burundi, Zambia, Nigeria, Malawi and Democratic Republic of Congo (DRC).

The increasing levels of micronutrient deficiencies such as Se are thought to be due to, among others, modern crop breeding which has focused on high yield rather than nutritional quality (Morris and Sands, 2006) and this may have led to a lower density of micronutrients in the seed. Cereals remain an important part of the diet and source of micronutrients for most populations.

Very low Se status in humans has been associated with a juvenile, multifocal myocarditis called Keshan disease and a chondrodystrophy called Kaschin-Beck disease (FAO/WHO, 2004). The Se levels result in increased oxidative stress and apoptosis of infected cells, thereby activating the virus to replicate at higher rates. Baum *et al.* (1998) showed that Se-deficient HIV patients were nearly 20 times more likely ($p<0{\cdot}001$) to die from HIV-related causes than those with adequate levels.

Good nutrition is critical for HIV/AIDS patients and a Se-rich diet may have far reaching positive outcomes.

2.9 Crop biofortification

Crop biofortification is a process which nutritionally enhances staple crop varieties with higher levels of bio-available vitamins and minerals (Bouis, 2003). The process holds great potential to improve the health of the poor in developing countries, particularly in rural areas where people have no access to supplements. The added benefit of biofortification is its cost- effectiveness and long term sustainability. Because, once Se-rich plants are developed, there will be less costs each year in buying fortificants and adding them to food supply during processing and farmers will be driven by a good profit (Gibson, 1994).

Increased micronutrient and vitamin density in grain meant for human consumption may alleviate deficiencies that affect the majority of the world's population. While biofortification of staple foods to address nutrient deficiencies is an appealing concept, there is much to understand about the potential impact that such efforts might have on other important traits (Morris and Sands, 2006). For instance, it is not clear whether selection for increased mineral micronutrient content might negatively affect yield or other important agronomic and end use characteristics. This could occur if genes that increase mineral content are linked to genes that have a deleterious effect on other desired traits, or it could occur as a consequence of trait associations (Morris and Sands, 2006).

2.10 Plant breeding for enhanced selenium accumulation

The development of an effective breeding programme is dependent upon the existence of genetic variability. Pearl millet is endowed with a rich reservoir of genetic variability for various yield components, adaptation and quality traits (Berwal and Khairwal, 1997). Exploitation of the genetic variability in the available germplasm holds promise for producing high grain and Se concentration. The more diverse the parents, the greater are the chances of obtaining new combinations of genes and, therefore, increasing the probability for crop improvement (Berwal and Khairwal, 1997).

Common cereal crops such as wheat, rice, maize, barley and oats were assessed for Se accumulation, though not sufficiently; however, paucity of information is a challenge on the accumulation of Se in other cereals including pearl millet. Surveys indicate that wheat is the most efficient Se accumulator of the common cereal crops (wheat, rice, maize, barley, oats) and is one of the most important Se sources for humans. Studies indicated that wide variations in wheat grain Se levels were observed, but most of the world's wheat falls within the 0.020 - 0.600 mg kg^{-1} range (Alfthan and Neve, 1996). However, very little work was done in the improvement of nutritional qualities of cereals.

Breeding for improved Se uptake by plants may be an effective, sustainable strategy. Preliminary studies have found a 15-fold variation in Se accumulating ability among brassica vegetables (Combs, 2001), and a Se-accumulating soybean cultivar has been identified (Wei, 1996). In a study done in rapid-cycling *Brassica oleracea* population, results indicated that significant variation exists and the range of Se accumulation in leaf tissue of *Brassica oleracea* population was 120 - 988 $\mu g.g^{-1}$ for longer photoperiods and warmer temperatures.

Substantial variability exists within cereal crop varieties for zinc, iron and other nutrients (Graham *et al.*, 1999). These findings suggest that it should be possible to breed cultivars with enhanced Se uptake or to use genetic engineering to enhance Se levels (and even specific Se metabolites) in food crops.

As cereals are eaten in large quantity by practically everyone, they are the ideal vehicles for changing the balance of nutrient intake of the whole human population. Developing cereals that are genetically enriched in micronutrients using genetics and genomics tools are considered as promising and cost-effective approaches to reducing malnutrition [Uauy *et al.*, 2006, Welch and Graham, 2004, Ghandilyan *et al*., 2006).

Moreover, the existence of large genetic variation in grain micronutrients is essential for a successful breeding programme aimed at the development of new micronutrient-rich plant genotypes. Substantial genetic (genotypic) variation has been found in cereals for Zn, Fe and vitamin A (Graham *et al.*, 2001), which means

that varieties high in these nutrients can be bred. In studies done on barley, Valen, (1965) reported that the environment is a key contributor to the range of morphological variation found in various organisms. In addition, Spearman correlation analysis showed that high temperature and evaporation and low latitude, altitude, rainfall, and humidity at the site in which *H. spontaneum* originated are favorable to wild barley having gained a strong ability to take up Se from the soil and to accumulate Se in its grains during evolution.

The accumulation of minerals including Se in grain (edible tissue) is a complex phenomenon which is controlled by a number of genes. The movement of mineral elements from soils to seeds involves their mobilisation from soils, uptake by roots, translocation to the shoot, redistribution within the plant and deposition in seeds (Grusak and DellaPenna, 1999; White and Broadley, 2009).

2.11 Inheritance of Se accumulation in crops

2.11.1 Gene Action

Genes located on chromosomes represent the basic units of inheritance and control the expression of characters, individually or in combinations. Gene action is the way genes express themselves (Welsh, 1981). In quantitative genetics, genetic components are divided into additive and dominance variance and epistasis (Robinson *et al*., 1949; Falconer, 1981).

In the presence of additive gene action, characters of heterozygotes in the F2 generations are the intermediate of the two parents, because additive variation is associated with the average effects of the particular alleles (Falconer, 1981). The additive portion reflects the degree to which progenies are likely to resemble their parents, which is reflected in the narrow sense heritability.

Non-additive gene action is observed when the additive model cannot adequately explain the variation (Falconer, 1981). According to Robinson *et al*. (1949), the size of dominance relative to the additive variance indicates the degree of dominance.

Thus, levels of dominance in the progeny display a range from partial to over-dominance in relation to the mean of their parents. Negative variance component are not uncommon and are often found for dominance variance components (Hallauer and Miranda, 1981). When estimates of maternal components of variances are greater than paternal components of variances, it indicates the possible presence of maternal effects on the trait of interest (Mather and Jinks, 1971).

2.11.2 Heritability

Heritability is one of the most important genetic parameters widely used in plant and animal breeding genetic improvement studies. An important function of heritability is its role in predicting the breeding value of an individual as well as in predicting the genetic improvement expected as a result of the adoption of a particular scheme of selection. If heritability is high, the strategy would be to go for individual selection and if heritability is low to go for family selection.

Narrow sense heritability expresses the extent to which phenotypes are determined by the gene transmitted from the parents (Falconer and Mackay, 1996). The heritability estimates of > 70 % is considered very high; 50-70 % high; 30-50 % moderate and < 30 % low (Hallauer and Miranda, 1981). Knowledge of heritability indicates to the breeder the possibility to which genetic improvement is possible through selection. McQuinn *et al.* (1991) stated that investigation into the genetic variation for Se content in tall fescue revealed that progress from selection for Se content is possible and that the Se accumulation trait is heritable. Kopsell and Randle (2001) also found out that narrow-sense heritability estimates for Se accumulation in a rapid-cycling *Brassica oleracea* L. population were moderate (0.55) and gains from selection were 4.8 and 4.0 per selection cycle for high and low Se accumulators, respectively.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental sites

The study was conducted in three phases. Phase I and III experiments were carried out at University of Zambia (UNZA) Field Station, in Lusaka (Latitude 28º 22´S and Longitude 15º 22´E) during 2011/2012 and 2012/2013 rain seasons (January to May), respectively. While Phase II experiment was conducted at National Irrigation Research Station (NIRS), Nanga, in Mazabuka (Latitude 15º 46´S and Longitude 27º 55´E), Southern Province of Zambia during 2012 off-season (August to December).

Since the experiments were carried out at different locations with varying soil chemical properties, a composite soil sample was collected prior to planting at each site. The soil samples were analysed for Se, nitrogen (N), available phosphorus (P), potassium (K), sulphur (S), organic matter (OM) and soil reaction (pH) (Table 2). Se was determined using the Atomic Absorption Spectrophotometer (AAS) method using the wet digestion method at Zambia Bureau of Standards, Lusaka. Potassium, OM and N were determined using the AAS, Black and Walkey method and Macro Kjeldahl method, respectively in the Soil Science Laboratory of the School of Agricultural Sciences, University of Zambia.

Table 2: Selected chemical properties of the soils at experimental sites

Location	pH $CaCl_2$	OM %	N %	P mg/kg	K me/100g	S mg/kg	Se µg/g
UNZA Field Station 1	7.03	3.52	0.19	2.07	0.64	12.8	0.16
UNZA Field Station 2	6.86	2.00	0.13	4.76	0.58	16.1	0.11
NIRS, Nanga	5.93	2.32	0.09	8.65	1.13	31.16	0.17

UNZA Field Station site 1= where the experiment on screening the parents was planted

UNZA Field Station site 2 = where the experiment on evaluation of crosses was planted

3.2 Plant materials

The experimental material for the study consisted of thirty seven diverse genotypes of pearl millet, which included local landraces, open pollinated varieties (OPV), OPV restorers and populations. All the genotypes used in the study were provided by Zambia Agriculture Research Institute (ZARI) – Mongu, Western Province. These genotypes are among the ones used in the Southern Africa Development Community (SADC) pearl millet breeding programme and they originated from countries such as Zambia, Namibia, Togo, Mali, Ghana, Ivory Coast, Niger, Nigeria, India and USA. This was a combination of early, medium, late and very late genotypes (Table 3). These genotypes had not been bred for Se accumulation, but were selected on the basis of high yielding potential and other important agronomic traits.

Table 3: Characteristics of 37 pearl millet genotypes used in a North Carolina design II mating scheme and those analysed for grain Se Accumulation

GENOTYPE	DESCRIPTION	ORIGIN	STATUS
MANGANARA	Early grey grain	Ghana	OPV
ARROW	Early cream white grain	Ghana	OPV
NCD_2 - CO BULK	Early grey grain	India	OPV
ZPMDC	Zambian Composite	India	D2 gene Dwarf
ICMV 155 Br	Early bristled grey grain	India	OPV
LAGRAP	Early large seeded grey grain	India	OPV
HHVBC TALL	Early dwarf grey grain	India	OPV
HHVBC DWARF	Early	India	OPV
570028 R1w	Very late	Lincoln , USA	OPV Restorer
TORONIO	Very late	Mali	Local
TARAM	Medium Early grey grains	Mali	OPV
SOSANK	Very late	Mali	Local
BOBONI	Very late	Mali	Local
KANGARA	Early cream grain	Namibia	Released in Namibia
GWAGWA	Late	Nigeria	Local
SDMV 59009	Late Experimental	SADC	OPV
OKASHANA 1	Early grey grain	Togo	Released in several SADC countries and in Sudan
OKOA	Late grey grain	West Africa	Released in Tanzania
ZPMV 20402	Early grey grain	Zambia	OPV Zambian Experimental
NLBC-C_3	Late Zambian composite	Zambia	Population
LISELI	Medium Early grey grain	Zambia	OPV released in Zambia

NAMALOBA LOCAL	Late	Zambia	Local
ZPMV 21601	Late	Zambia	OPV Zambian Experimental
$NL_0C - C_4$	Late Zambian composite	Zambia	Population
$NLC - C_3$	Late Zambian composite	Zambia	Population
ZPMV 28402	Early grey grain	Zambia	OPV Zambian Experimental
ZPMV 24802	Late	Zambia	OPV Zambian Experimental
SEPO	Late grey/cream grain	Zambia	OPV released in Zambia
ZPMV 20502	Late	Zambia	OPV Zambian Experimental
KUOMBOKA	Late grey grain	Zambia	OPV released in Zambia
LCIC 9702	Early	Zambia	Local
TUSO	Late grey grain	Zambia	OPV released in Zambia
KAUFELA	Medium Early grey grains	Zambia	OPV released in Zambia
ZPMV 28401	Early grey grain	Zambia	OPV Zambian Experimental
$NEC - C_3$	Early Zambian Composite	Zambia	Population
ICMV – IS- 9031	Medium Early	Sahel	OPV
ZPMV 24801	Late Zambian Experimental	Zambia	OPV

3.3 Experimental design and treatments

Phase I: Screening of pearl millet genotypes for Se accumulation

Thirty seven pearl millet genotypes were screened for Se accumulation in the field experiment conducted at the University of Zambia (UNZA) Field Station in Lusaka during 2011/2012 (February to June) rain season. Genotypes were planted in a single plant replication with ten plants per treatment, using a split-plot design, whereby the main plot (factor A) was the genotype and the sub-plot (factor B) was either sprayed with Se or not sprayed. Two rows of 4 m each (2m was sprayed and 2m not sprayed) were planted per genotype. These genotypes were exposed to Se in a form of sodium selenate (Na_2SeO_4) through foliar spray at a rate of 2 mg L^{-1} (288 m^3 ha^{-1}) during the vegetative stage, thirty days after planting. The foliar application was done late in the day, when there was no wind to avoid blow over and a sticker was added to the chemical to ensure good adhesion to the leaves.

Agronomic practices such as weeding, thinning and fertiliser application were done homogeneously for all genotypes. The crop was harvested at maturity, threshed and winnowed to separate the grain and chaff but only grain samples were analysed for Se. The Se concentration in grain samples was determined in parts per billion (ppb – µg g^{-1}dry weight) using the Shimadzu Cookbook section 6.10 Atomic Absorption Spectrophotometer with Graphite furnace (AAS - GF) by the Zambia Bureau of Standards (ZABS) in Lusaka.

Phase II: Development of population crosses

Pearl millet crossing blocks were planted on a 500 m^2 plot at National Irrigation Research Station, Nanga, in Mazabuka District, Southern Province, Zambia, from August to December 2012, with the aim of crossing the good with the moderate or low accumulators from phase I. This was done in order to study the genetics of Se accumulation by determining the gene action and heritability of the Se accumulation trait. However, there was a delay in obtaining the Se analysis results before hybridisation could commence.

Therefore, all 37 genotypes were planted, in which 12 and 25 were randomly selected as male and female parents, respectively and not on the basis of whether is high or low accumulator but on the basis of the amount of pollen. Two rows were planted per genotype and crosses were made according to North Carolina Design II Mating Scheme (Robinson and Comstock, 1948; Hallauer and Miranda, 1988).

Genotypes were staggered planted to synchronise flowering at an interval of 5 days in between. Since pearl millet is a cross–pollinated crop, shoot bags (pollination bags) were used to avoid cross pollination. Plants were covered with white transparent water proof paper bags upon panicle emergence and a brown pollination bag was used after pollination until maturity (Fig. 1 and 2). A total of 300 crosses were made, however, not all of them were successful. This was due to several problem experienced during hybridisation experiment such as; the outbreak of the spotted stem borer pest which was feeding on the already pollinated panicles, windstorm which was experienced and due to incompatibility of some crosses. Therefore, in order to meet the NCDII requirements of each male mated to each female, successful crosses were grouped into three independent experiments (sets).

Figure 1: Panicles covered with white transparent paper bags before pollination

Figure 2: Pollinated panicles covered with brown pollination paper bags

Phase III: Evaluation of population crosses

The harvested seeds from the NCD II mating scheme crossing blocks were planted at UNZA Field Station during 2012/2013 rain season for evaluation of progenies of population crosses for Se accumulation and other agronomic traits including yield.

The successful crosses were grouped into three independent experiments (sets) (Table 4-6). These experiments were laid out in a Randomised Complete Block Design (RCBC) with three replicates. One set had 24 entries (crosses) while the other two had 15 entries (crosses) each. Two rows of 3m long were planted per cross, with inter row spacing of 0.75m and intra row spacing of 0.4m. Compound D (10N: 20P: 10K) fertilizer was used as basal dressing at a rate of 200 kg ha^{-1} while Urea (46%N) was applied as top dressing at a rate of 150 kg ha^{-1}. Weeding was done manually were necessary during the growing period.

The progenies were also sprayed with Se in a form of sodium selenate (Na_2SeO_4) during the vegetative stage, thirty days after emergence, through foliar application at a rate of 2mg L^{-1} solution. Grain samples were also analysed for Se accumulation using the same procedure as for parents.

Table 4: Successful crosses (males and females) mated in the NCD II mating scheme for Set I

FEMALE GENOTYPES	MALE GENOTYPES			
	SOSANK	SEPO	ZPMV 24801	ZPMDC
NEC-C_3	X	x	x	x
LAGRAP	X	x	x	x
LCIC 9702	X	x	x	x
KUOMBOKA	X	x	x	x
OKASHANA 1	X	x	x	x
NL_0C - C_4	X	x	x	x

Table 5: Successful crosses (males and females) mated in the NCD II mating scheme for Set II

FEMALE GENOTYPES	MALE GENOTYPES				
	GWAGWA	ZPMV 20502	NLBC-C_3	570028 R1w	ICMV-155 Br
NEC-C_3	x	x	x	X	x
TARAM	x	x	x	X	x
LCIC 9702	x	x	x	X	x

Table 6: Successful crosses (males and females) mated in the NCD II mating scheme for Set III

FEMALE GENOTYPES	MALE GENOTYPES		
	ICMV-IS-9031	ZPMV 24802	OKOA
KUOMBOKA	x	x	x
NL_0C - C_4	x	x	x
TUSO	x	x	x
ZPMV 28402	x	x	x
KAUFELA	x	x	x

3.4 Data collection

Data on phenological traits included 50 percent emergence date, days to 50 percent flowering, days to maturity and grain yield. Morpho-physiological traits measured included; plant height, panicle length, number of productive tillers and 1000 seed weight. Plant stands were counted before harvesting to determine the number of plants harvested. Measurement of the parameters was done as follows:

(i) **50 percent emergence date** – this was recorded on the day 50 percent of the seedlings emerged after planting.

(ii) **Days to 50 percent flowering** – Number of days from emergence to 50 percent of panicles flowered. This was necessary in order to synchronise flowering during hybridisation.

(iii) **Days to maturity** – Number of days from emergence to when panicles attain their physiological maturity.

(iv) **Plant height (cm)** – It was measured using a calibrated ruler in centimetres, from the ground level to the flag leaf of 10 randomly selected plants.

(v) **Panicle length (cm)** – This was done with a ruler from the base to the tip of the panicle from the 10 randomly selected plants.

(vi) **Number of productive tillers** – The number of productive tillers per plant were counted from the randomly selected 10 plants

(vii) **Harvest count** – The number of plant stands harvested were counted. This was recorded in order to determine the yield per plot.

(viii) **Thousand seed weight (g) –** Thousand seeds were counted and weighed to determine the weight.

(ix) **Selenium analysis in the grains (µg g^{-1}) -** Data on Se concentration in pearl millet grains was obtained following the procedure described by Shimadzu Cookbook Section 6.10 (Shimadzu, 2009). A four gram (4 g) sample of ground grains was digested in HNO_3 and $HClO_4$. The clear digest was analysed for Se at the Zambia Bureau of Standards (ZABS) using atomization Graphite furnace - Atomic Absorption Spectrophotometer (GF-AAS) in ppb (µg kg^{-1}dry weight). Complementary Se analyses on the same samples were obtained from Sabanci University, Turkey. The results were consistent and, therefore, only one set was used.

3.5 Statistical Analysis

Data were subjected to ANOVA techniques in Genstat 14th Version and Microsoft Excel. Paired t-test was used to determine significant differences in Se accumulation between the sprayed and non-sprayed genotypes. Coefficient of variation was calculated to determine the variation in the ability to accumulate Se among parental genotypes and crosses. Correlation of Se accumulation to morpho-physiological and phenological parameters was determined and significance was tested using a statistical table in Gomez and Gomez (1984).

Genetic analyses of crosses from NCD II mating scheme was done using line x tester as described by Singh and Chaundhary (1985). The following linear model was followed: $\boldsymbol{Yijkl = \mu + rl + mi + fj + (mf)ij + eijkl,}$ where ***Yijk*** is the observed value of the progeny of the i^{th} male crossed with j^{th} female in the k^{th} replication.
The terms are defined as follows: µ = overall population mean, *mi* = general combining ability of the *ith female*, *fj* = general combining ability of the j^{th} male, *(mf)ij* = specific combining ability of the i^{th} *X j cross*, *rl* = replication effect and *eijk* = experimental error.

Genetic variances were estimated by equating the variances to the respective mean square from ANOVA (Table 7) and they were as follows: σ^2_m = (MSm –MSmf)/fr = covHS =1/4 σ^2A, σ^2_F = (MS_f –MS_{mf})/mr = covHS =1/4 σ^2A, σ^2_{mf} = (MS_{mf} – MS_e)/r = covFS-2covHS=1/4σ^2D.

Where σ^2_m = male variance, σ^2_f = female variance, σ^2_e= random error variance; σ^2_{mf} = male x female variance; r = number of replications; σ^2A = additive variance and σ^2D = dominance variance.

Baker's ratio was used to determine the additive and non-additive variances (Baker, 1978). Baker's ratio = (σ^2gca male + σ^2gca female)/ (σ^2gca male + σ^2gca female + σ^2sca). General and specific combining ability were determined using Griffings (1956b) model one or the fixed effect model.

Crosses were classified on the basis of Se accumulation using the following categories as described by Abdel-Ghani *et al.* (2012)

($\dot{x}$ - SD) = low, ($\dot{x}$ + SD) = medium, ($\dot{x}$ +2SD) = high

Where **$\dot{x}$ =** sample mean, **SD =** standard deviation

Table 7: ANOVA of NCD II (Comstock and Robinson, 1948) used in all sets for Se accumulation trait in pearl millet genotypes

Source of Variation	**d.f**	MS	Expected Mean Square (EMS)
Rep	r-1		
Males	m-1	MS_m	$\sigma^2_e + r\sigma^2_{fm} + rf\sigma^2_m$
Females	f-1	MS_f	$\sigma^2_e + r\sigma^2_{fm} + rm\sigma^2_f$
Male x Female	(m-1)(f-1)	MS_{fm}	$\sigma^2_e + r\sigma^2_{fm}$
Error	mf(r-1)	MS_e	σ^2_e
Total			

KEY: m, f, and r refer to males, females, and replications, respectively. $\sigma^2_m = \sigma^2_f$ = covariance of half-sibs = 1/4 σ^2 A. σ2mf = (covariance of full-sibs)– (covariance of half-sibs of males and females) = 1/4 σ^2 , D. σ^2me = σ^2fr = interaction of covariance of half-sibs per replication = 1/4 σ^2D. σ^2fmr = interaction of females × males per replications = 1/4 σ^2DL. σ^2_e = experimental error.

Heritability estimates were calculated using variance ratios as follows:

Narrow sense heritability was estimated by $h^2 = V_A/V_P$, where V_A= additive variance due to male and female and V_P is the total variance ($V_A + V_D + V_E$)

V_D= Variation due to dominance, V_E = Variation due to environment

CHAPTER FOUR

4.0 RESULTS

4.1 Variation in Se accumulation of parental genotypes

The results of grain Se concentration of 37 pearl millet parental genotypes are presented in Table 8. Results showed that pearl millet genotypes accumulated Se naturally from the soil and through leaves after foliar spraying differently. The grain Se concentration among the genotypes that were not sprayed ranged from 0.00µg g^{-1} to 0.09 µg g^{-1}, with the overall mean of 0.01 µg g^{-1}, while the concentrations among the sprayed genotypes ranged from 0.01µg g^{-1} to 0.63 µg g^{-1} with the overall mean of 0.08 µg g^{-1}.

The highest grain Se accumulation among the sprayed genotypes was observed for NLC-C_3 (0.63 µg g^{-1}) followed by SDMV 59009 (0.36 µg g^{-1}) while among the non-sprayed, the highest was SDMV 59009 (0.09 µg g^{-1}) followed by LCIC 9702 with 0.08 µg g^{-1} (Table 8). The lowest Se accumulating genotype among the sprayed was 570028 R1w (0.01 µg g^{-1}) while among the non-sprayed, results indicated that, eight genotypes (22%) could not accumulate Se enough to be detected by the method used (AAS-GF). These genotypes were 570028 R1w, ZPMV 20402, MANGANARA, NLBC-C_3, ICMV 155 Br, TORONIO, NCD_2 _CO BULK and LISELI all with 0.00 µg g^{-1}. These genotypes were considered to have a very poor remobilisation efficiency given the amount of Se in the soil (0.16 µg g^{-1}) at UNZA site 2 (Table 2) where the experiment was planted.

Results further indicated that spraying the crop with a 2 mg L^{-1} solution of Se (Na_2SeO_4) increased grain Se content by 6.19% on average. The paired t-test that was performed on these data showed that there was strong evidence (P=0.004) of the positive effect of spraying Se on enhancing Se accumulation. The range for response to spraying varied from 0.00% to 61%.

Table 8: Grain Se concentration of 37 pearl millet parental genotypes from both non-sprayed and sprayed treatments. *(ranked by sprayed).*

Genotype	Origin	Non-sprayed (µg $g^{-1)}$	RE (%) (0.16 µg g^{-1})	Sprayed (µg g^{-1})	Response (%)
NLC - C_3	Zambia	0.02	12.50	0.63	61.26
SDMV 59009	SADC	0.09	56.25	0.36	27.03
ICMV - IS -9031	India	0.01	6.25	0.31	29.79
ZPMV 28402	Zambia	0.01	6.25	0.12	10.75
HHVBC TALL	India	0.01	6.25	0.09	7.44
LCIC 9702	Zambia	0.08	0.50	0.08	0.00
OKASHANA 1	Togo	0.01	6.25	0.08	7.11
ZPMV 20502	Zambia	0.01	6.25	0.07	6.72
OKOA	West Africa	0.01	6.25	0.07	6.03
SOSANK	Mali	0.01	6.25	0.07	6.26
KUOMBOKA	Zambia	0.01	6.25	0.07	5.84
SEPO	Zambia	0.01	6.25	0.06	5.45
ZPMV 24802	Zambia	0.02	12.50	0.06	4.32
TUSO	Zambia	0.01	6.25	0.05	4.83
ZPMV 24801	Zambia	0.02	12.50	0.04	2.10
ARROW	Ghana	0.02	12.50	0.04	2.26
NL_0C - C_4	Zambia	0.01	6.25	0.04	3.48
HHVBC DWARF	Zambia	0.01	6.25	0.04	2.94
KAUFELA	Zambia	0.02	12.50	0.04	1.99
BOBONI	Mali	0.02	12.50	0.04	1.72
TORONIO	Mali	0.00	0.00	0.04	3.13
LAGRAP	India	0.01	6.25	0.03	2.87
NEC - C_3	Zambia	0.01	6.25	0.03	2.07
GWAGWA	West Africa	0.01	6.25	0.03	1.91
ZPMV 28401	Zambia	0.03	18.75	0.03	0.00
ICMV 155 Br	India	0.00	0.00	0.03	2.75
LISELI	Zambia	0.00	0.00	0.03	2.60
NAMALOBA L.	Zambia	0.01	6.25	0.03	2.24
KANGARA	Namibia	0.01	6.25	0.02	1.13
ZPMV 21601	Zambia	0.01	6.25	0.02	1.60
ZPMV 20402	Zambia	0.00	0.00	0.02	1.96
TARAM	Mali	0.01	6.25	0.02	1.33
NLBC-C_3	Zambia	0.00	0.00	0.02	1.70
NCD_2-CO BULK	India	0.00	0.00	0.02	1.39
MANGANARA	Ghana	0.00	0.00	0.02	1.52
ZPMDC	India	0.01	6.25	0.02	0.97
570028 R1w	USA	0.00	0.00	0.01	1.43
Mean (Ẋ)		**0.01**	**7.45**	**0.08**	**6.19**

RE = Remobilisation efficiency

Out of 37 genotypes analysed, 35 were accumulators while two LCIC 9702 and ZPMV 24801 both with (0.00%) from Zambia were considered non-accumulators from spraying through leaves. The highest accumulators were NLC-C_3 (61.26%) from Zambia, ICMV-IS-9031 (29.79%) from India, SDMV 59009 (27.03%) from SADC and ZPMV 28402 (10.75%) from Zambia while the lowest accumulator was ZPMDC with 0.97% from India (Table 8).

Genotypes used in this study showed inconsistency in Se accumulation from the soil and through the leaves. This was demonstrated by the fact that differential Se accumulation was observed among genotypes; the highest Se accumulators in the non-sprayed regime were not necessarily the best in the sprayed regime. Only four genotypes, SDMV 59009 (0.36 µg g^{-1}), LCIC 9702 (0.08 µg g^{-1}) and NLC-C_3 (0.63µg g^{-1}) and 570028 R1w (0.01 µg g^{-1}) maintained their position as high and low accumulators, respectively. However, results of correlation showed a significant ($P<0.05$) positive correlation ($r=0.37$) between sprayed and non-sprayed treatments, indicating the relationship between the two treatments.

The summary of descriptive statistics of sprayed and non-sprayed treatments is presented in Table 9. Wide variation was observed for Se accumulation among genotypes. Results showed that high coefficient of variation (CV) values of 135% and 156% were obtained from non-sprayed and sprayed treatments, respectively.

T**able 9:** Summary of descriptive statistics of sprayed and non-sprayed pearl millet genotypes for grain Se accumulation

	Se (µg g^{-1} dry weight)	
Parameter	**Non-sprayed**	**Sprayed**
Mean	0.01	0.08
Range	0.00 - 0.09	0.01-0.63
STDEV	0.02	0.12
SE	0.003	0.019
CV (%)	135	156

STDDEV = Standard deviation, SE = Standard Error, CV = Coefficient of Variation

The graph on characterization of the genotypes from the non-sprayed, showed that 22% did not accumulate Se from the soil, 73% of the genotypes accumulated Se between 0.01– 0.04 µg g^{-1} and 5% accumulated Se between 0.05-0.10 µg g^{-1} (Fig. 3).

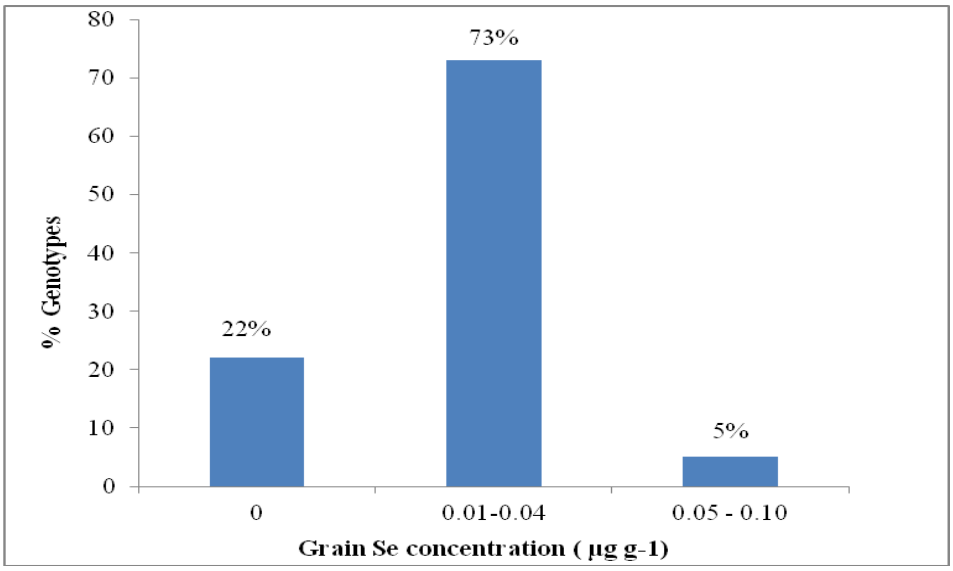

Figure 3: Variability in grain Se accumulation of 37 pearl millet parental genotypes not sprayed, planted in 2011/2012 cropping season at UNZA

For the sprayed genotypes, 62% accumulated Se within the range of 0.01- 0.04 µg g^{-1}, 27% accumulated from 0.05 – 0.10 µg g^{-1}, while 11% accumulated between 0.11-0.63 µg g^{-1} of Se (Fig. 4).

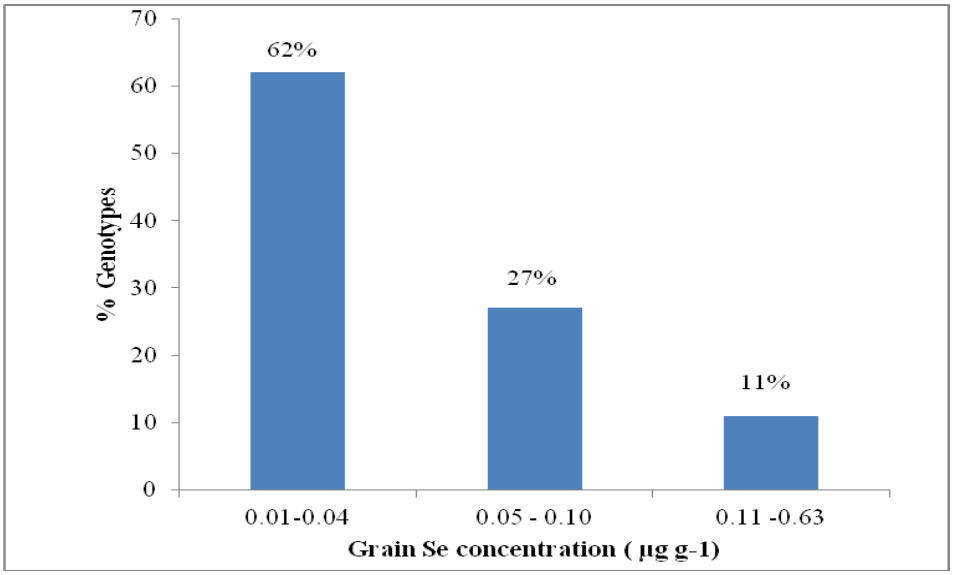

Figure 4: Variability for grain Se accumulation of 37 pearl millet parental genotypes sprayed with 2mg L^{-1} Na_2SeO_4 planted in 2011/12 cropping season at UNZA

4.2 Evaluation of NCD II crosses for Se accumulation and agronomic traits

4.2.1 Crosses performance

SET I

The analysis of variance (Table 10) for Set I shows significant ($P<0.05$) differences among crosses for days to 50% flowering (DF), days to maturity (M), plant height (PH), panicle length (PL), number of productive tillers (NT) and thousand seed weight (TSW). There were no significant differences for Se accumulation in this set. The means for genotypes' grain Se accumulation and other agronomic traits are presented in Table 11.

Days to 50% flowering (DF): The latest flowering crosses were ZPMDC x NL_0C-C_4 (65 days), SOSANK x NL_0C-C_4 (64 days), ZPMV 24801 x KUOMBOKA and SOSANK x NEC-C_3 both with 63 days, while the earliest flowering crosses were ZPMV 24801 x OKASHANA 1 (47 days), SEPO x OKASHANA 1 (48 days) and ZPMDC x OKASHANA 1 (52 days). Overall, about 50% of the crosses flowered within 57 days.

Days to maturity (M): The crosses which matured late were ZPMDC x NL_0C-C_4, SOSANK x NL_0C-C_4, ZPMV 24801 x KUOMBOKA and SOSANK x NEC-C_3, all with 97 days, while the earliest maturing crosses were ZPMV 24801 x OKASHANA 1 (88 days), SEPO x OKASHANA 1 (91 days) and SOSANK x LAGRAP (92 days). On average, 55% of the crosses attained their physiological maturity within 94 days.

Plant height (PH): The plant height for crosses ranged from 176.80 to 221.60 cm. The tallest crosses were SEPO x KUOMBOKA (221.60 cm), SOSANK x NL_0C-C_4 (214.40 cm) and ZPMDC x NL_0C-C_4 (211.00 cm) while the shortest crosses in this set were SOSANK x OKASHANA 1 (176.80 cm), SOSANK x NEC-C3 (179.80 cm), ZPMV 24801 x NEC-C_3 (184.20 cm). The average plant height for crosses was 196.38 cm and 55% of crosses plant heights were below average.

Table 10: Mean squares for grain Se accumulation and other agronomic traits (Set I) of pearl millet evaluated during 2012/2013 cropping season at UNZA

Source of Variation	**d.f**	**Se**	**GY**	**DF**	**M**	**PH**	**PL**	**NT**	**TSW**
Rep	2	1.83	0.52	95.43	50.17	533.6	1.36	1.49	2.21
Crosses	23	0.05	0.21	62.17***	13.6**	366.2*	20.55***	0.48	7.90**
GCA male	3	0.04	0.04	64.75*	8.05	157.5	5.68	0.56	3.47
GCA female	5	0.05	0.39	170.75***	41.69***	816.8**	36.68***	1.13**	26.89***
SCA	15	0.04	0.18	25.47	5.35	257.7	18.15***	0.25	2.45
Error	**46**	0.04	0.27	20.23	5.56	245.9	4.57	0.33	3.11
CV%		**31.4**	**10.8**	**3.4**	**1.5**	**2.4**	**4.57**	**6.2**	**2.1**

KEY: **Se:** Grain Se concentration, **GY**: Grain yield, **DF:** Days to 50% flowering, **M:** Days to Maturity, **PH**: Plant height, **PL**: Panicle length, **NT**: No. of productive tillers/plant, **TSW**: 1000 seed weight
*,**, *** significant at 0.05, 0.01 and 0.001 probability levels, respectively

Table 11: Mean performance of crosses for grain Se accumulation and other agronomic traits (Set I) evaluated during 2012/2013 cropping season at UNZA *(ranked by Se accumulation)*

CROSSES	Se (µg g^{-1})	GY (t ha^{-1})	DF (days)	M (days)	PH (cm)	PL (cm)	NT (No.)	TSW (g)
ZPMDC x NEC-C_3	0.62	1.43	56.33	95.00	190.2	22.83	3.75	13.8
ZPMDC x LCIC 9702	0.56	1.39	59.00	95.33	191.5	28.75	3.87	15.6
SOSANK x NL_0C-C_4	0.50	1.48	64.33	97.33	214.4	24.67	3.41	14.4
ZPMV 24801 x KUOMBOKA	0.50	1.23	63.67	97.00	204.6	26.33	4.66	11.7
ZPMV 24801 x NL_0C-C_4	0.50	1.43	56.67	94.33	195.2	27.67	4.04	13.2
SEPO x LCIC 9702	0.49	1.99	59.33	94.67	204.2	26.17	4.37	15.3
SEPO x OKASHANA 1	0.49	1.99	48.33	91.00	187.2	21.33	4.71	16.2
ZPMV 24801 x NEC-C_3	0.48	1.24	55.67	94.67	184.2	23.33	3.83	12.6
SEPO x NEC-C_3	0.46	1.57	55.67	94.67	195.8	25.75	3.50	14.4
ZPMV 24801 x OKASHANA 1	0.41	1.81	47.67	88.67	187.3	22.67	4.37	16.2
ZPMDC x LAGRAP	0.40	1.37	56.00	94.00	197.5	24.50	3.87	15.8
ZPMDC x NL_0C-C_4	0.38	1.46	65.33	97.67	211.0	25.50	4.09	12.4
SOSANK x NEC-C_3	0.37	1.12	63.67	97.00	179.8	21.92	3.87	12.0
ZPMDC x KUOMBOKA	0.32	1.21	62.67	96.67	197.4	22.92	4.63	12.9
SOSANK x LAGRAP	0.31	1.68	54.67	92.00	189.6	29.00	3.62	16.0
ZPMDC x OKASHANA 1	0.31	1.50	52.33	93.33	195.5	23.25	4.20	17.1
SOSANK x LCIC 9702	0.30	1.43	59.33	94.00	208.8	21.67	3.20	16.9
SEPO x KUOMBOKA	0.28	0.85	60.00	96.33	221.6	26.17	4.02	13.8
SEPO x LAGRAP	0.25	1.54	59.00	96.00	202.6	23.92	3.50	15.4
ZPMV 24801 x LCIC 9702	0.24	1.55	54.67	93.67	201.9	21.92	4.13	15.7

Table 11 continued

CROSSES	Se ($\mu g\ g^{-1}$)	GY ($t\ ha^{-1}$)	DF (days)	M (days)	PH (cm)	PL (cm)	NT (No.)	TSW (g)
SOSANK x KUOMBOKA	0.21	1.50	61.33	96.67	201.3	27.92	4.29	15.2
SEPO x NL_0C-C_4	0.21	1.10	58.67	95.67	189.1	28.58	3.79	12.7
ZPMV 24801 x LAGRAP	0.21	1.32	56.67	94.33	185.6	22.83	4.12	14.8
SOSANK x OKASHANA 1	0.19	1.48	57.33	93.00	176.8	19.67	4.25	16.1
GRAND MEAN	**0.37**	**1.44**	**57.85**	**94.71**	**196.4**	**24.55**	**4.01**	**14.60**
LSD (5%)	**0.33**	**0.85**	**7.39**	**3.88**	**25.8**	**3.5**	**0.95**	**2.90**

KEY: **Se:** Grain Se concentration, **GY**: Grain yield, **DF:** Days to 50% flowering, **M:** Days to Maturity, **PH**: Plant height, **PL**: Panicle length, **NT**: No. of productive tillers/plant, **TSW**: 1000 seed weight

Panicle length (PL): The crosses with the longest panicles in this set were SOSANK x LAGRAP (29.00 cm), ZPMDC x LCIC 9702 (28.75 cm) and SEPO x NL_0C-C_4 (28.58 cm), while the shortest panicles were obtained in SOSANK x OKASHANA 1 (19.67cm), SEPO x OKASHANA 1 (21.33 cm) and SOSANK x LCIC 9702 (21.67 cm). Overall, the average panicle length for the crosses was 24.55 cm and only 45% of crosses had longer panicles than the average (24.55 cm).

Number of productive tillers per plant (NT): The number of productive tillers per plant in this set ranged from 3.20 to 4.71. The crosses with the highest NT were SEPO x OKASHANA 1(4.71), ZPMV 24801 x KUOMBOKA (4.66) and ZPMDC x KUOMBOKA (4.63) while the crosses with few numbers of productive tillers were SOSANK x LCIC 9702 (3.20), SEPO x NEC-C3 (3.50) and SEPO x LAGRAP (3.50). About 54% of the crosses produced more tillers than the average (4.0).

Thousand seed weight (TSW): The crosses with the heaviest TSW were ZPMDC x OKASHANA 1 (17.10 g) followed by SOSANK x LCIC 9702 (16.9) and ZPMV 24801 x OKASHANA 1 and SEPO x OKASHANA 1 both with 16.20 g while the crosses which recorded the lightest TSW were ZPMV 24801 x KUOMBOKA (11.70 g), SOSANK x NEC-C3 (12.0 g) and ZPMDC x NL_0C-C_4 (12.40 g). The average TSW for crosses in this set was 14.5g and 54% of the crosses had higher thousand seed weight than the average 14.5 g.

SET II

The analysis of variance (Table 12) for Set II shows highly significant ($P<0.01$) differences among crosses for Se accumulation (Se), grain yield (GY), days to 50% flowering (DF), days to maturity (M), plant height (PH) and panicle length (PL). The means for grain Se accumulation and other agronomic traits are presented in Table 13.

Se accumulation (Se): The highest Se accumulating crosses were 570028 R1w x NEC-C_3 (0.19 µg g^{-1}), ZPMV 20502 x TARAM (0.15 µg g^{-1}) and 570028 R1w x TARAM (0.14 µg g^{-1}) whereas the lowest Se accumulating crosses were NLBC – C_3 x TARAM (0.08 µg g^{-1}), GWAGWA x NEC-C_3 (0.09 µg g^{-1}) and ZPMV 20502 x NEC-C_3, ICMV – 155 Br x NEC-C_3 and GWAGWA x LCIC 9702 all with 0.11 µg g^{-1}. Results indicated that only 67% of the crosses accumulated Se above the average (0.12 µg g^{-1}).

Grain Yield (GY): High grain yield in this set was obtained from GWAGWA x LCIC 9702 (2.63 t ha^{-1}) followed by 570028 R1w x NEC-C_3 (2.14 t ha^{-1}) and ICMV–155Br x LCIC 9702 (1.94 t ha^{-1}) while the lowest grain yield obtained from ZPMV 20502 x TARAM (1.06 t ha^{-1}), ZPMV 20502 x NEC-C_3 (1.26 t ha^{-1}) and NLBC-C_3 x NEC-C_3 (1.28 t ha^{-1}). On average, 53% of the crosses yielded more than 1.75 t ha^{-1}.

Days to 50% flowering (DF): The latest flowering crosses were NLBC-C_3 x NEC-C_3 (65 days), ZPMV 20502 x TARAM (64 days) and NLBC-C_3 x TARAM (62 days) while the early flowering observed in ICMV-155 Br x LCIC 9702 (44 days), GWAGWA x LCIC 9702 (49 days) and GWAGWA x TARAM (53 days). About 53% of the crosses took more than 56 days to flower.

Day to maturity (M): The late maturing crosses in this set were NLBC-C_3 x NEC-C_3 and 570028 R1w x TARAM both took 98 days followed by ZPMV 20502 x NEC-C_3 and ICMV-155 Br x TARAM with 96 days. The earliest maturing crosses were ICMV-155Br x LCIC 9702 (86 days), GWAGWA x LCIC 9702 (91 days) followed by 570028 R1w x NEC-C_3 and GWAGWA x TARAM with 93 days. Results showed that 67% of the crosses matured within 94 days.

Table 12: Mean squares for grain Se accumulation and other agronomic traits (Set II) evaluated during 2012/2013 cropping season at UNZA

Source of Variation	d.f	Se (x10^3)	GY	DF	M	NT	PH	PL	TSW
Rep	2	0.15	0.57	62.49	10.76	0.3	1796.7	9.46	3.26
Crosses	14	1.98**	0.45***	90.14***	26.98***	0.28	855.50***	28.23***	2.87
GCA males	4	3.32***	0.89***	118.14***	31.13***	0.23	1444.1***	44.43***	1.51
GCA females	2	0.05	0.55**	219.36***	61.76***	0.17	1638.7***	46.67***	2.86
SCA	8	1.79**	0.20*	43.83**	16.20***	0.33	365.5*	15.51**	3.55
Error	28	0.55	0.09	17.25	3.49	0.34	154.6	4.61	4.12
CV%		**2.6**	**11.1**	**3.6**	**0.9**	**3.7**	**5.0**	**3.0**	**2.8**

KEY: **Se:** Grain Se concentration, **GY**: Grain yield, **DF:** Days to 50% flowering, **M:** Days to Maturity, **PH**: Plant height, **PL**: Panicle length, **NT**: No. of productive tillers/plant, **TSW**: 1000 seed weight

*,**, *** significant at 0.05, 0.01 and 0.001 probability levels, respectively

Table 13: Mean performance of crosses for grain Se accumulation and other agronomic traits (Set II) evaluated during 2012/2013 cropping season at UNZA *(ranked by Se accumulation)*

CROSSES	Se ($\mu g\ g^{-1}$)	GY ($t\ ha^{-1}$)	DF (days)	M (days)	PH (cm)	PL (cm)	NT (No.)	TSW (g)
570028 R1w x NEC-C_3	0.19	2.14	55.00	93.33	221	27	3.9	17.1
ZPMV 20502 x TARAM	0.15	1.06	64.33	94.00	228	34	3.8	17.6
570028 R1w x TARAM	0.14	1.92	57.00	98.33	231	28	3.6	16.1
ZPMV 20502 x LCIC 9702	0.13	1.90	55.67	93.67	238	26	3.6	16.6
570028 R1w x LCIC 9702	0.13	1.83	58.00	95.33	209	27	3.3	16.9
NLBC - C_3 x NEC-C_3	0.12	1.28	65.33	98.67	238	30	3.5	15.6
GWAGWA x TARAM	0.12	1.84	53.67	93.33	213	25	3.9	17.1
ICMV-155 Br x TARAM	0.12	1.73	60.33	96.00	201	26	4.0	16.4
NLBC - C_3 x LCIC 9702	0.12	1.55	54.67	94.00	211	27	3.9	15.4
ICMV - 155 Br x LCIC 9702	0.12	1.94	44.33	86.00	176	23	4.3	16.2
ZPMV 20502 x NEC-C_3	0.11	1.26	60.67	96.00	231	26	4.1	15.2
ICMV - 155 Br x NEC - C_3	0.11	1.77	54.33	94.00	222	26	3.7	17.1
GWAGWA x LCIC 9702	0.11	2.63	49.67	91.96	197	20	3.6	14.2
GWAGWA x NEC-C_3	0.09	1.88	57.33	93.67	223	26	4.2	17.8
NLBC-C_3 x TARAM	0.08	1.51	62.33	93.67	224	27	4.3	16.1
Grand mean	**0.12**	**1.75**	**56.84**	**94.42**	**218**	**27.00**	**3.80**	**16.40**
LSD (5%)	**0.04**	**0.51**	**6.95**	**3.13**	**20.8**	**3.60**	**0.98**	**3.39**

KEY: **Se:** Grain Se concentration, **GY**: Grain yield, **DF:** Days to 50% flowering, **M:** Days to Maturity, **PH**: Plant height, **PL**: Panicle length, **NT**: No. of productive tillers/plant, **TSW**: 1000 seed weight

*,**, *** significant at 0.05, 0.01 and 0.001 probability levels, respectively

Plant height (PH): Tallest plant height of 238 cm in this set was observed for NLBC-C_3 x NEC-C_3 and ZPM 20502 x LCIC 9702 followed by ZPMV 20502 x TARAM with 228 cm while the shortest plant heights were recorded from ICMV-155Br x LCIC 9707 (176 cm), GWAGWA x LCIC 9702 (197 cm) and ICMV 155Br x TARAM (201 cm). The average plant height was 218cm and about 60 % of the crosses were taller than the average.

Panicle length (PL): The crosses with the longest panicles were ZPMV 20502 x TARAM (34 cm), NLBC-C_3 x NEC-C_3 (30 cm) and 570028 R1w x TARAM (28 cm) while the crosses which had shortest panicles were GWAGWA x LCIC 9702 (20 cm), ICMV 155Br x LCIC 9702 (23 cm) and GWAGWA x TARAM (25 cm). Most of the crosses (53%) had panicles longer than 27 cm.

SET III

The analysis of variance (Table 14) for Set III shows significant ($P<0.05$) differences among crosses for grain yield (GY), days to 50% flowering (DF), panicle length (PL), number of productive tillers per plant (NT) and 1000 seed weight (TSW). In this set, it is of importance to note that crosses did not show any significant differences for Se accumulation. The means for grain Se accumulation and other agronomic traits are presented in Table 15.

Grain yield (GY): The crosses which had the highest grain yield in this set were ICMV-IS-9031 x ZPMV 28402 (1.62 t ha^{-1}), ICMV-IS-9031 x TUSO (1.42 t ha^{-1}) and ICMV-IS-9031 x KAUFELA, ZPMV 24802 x ZPMV 28402 both with (1.26 t ha^{-1}). The lowest yield was obtained in OKOA x KUOMBOKA (0.69 t ha^{-1}), ZPMV 24802 x KAUFELA (0.84 t ha^{-1}) and ZPMV 24802 x NL_0C-C_4 (0.84 t ha^{-1}). On average, 60 % of the crosses yielded below than 1.04 t ha^{-1}.

Days to 50% flowering (DF): The latest flowering crosses were ZPMV 24802 x NL_0C-C_4 (64 days), ICMV-IS-9031 x KUOMBOKA (62 days) and ICMV-IS-9031 x TUSO and ZPMV 24802 x KUOMBOKA both with 61 days while the earliest crosses were ICMV-IS-9031 x KAUFELA (54 days), ICMV-IS-9031 x ZPMV 28402 (56 days) and ICMV-IS-9031 x NL_0C-C_4 (58 days). About 54% of the crosses flowered within the average (59 days).

Panicle length (PL): The longest panicles in this set were recorded in ZPMV 24802 x TUSO (35.00 cm) and OKOA x KAUFELA (32.08 cm), while shortest panicles were observed in ICMV-IS-9031 x NLoC-C4 (24.17 cm) and OKOA x KUOMBOKA (25.42 cm). The average panicle length was 28.90 cm and 60% of the crosses had longer panicles than the average.

Number of productive tillers per plant (NT): The number of productive tillers per plant in this set ranged from 3.04 to 4.33. The crosses with the highest number of productive tillers per plant were ICMV-IS-9031 x NL_0C-C_4 (4.33), OKOA x NL_0C-C_4 (4.25) and OKOA x TUSO (4.13) while the crosses with few numbers of productive tillers were ICMV-IS-9031 x TUSO (3.04) and OKOA x KAUFELA (3.07). About 46% of the crosses produced more tillers than the average (3.8).

Thousand seed weight (TSW): The crosses with the heaviest TSW were ICMV-IS-9031 x KAUFELA (17.00 g), ZPMV 24802 x KAUFELA (16.97 g) and OKOA x KAUFELA (16.87 g) while the crosses which recorded the lightest TSW were ICMV-IS-9031 x ZPMV 28402 (13.87 g) and ZPMV 24802 x NL_0C-C_4 (14.00). The average TSW for crosses in this set was 15.4g and 53% of the crosses had lighter thousand seed weight than the average.

Table 14: Mean squares for grain Se accumulation and other agronomic traits (Set III) evaluated during 2012/2013 cropping season at UNZA

Source of Variation	d.f	Se	GY	DF	M	PH	PL	NT	TSW
Rep	2	0.001	0.79	34.07	12.87	870.00	4.12	0.16	4.39
Crosses	14	0.003	0.21**	16.75*	1.87	521.60	22.65**	0.41*	0.125**
GCA male	2	0.005	0.63***	30.20**	1.80	784.00	25.46**	0.58	3.36
GCA female	4	0.002	0.26**	23.52**	2.81	204.70	41.71***	0.56*	8.15**
SCA	8	0.002	0.09	10.01	1.41	614.30	12.41	0.30	2.49
Error	**28**	0.002	0.09	7.73	2.32	415.30	8.33	0.24	2.56
CV%		**3.4**	**22.1**	**2.5**	**1.0**	**3.5**	**1.8**	**2.7**	**3.5**

KEY: **Se:** Grain Se concentration, **GY**: Grain yield, **DF:** Days to 50% flowering, **M:** Days to Maturity, **PH**: Plant height, **PL**: Panicle length, **NT**: No. of productive tillers/plant, **TSW**: 1000 seed weight
*,**, *** significant at 0.05, 0.01 and 0.001 probability levels, respectively

Table 15: Mean performance of progenies for grain Se accumulation and other agronomic traits (Set III) evaluated during 2012/2013 cropping season at UNZA *(ranked by Se accumulation)*

CROSSES	Se ($\mu g\ g^{-1}$)	GY ($t\ ha^{-1}$)	DF (days)	M (days)	PH (cm)	PL (cm)	NT (No.)	TSW (g)
ZPMV 24802 x ZPMV 28402	0.27	1.26	60.67	96.33	234.2	27.33	4.08	14.70
ZPMV 24802 x NL_0C-C_4	0.25	0.84	64.00	97.00	237.5	28.92	3.79	14.00
OKOA x TUSO	0.23	0.73	59.33	95.33	211.2	29.25	4.13	14.17
ZPMV 24802 x TUSO	0.22	1.11	61.67	96.00	221.0	35.00	3.21	14.17
ICMV-IS 9031 x NL0C-C4	0.21	0.91	58.67	95.33	191.2	24.17	4.33	15.57
OKOA x KUOMBOKA	0.21	0.69	59.67	95.67	210.4	25.42	4.00	14.83
ZPMV 24802 x KUOMBOKA	0.20	1.15	61.33	96.67	215.4	29.50	3.47	14.90
OKOA x ZPMV 28402	0.20	1.00	59.33	95.67	226.2	27.58	4.04	14.67
ICMV-IS 9031 x KUOMBOKA	0.19	1.00	62.33	97.33	208.2	27.08	3.99	16.07
ICMV-IS 9031 x ZPMV 28402	0.19	1.62	56.33	95.33	203.9	26.33	3.63	13.87
ICMV-IS 9031 x KAUFELA	0.19	1.26	54.33	94.33	234.5	31.33	3.79	17.00
ZPMV 24802 x KAUFELA	0.19	0.84	59.33	95.67	210.0	30.50	3.62	16.97
ICMV-IS 9031 x TUSO	0.18	1.42	61.33	96.33	215.0	29.33	3.04	16.37
OKOA x NL_0C-C_4	0.18	0.78	60.67	96.67	200.8	29.67	4.25	16.43
OKOA x KAUFELA	0.16	0.96	59.00	95.33	209.9	32.08	3.07	16.87
Grand mean	**0.20**	**1.04**	**59.87**	**95.93**	**215.40**	**29.00**	**3.80**	**15.40**
LSD (5%)	**0.08**	**0.51**	**4.65**	**2.55**	**34.00**	**4.83**	**0.82**	**2.68**

KEY: **Se:** Grain Se concentration, **GY**: Grain yield, **DF:** Days to 50% flowering, **M:** Days to Maturity, **PH**: Plant height, **PL**: Panicle length, **NT**: No. of productive tillers/plant, **TSW**: 1000 seed weight

*,**, *** significant at 0.05, 0.01 and 0.001 probability levels respectively

4.2.2 Classification of crosses based on Se accumulation

SET I

Results indicated that 25% of the 24 crosses in Set I accumulated Se between 0.19-0.25 µg g^{-1} and were classified as low accumulators (LA), 67% of the crosses accumulated Se within the range of 0.26-0.50 µg g^{-1} and were classified as medium accumulators (MA), while only 8% of the 24 crosses accumulated Se from 0.51-0.62 µg g^{-1} and were regarded as high accumulators (HA) in this study (Figure 5).

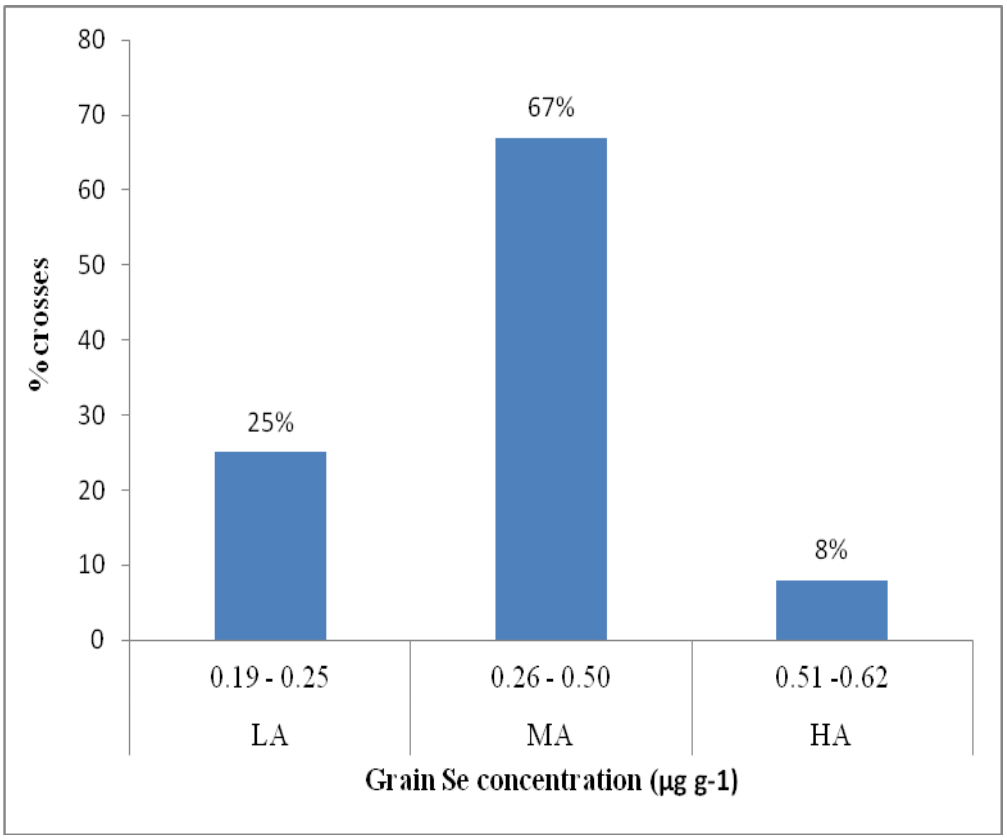

Fig. 5: Distribution of 24 crosses in Set I for Se accumulation sprayed with 2mg L^{-1} solution of Na_2SeO_4 during 2012/13 rain season at UNZA
(LR = Low Accumulator, MR =Medium Accumulator, HR = High Accumulator)

SET II

Results showed that, 80% of the 15 crosses in Set II accumulated Se within the range of 0.11- 0.15 µg g^{-1} and were classified as medium accumulators (MA) and only 7% of the 15 crosses were classified as high accumulators (HA) after accumulated Se between 0.16 – 0.19 µg g^{-1}. The 13% was for the low accumulators (LA) which accumulated Se within the range of 0.08 – 0.10 µg g^{-1} (Fig. 6).

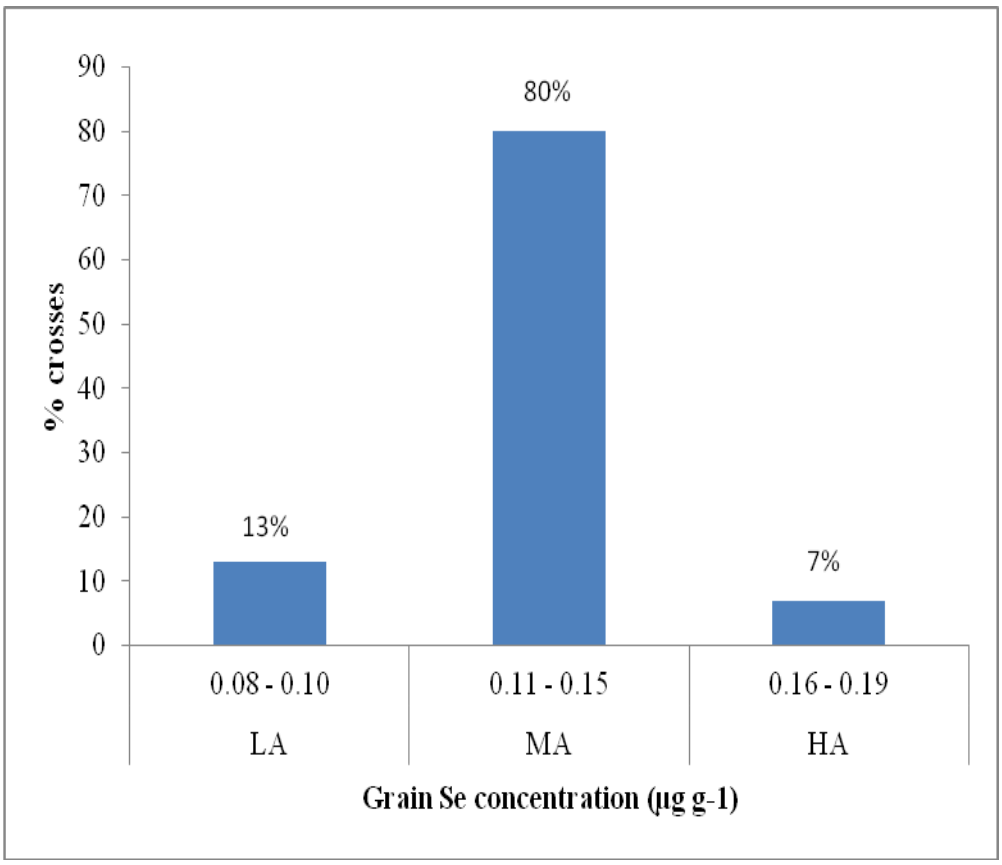

Fig. 6: Distribution of 15 crosses in Set II for Se accumulation sprayed with 2mg L^{-1} solution of Na_2SeO_4 in 2012/13 rain season at UNZA (*LA = Low Accumulator, MA = Medium Accumulator, HA = High Accumulator*)

SET III

Results demonstrated that 13% of the 15 crosses from Set III accumulated Se within the range of 0.25 – 0.27 and were classified as high accumulators (HA) while 67% of the 15 crosses accumulated Se between 0.19 – 0.23 µg g^{-1} and were classified as medium accumulators (MA). The 20% of the crosses accumulated Se from 0.16 – 0.18 µg g^{-1} and they fall under the low accumulators (LA) class (Fig. 7).

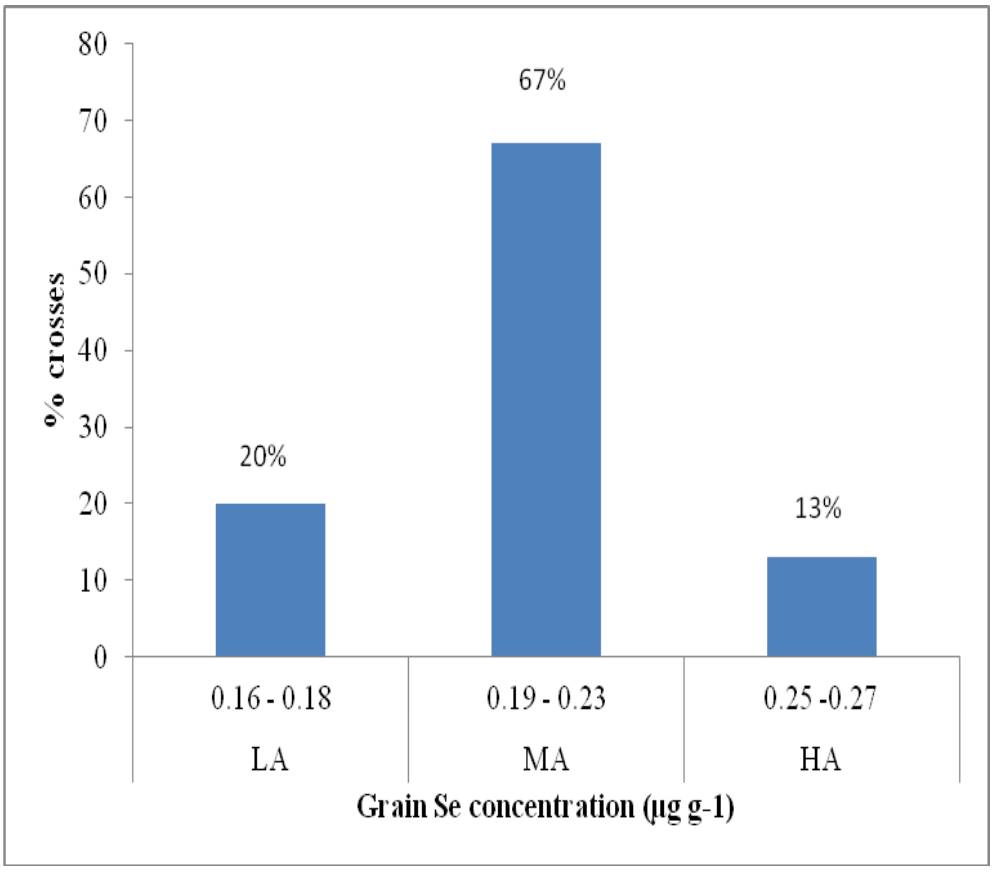

Fig. 7: Distribution of 15 crosses in Set II for Se accumulation sprayed with 2mg L^{-1} solution of Na_2SeO_4 in 2012/13 rain season at UNZA
(LA = Low Accumulator, MA = Medium Accumulator, HA = High Accumulator)

4.2.3 Correlation of agronomic traits and Se accumulation in pearl millet

Phenotypic correlation coefficients among agronomic traits and Se accumulation are presented in tables 16 – 18 for Set I, II and Set III, respectively**.**

SET I

A highly significant (P<0.01) strong positive correlation (r = 0.579) of Se accumulation was observed with days to 50% flowering while a highly significant strong negative correlation (r = -0.910) of Se accumulation was found with days to maturity (Table 16). No significant correlation was found in this set between Se accumulation and grain yield, plant height, panicle length, number of productive tillers and thousand seed weight. Results in Table 16 also showed strong and weak inter-component correlations. Highly significant (P<0.01) strong negative correlation (r = -0.786) was recorded between days to 50% flowering and days to maturity. There were also significant but weak negative correlations between grain yield and plant height (r = -0.357) grain yield and thousand seed weight (r = -0.236), days to maturity and plant height (r = -0.241) and days to maturity with number of productive tillers (r = -0.273).

SET II

In this set, Se accumulation showed highly significant (P<0.01) strong positive correlation (r = 0.630) with days to 50% flowering and adversely, highly significant strong negative correlation (r = -0.912) with days to maturity (Table 17). No significant correlation was found between Se accumulation and grain yield, plant height, panicle length, number of productive tillers and thousand seed weight. Results further indicated strong and weak correlations among agronomic traits. Highly significant (P<0.01) strong positive correlation were recorded between grain yield and days to 50% flowering (r = 0.522) and between grain yield and panicle length (r = 0.441).

Table 16: Phenotypic correlation coefficients estimates among agronomic traits and Se accumulation for SET I

	Se	**GY**	**DF**	**M**	**PH**	**PL**	**NT**	**TSW**
Se	1.00							
GY	-0.185	1.00						
DF	0.579**	0.169	1.00					
M	-0.910**	0.145	-0.786**	1.00				
PH	0.117	-0.357**	-0.014	-0.241*	1.00			
PL	0.050	-0.083	0.120	-0.200	-0.175	1.00		
NT	0.092	-0.043	0.163	-0.273*	0.144	0.177	1.00	
TSW	-0.219	-0.236*	0.160	0.007	-0.141	0.164	0.042	1.00

KEY: **Se:** Grain Se concentration, **GY**: Grain yield, **DF:** Days to 50% flowering, **M:** Days to maturity, P**H**: Plant height, **PL**: Panicle length, **NT**: No. of productive tillers/plant, **TSW**: 1000 seed weight

*,**, significant at 0.05 and 0.01 probability levels, respectively

Table 17: Phenotypic correlation coefficient estimates among agronomic traits and Se accumulation for SET II

	Se	**GY**	**DF**	**M**	**PH**	**PL**	**NT**	**TSW**
Se	1.00							
GY	-0.001	1.00						
DF	0.630**	0.522**	1.00					
M	-0.912**	-0.317*	-0.827**	1.00				
PH	0.062	0.007	-0.085	-0.150	1.00			
PL	0.272	0.441**	0.264	-0.401**	-0.174	1.00		
NT	-0.261	0.120	0.012	0.089	0.002	-0.003	1.00	
TSW	-0.241	-0.065	-0.068	0.161	-0.194	-0.290	-0.088	1.00

KEY: **Se:** Grain Se concentration, **GY**: Grain yield, **DF:** Days to 50% flowering, **M:** Days to maturity, P**H**: Plant height, **PL**: Panicle length, **NT**: No. of productive tillers/plant, **TSW**: 1000 seed weight

*,**, significant at 0.05 and 0.01 probability levels, respectively

Table 18: Phenotypic correlation coefficient estimates among agronomic traits and Se accumulation for SET III

	Se	**GY**	**DF**	**M**	**PH**	**PL**	**NT**	**TSW**
Se	1.00							
GY	0.262	1.00						
DF	0.749**	0.329*	1.00					
M	-0.958**	-0.326*	-0.889**	1.00				
PH	-0.411**	-0.310*	-0.235	0.323*	1.00			
PL	0.009	0.090	0.109	-0.096	-0.337*	1.00		
NT	-0.018	0.237	0.265	-0.151	-0.055	0.393**	1.00	
TSW	-0.146	0.145	0.01	0.035	0.190	-0.141	-0.024	1.00

KEY: **Se:** Grain Se concentration, **GY**: Grain yield**, DF:** Days to 50% flowering, **M:** Days to maturity, P**H**: Plant height, **PL**: Panicle length, **NT**: No. of productive tillers/plant, **TSW**: 1000 seed weight

*,**, significant at 0.05 and 0.01 probability levels, respectively

Inversely, highly significant (P<0.01) strong negative correlation was observed between days to 50% flowering and days to maturity (r = -0.827) and between days to maturity and panicle length (r = -0.401). In addition, a significant (P<0.05) negative but weak correlation (r = -0.317) was also found between grain yield and days to maturity.

SET III

Results indicated that highly significant (P<0.01) strong positive correlation (r = 0.749) was recorded for Se accumulation with days to 50% flowering while highly significant (P<0.01) strong negative correlation was found for Se accumulation with days to maturity (r = -0.958) as well as with plant height (r= -0.411) (Table 18). No significant correlation was found for Se accumulation with grain yield, panicle length, number of productive tillers and thousand seed weight.

Results further showed significant (P<0.05) weak but positive correlation between grain yield and days to 50% flowering (r = 0.329), days to maturity and plant height (r = 0.323) and between panicle length and number of productive tillers (r = 0.393). Inversely, significant (P<0.05) weak negative correlation were observed between grain yield and days to maturity (r = -0.326), grain yield and plant height (r = -0.310) and plant height and panicle length (r = -0.337).

4.3 Analysis of General and Specific Combining Ability (GCA) and (SCA)

SET I

The analysis for combining ability (Table 10) indicated significant ($P<0.05$) differences among GCA males, GCA females and SCA. The individual GCA and SCA effects are presented in Table 19 and Table 20, respectively. Significant GCA effects were observed for some parents in some of the traits (Table 19).

Parent KUOMBOKA was the only one that had significant ($P=0.01$) but negative GCA (-0.05) as a female source for Se accumulation. Significant ($P<0.05$) positive GCA effects were observed for parents SEPO (0.06) and LCIC 9702 (0.15) as a male and female sources, respectively, for grain yield, while NEC-C_3 displayed significant negative GCA effects (-0.10) for the same trait as a female source. LAGRAP had highly significant ($P<0.01$) negative GCA effects (-1.26, -0.63 and -2.57), for days to 50% flowering, days to maturity and plant height, respectively. It also had, on the other hand, highly significant ($P<0.001$) positive GCA effects (0.51) for panicle length as a female source.

SEPO manifested highly significant ($P=0.001$) negative GCA effects (-1.01) for days to 50% flowering as a male source and positive significant GCA effects for plant height (3.68) while ZPMV 24801 had significant ($P<0.001$) GCA effects for plant height (-3.24). ZPMDC had highly significant ($P<0.01$) positive GCA effects for days to 50% flowering (0.76) as a male source and significant positive GCA effects for number of productive tillers per plant (0.06).

SOSANK and NEC-C_3 had significant ($P<0.05$) positive GCA effects (0.29 and 0.63) for days to maturity as a male and female source, respectively. LCIC 9702 and OKASHANA 1 had significant ($P<0.01$) positive GCA effects (1.38 and 1.58) as female source, for thousand seed weight whereas NEC-C_3 and NL_0C-C_4 showed significant ($P<0.05$) negative GCA effects (-1.60 and -1.17) for the same trait.

Table 19: GCA effects of 10 pearl millet parental genotypes for Se accumulation and agronomic traits in Set I

Males	Se ($\mu g\ g^{-1}$)	GY ($t\ ha^{-1}$)	DF (days)	M (days)	PH (cm)	PL (cm)	NT (No.)	TSW (g)
SOSANK	-0.06	0.00	2.26	0.29*	-1.25	-1.00	-0.23	0.51
SEPO	-0.01	0.06*	-1.01***	0.01	3.68***	0.00	-0.02	0.05
ZPMV 24801	0.02	-0.01	-2.01	-0.93	-3.24***	1.00	0.19	-0.56
ZPMDC	0.06	-0.05	0.76**	0.63	0.82	0.00	0.06*	0.00
SE	**0.01**	**0.03**	**0.25**	**0.13**	**0.87**	**0.12**	**0.03**	**0.42**
Females								
NEC-C_3	0.10	-0.10**	-0.01	0.63**	-8.90	-1.09	-0.27	-1.60**
LAGRAP	-0.08	0.03	-1.26***	-0.63**	-2.57*	0.51***	-0.23	0.81
LCIC 9702	0.02	0.15***	0.24	-0.29	5.23***	0.07	-0.11*	1.38**
KUOMBOKA	-0.05**	-0.25	4.07	1.96	9.85	1.28	0.40	-1.00*
OKASHANA 1	-0.02	0.25	-6.43	-3.21	-9.67	-2.82	0.38	1.58**
NL_0C-C_4	0.02	-0.08	3.40	1.54	6.06	2.05	-0.17***	-1.17*
SE	**0.02**	**0.04**	**0.37**	**0.2**	**1.31**	**0.18**	**0.05**	**0.51**

KEY: **Se:** Grain Se concentration, **GY**: Grain yield**, DF:** Days to 50% flowering, **M:** Days to Maturity, P**H**: Plant height, **PL**: Panicle length, **NT**: No. of productive tillers/plant, **TSW**: 1000 seed weight, **SE: Standard Error**

*,**, ***significant at 0.05, 0.01and 0.001 probability levels, respectively

Table 20: SCA effects estimates for 24 pearl millet crosses used in evaluation experiment (Set I), during 2012/13 cropping season at UNZA

CROSSES	Se ($\mu g\ g^{-1}$)	GY ($t\ ha^{-1}$)	DF (days)	M (days)	PH (cm)	PL (cm)	NT (No.)	TSW (g)
SOSANK x NEC-C_3	-0.05	-0.23	3.57*	1.37	-6.48	-0.54	0.37	-1.53
SEPO x NEC-C_3	-0.01	0.17	-1.15	-0.68	4.59	2.29**	-0.22	1.40
ZPMV 24801 x NEC-C_3	-0.02	-0.08	-0.15	0.27	-0.07	-1.13	-0.09	0.14
ZPMDC x NEC-C_3	0.07	0.14	-2.26	-0.96**	1.95	-0.63	-0.18	0.78
SOSANK x LAGRAP	0.08	0.2	-4.18**	-2.38**	-2.98	4.94	0.07	0.12
SEPO x LAGRAP	-0.03	0.00	3.43*	1.90	5.09	-1.15	-0.26	-0.08
ZPMV 24801 x LAGRAP	-0.10	-0.15	2.10	1.18	-4.99	-3.23	0.16	-0.04
ZPMDC x LAGRAP	0.05	-0.06	-1.35	-0.71	2.87	-0.56	0.03	0.37
SOSANK x LCIC 9702	-0.04	-0.17	-1.01	-0.71	8.48	-1.96**	-0.46*	0.41
SEPO x LCIC 9702	0.10	0.34*	2.26	0.24	-1.11	1.54*	0.50**	-0.69
ZPMV 24801 x LCIC 9702	-0.17**	-0.02	-1.15	0.18	3.55	-3.71	0.05	0.28
ZPMDC x LCIC 9702	0.11	-0.15	0.15	0.29	-10.92*	4.13	-0.09	-0.38
SOSANK x KUOMBOKA	-0.06	0.30	-2.85	-0.29	-3.64	3.08	0.11	1.13
SEPO x KUOMBOKA	-0.03	-0.41*	-0.90	-0.35	11.68*	0.33	-0.35	0.20
ZPMV 24801 x KUOMBOKA	0.15**	0.05	3.76**	1.26	1.59	-0.5	0.07	-1.33
ZPMDC x KUOMBOKA	-0.06	0.06	-0.01	-0.62	-9.63	-2.92***	0.16	-0.69
SOSANK x OKASHANA 1	-0.10	-0.22	3.65**	1.21	-8.62	-1.06	0.09	-0.56
SEPO x OKASHANA 1	0.15*	0.23	-2.07	-0.51**	-3.22	-0.4	0.35	-0.02
ZPMV 24801 x OKASHANA 1	0.05	0.13	-1.74	-1.90	3.86	-0.06	-0.2	0.58
ZPMDC x OKASHANA 1	-0.10	-0.14	0.15	1.21	7.98	1.52*	-0.24	0.95

Table 20 continued

CROSSES	Se (µg g^{-1})	GY (t ha^{-1})	DF (days)	M (days)	PH (cm)	PL (cm)	NT (cm)	TSW (g)
SOSANK x NL_0C-C_4	0.16*	0.11	0.82	0.79	13.23**	-0.94	-0.19	0.43
SEPO x NL_0C-C_4	-0.18**	-0.33	-1.57	-0.59	-17.03**	1.98**	-0.02	-0.80
ZPMV 24801 x NL_0C-C_4	0.09	0.07	-2.57	-0.99	-3.95	0.06	0.02	0.37
ZPMDC x NL_0C-C_4	-0.07	0.15	3.32*	0.80	7.75	-1.1	0.19	-1.02
SE	**0.07**	**0.17**	**1.50**	**0.79**	**5.23**	**0.71**	**0.19**	**1.02**

KEY: **Se:** Grain Se concentration, **GY**: Grain yield, **DF:** Days to 50% flowering, **M:** Days to Maturity, P**H**: Plant height, **PL**: Panicle length, **NT**: No. of productive tillers/plant, **TSW**: 1000 seed weight; **SE: Standard Error**

*,**, ***significant at 0.05, 0.01 and 0.001 probability levels, respectively

Significant SCA effects were observed for some of the crosses in seven out of eight traits studied. Significant ($P \leq 0.05$) positive SCA effects for Se accumulation were observed for crosses SOSANK x NL_0C-C_4 (0.16), ZPMV 24801 x KUMBOKA (0.15) and SEPO x OKASHANA 1 (0.15) while ZPMV 24801 x LCIC 9702 and SEPO x NL_0C-C_4 had highly significant ($P<0.01$) negative SCA effects, -0.17 and -0.18, respectively for the same trait. For grain yield, SEPO x LCIC 9702 had significant ($P<0.05$) positive SCA effects (0.34) while SEPO x KUOMBOKA displayed significant ($P<0.05$) negative SCA effects (-0.41).

Six crosses had significant SCA effects for days to 50% flowering and of these, only SOSANK x LAGRAP had negative SCA effects (-4.18) for this trait. For days to maturity, highly significant ($P<0.01$) negative SCA effects were obtained for the following crosses SOSANK x LAGRAP (-2.38), ZPMDC x NEC-C_3 (-0.96) and SEPO x OKASHANA 1 (-0.51).

For plant height, ZPMDC x LCIC 9702 and SEPO x NL_0C-C_4 showed significant ($P \leq 0.05$) negative SCA effects (-10.92 and -17.03) while SEPO x KUOMBOKA and SOSANK x NL_0C-C_4 had significant ($P<0.05$) positive SCA effects (11.68 and 13.23) for the same trait.

Significant ($P<0.05$) positive SCA effects for panicle length were observed for four crosses (SEPO x NEC-C_3 (2.29), SEPO x NL_0C-C_4 (1.98), SEPO x LCIC 9702 (1.54) and ZPMDC x OKASHANA 1 (1.52), while two crosses SOSANK x LCIC 9702 and ZPMDC x KUOMBOKA had highly significant ($P<0.01$) negative SCA effects (-1.96 and -2.92) for the same trait. For number of productive tillers per plant, SEPO x LCIC 9702 had significant ($P<0.01$) positive SCA effects (0.50) while SOSANK x LCIC 9702 recorded significant ($P<0.05$) negative SCA effects (-0.46) for the same trait. None of the crosses displayed significant SCA effects for the thousand seed weight trait.

SET II

The analysis for combining ability (Table 12) showed significant ($P<0.05$) differences among GCA males, GCA females and SCA. Individual GCA and SCA effects are presented in Table 21 and Table 22, respectively. Highly significant GCA and SCA effects were observed in some parents and crosses, respectively, for some of the traits.

For Se accumulation, parents NLBC-C_3 and GWAGWA showed highly significant ($P<0.001$) but negative GCA effects (-0.02 and -0.01) as a male source. None of the female parents showed significant GCA effects for Se accumulation trait (Table 21). Highly significant ($P<0.001$) but negative GCA effect (-0.08) for grain yield was observed in NEC-C_3 as a female source.

For days to 50% flowering, parents ICMV 155 Br and GWAGWA had highly significant ($P<0.001$) negative GCA effects (-3.84 and -3.29) both as a male source while ZPMV 20502 and NLBC-C_3 showed significant ($P\leq0.01$) but positive GCA effects (3.38 and 3.93) as a male source also for the same trait. Parent 570028 R1w was the only one recorded significant ($P<0.01$) but positive GCA effects (0.47), as a male source for days to maturity.

For plant height, both 570028 R1w and TARAM recorded significant ($P<0.05$) positive GCA effects (2.82 and 1.75). Highly significant ($P<0.01$) positive GCA effects (1.04 and 0.38) for panicle length were observed in 570028 R1w and NEC-C_3 as male and female sources, respectively. For number of productive tillers per plant, significant ($P<0.05$) positive GCA effects (0.14 and 0.08) were observed in ICMV 155 Br and TARAM while highly significant ($P<0.01$) negative GCA effects (-0.27 and -0.12) recorded in 570028 R1w and LCIC 9702, as female and male source, respectively. NLBC-C_3 and LCIC 9702 had highly significant ($P<0.01$) negative GCA effects (-0.69 and -0.50) for 100 seed weight as male and female sources, respectively, whereas, TARAM had significant positive GCA effects (0.31) for the same trait.

Table 21: GCA effects for 8 pearl millet parental genotypes for Se accumulation and agronomic traits (Set II)

Males	**Se ($\mu g\ g^{-1}$)**	**GY ($t\ ha^{-1}$)**	**DF (days)**	**M (days)**	**PH (cm)**	**PL (cm)**	**NT (No.)**	**TSW (g)**
ZPMV 20502	0.01	-0.34	3.38**	1.58	14.85	2.37	-0.01	0.11
NLBC-C_3	-0.02***	-0.30	3.93***	1.80	6.91	1.18	0.09	-0.69**
570028 R1w	0.03	0.21	-0.18	0.47*	2.82*	1.04***	-0.27***	0.37
ICMV 155 Br	0.01	0.06	-3.84***	-2.42	-17.93	-1.54	0.14*	0.20
GWAGWA	-0.01***	0.37	-3.29***	-1.42	-6.65	-3.04	0.06	0.02
SE	**0.003**	**0.03**	**0.46**	**0.21**	**1.38**	**0.24**	**0.07**	**0.23**
Females								
NEC-C_3	0.00	-0.08***	1.69	0.71	9.47	0.38**	0.04	0.19
LCIC 9702	0.00	0.22	-4.38	-2.29	-11.22	-1.92	-0.12**	-0.50***
TARAM	0.00	-0.14	2.69	1.58	1.75*	1.54	0.08*	0.31**
SE	**0.002**	**0.02**	**0.28**	**0.12**	**0.83**	**0.14**	**0.04**	**0.14**

KEY: **Se:** Grain Se concentration, **GY**: Grain yield, **DF:** Days to 50% flowering, **M:** Days to Maturity, **PH**: Plant height, **PL**: Panicle length, **NT**: No. of productive tillers/plant, **TSW**: 1000 seed weight

*,**, *** significant at 0.05, 0.01 and 0.001 probability levels, respectively

Table 22: SCA effects for 15 pearl millet crosses used in evaluation experiment (Set II) during 2012/13 cropping season at UNZA

PROGENIES	Se (µg g-1)	GY (t ha-1)	DF (days)	M (days)	PH (cm)	PL (cm)	NT (No.)	TSW (g)
ZPMV 20502 x NEC-C_3	-0.03*	-0.06	-1.24	-0.71	-10.47**	-2.82***	0.21	-1.49*
NLBC-C3 x NEC-C_3	0.02	-0.08	2.87*	1.74**	3.98	1.62*	-0.43*	-0.29
570028 RIW x NEC-C_3	0.03**	0.26**	-3.36*	-2.27***	-9.11*	-0.91	0.26	0.14
ICMV 155 BR x NEC-C_3	-0.01	0.04	-0.36	1.29*	13.06**	0.51	-0.34	0.38
GWAGWA x NEC-C_3	-0.02	-0.16	2.09	-0.04	2.53	1.59*	0.29	1.26
ZPMV 20502 x LCIC 9702	0.00	0.28**	-0.18	-0.04	17.13***	-0.69	-0.14	0.67
NLBC-C_3 x LCIC 9702	0.02	-0.12	-1.73	0.07	-1.84	0.84	0.13	0.20
570028 R1w x LCIC 9702	-0.02	-0.35***	5.71***	2.73***	-0.01	1.64*	-0.20	-0.07
ICMV 155 Br x LCIC 9702	0.00	-0.09	-4.29**	-3.71	-12.26**	0.14	0.41*	-1.82**
GWAGWA x LCIC 9702	0.00	0.29**	0.49	0.96	-3.03	-1.94**	-0.19	-1.64*
ZPMV 20502 x TARAM	0.02	-0.21*	1.42	0.75	-6.67	3.51	-0.07	0.83
NLBC-C_3 x TARAM	-0.03**	0.20	-1.13	-1.80**	-2.14	-2.46***	0.30	0.09
570028 R1w x TARAM	-0.01	0.10	-2.36	-0.47	9.11*	-0.74	-0.06	-0.81
ICMV 155 Br x TARAM	0.00	0.05	4.64**	2.42***	-0.81	-0.66	-0.06	-0.50
GWAGWA x TARAM	0.01	-0.14	-2.58	-0.91	0.50	0.34	-0.10	0.38
SE	**0.01**	**0.10**	**1.38**	**0.62**	**4.14**	**0.72**	**0.20**	**0.68**

KEY: **Se:** Grain Se concentration, **GY**: Grain yield, **DF:** Days to 50% flowering, **M:** Days to Maturity, **PH**: Plant height, **PL**: Panicle length, **NT**: No. of productive tillers/plant, **TSW**: 1000 seed weight; **SE: Standard Error**

*,**, *** significant at 0.05, 0.01 and 0.001 probability levels, respectively

Results further indicated that significant SCA effects were observed among some crosses for all the traits studied (Table 22). Highly significant (P<0.01) positive SCA effects (0.03) for Se accumulation was observed in 570028 R1w x NEC-C_3 while significant (P<0.05) negative SCA effects (-0.03 and -0.03) for the same trait was recorded in ZPMV 20502 x NEC-C_3 and NLBC-C_3 x TARAM.

For grain yield, GWAGWA x LCIC 9702, ZPMV 20502 x LCIC 9702 and 570028 R1w x NEC-C_3 had significant (P<0.01) positive SCA effects (0.29, 0.28 and 0.26) whereas 570028 R1w x LCIC 9702 and ZPMV 20502 x TARAM showed significant (P<0.05) negative SCA effects (-0.35 and -0.21) for the same trait.

Significant (P<0.05) negative SCA effects (-4.29 and -3.36) for days to 50% flowering were observed in ICMV 155 Br x LCIC 9702 and 570028 R1w x NEC-C_3, respectively, while highly significant (P<0.01) positive SCA effects (5.71, 4.64 and 2.87) were recorded in 570028 R1w x LCIC 9702, ICMV 155 Br x TARAM and NLBC–C_3 x NEC-C_3 for the same trait. For days to maturity, 570028 R1w x NEC-C_3 and NLBC-C_3 x TARAM had highly significant (P<0.01) negative SCA effects (-2.27 and -1.80) whereas 570028 R1w x LCIC 9702 (2.73), ICMV 155 Br x TARAM (2.42), NLBC-C_3 x NEC-C_3 (1.74) and ICMV 155 Br x NEC-C_3 (1.29), recorded significant positive SCA effects.

Six crosses exhibited significant SCA effects for plant height. Of these, three ICMV 155 Br x LCIC 9702, ZPMV 20502 x NEC-C_3 and 570028 R1w x NEC-C_3 showed highly significant (P<0.01) negative SCA effects (-12.26, -10.47 and -9.11) while other three, ZPMV 20502 x LCIC 9702, ICMV 155 Br x NEC-C_3, and 570028 R1w x TARAM had highly significant positive SCA effects (17.13, 13.06 and 9.11) for the same trait. ZPMV 20502 x NEC-C_3, NLBC-C_3 x TARAM and GWAGWA x LCIC 9702 showed highly significant (P<0.01) negative SCA effects (-2.82, -2.46 and -1.94) for panicle length, whereas, 570028 R1w x LCIC 9702, NLBC-C_3 x NEC-C_3 and GWAGWA x NEC-C_3 recorded significant (P<0.05) positive SCA effects (1.64, 1.62 and 1.59) for the same trait.

For number of productive tillers per plant, only two crosses had significant SCA effects, NLBC-C_3 x NEC-C_3 with negative SCA effects (-0.43) and ICMV 155 Br x LCIC 9702 with positive SCA effects (0.41). Three crosses, ICMV 155 Br x LCIC 9702, GWAGWA x LCIC 9702 and ZPMV 20502 x NEC-C_3 had significant negative SCA effects, (-1.82, -1.64 and -1.49) for thousand seed weight trait.

SET III

The analysis for combining ability (Table 14) indicated significant ($P<0.05$) differences among GCA males, females and SCA. The individual GCA and SCA effects are presented in Table 23 and Table 24, respectively. For Se accumulation, parent ZPMV 28402 showed significant ($P<0.01$) positive GCA effect (0.02) as a female source while ICMV-IS-9031 and OKOA had highly significant ($P<0.01$) negative GCA (-0.01 and -0.01) for the same trait both as male sources. KUOMBOKA was the only parent that showed significant but negative GCA effects (-0.09) for grain yield.

Highly significant ($P<0.01$) positive GCA effects (1.25, 1.24 and 0.91) for days to 50% flowering were observed in NL_0C-C_4, KUOMBOKA and TUSO, all as female parents while highly significant ($P<0.001$) negative GCA effects (-1.09) was obtained in ZPMV 28402 as a female parent as well. For days to maturity, ICMV-IS-9031 had significant ($P<0.05$) negative GCA effects (-0.20) while KUOMBOKA, ZPMV 24802 and NL_0C-C_4 showed significant but positive GCA effects (0.62, 0.40 and 0.40) for the same trait. Significant ($P<0.01$) negative GCA effects (-5.43, -4.73 and -3.59) for plant height were observed in NL_0C-C_4, ICMV-IS-9031 and OKOA whereas ZPMV 28402 had significant but positive GCA effects (6.12) for the same trait.

Table 23: GCA effects of 8 pearl millet parental genotypes for Se accumulation and agronomic traits (Set III)

Males	**Se ($\mu g\ g^{-1}$)**	**GY ($t\ ha^{-1}$)**	**DF (days)**	**M (days)**	**PH (cm)**	**PL (cm)**	**NT (No.)**	**TSW (g)**
ICMV-IS-9031	-0.01***	0.20	-1.27	-0.20*	-4.73***	-1.25	-0.04	0.48***
ZPMV 24802	0.02	0.00	1.53	0.40***	8.32	1.35	-0.17	-0.46***
OKOA	-0.01**	-0.21	-0.27	-0.20	-3.59**	-0.10	0.22	-0.02
SE	**0.003**	**0.02**	**0.19**	**0.10**	**1.36**	**0.19**	**0.03**	**0.11**
Females								
KUOMBOKA	0.00	-0.09**	1.24***	0.62***	-3.96	-1.57	0.02	0.06
NL0C-C4	0.01	-0.19	1.25***	0.40*	-5.43*	-1.32***	0.32	-0.08
TUSO	0.01	0.05	0.91**	-0.05	0.43	2.29	-0.34	-0.51**
ZPMV 28402	0.02**	0.26	-1.09***	-0.16	6.12**	-1.82	0.11*	-1.00
KAUFELA	-0.03	-0.02	-2.31	-0.82	2.84	2.41	-0.11*	1.53
SE	**0.01**	**0.03**	**0.31**	**0.17**	**2.26**	**0.32**	**0.05**	**0.18**

KEY: **Se:** Grain Se concentration, **GY**: Grain yield, **DF:** Days to 50% flowering, **M:** Days to Maturity, **PH**: Plant height, **PL**: Panicle length, **NT**: No. of productive tillers/plant, **TSW**: 1000 seed weight, **SE**: Standard Error

*,**, *** significant at 0.05, 0.01 and 0.001 probability levels respectively

Table 24: SCA effects for 15 pearl millet crosses planted in evaluation experiment (Set III) during 2012/13 cropping season at UNZA

PROGENIES	Se ($\mu g\ g^{-1}$)	GY ($t\ ha^{-1}$)	DF (days)	M (days)	PH (cm)	PL (cm)	NT (No.)	TSW (g)
ICMV-IS 9031 x KUOMBOKA	0.00	-0.15	2.49**	0.98	1.56	1.00	0.22	0.72
ICMV-IS 9031 x NL_0C-C_4	0.01	-0.14	-1.18	-0.80	-13.88*	-2.17*	0.25	-0.25
ICMV-IS 9031 x TUSO	-0.02	0.13	1.82*	0.65	4.01	-0.61	-0.37*	0.98
ICMV-IS 9031 x ZPMV 28402	-0.02	0.12	-1.18	-0.24	-12.77	4.35***	-0.24	-1.03
ICMV-IS 9031 x KAUFELA	0.02	0.04	-1.96*	-0.58	21.09**	1.28	0.14	-0.43
ZPMV 24802 x KUOMBOKA	-0.02	0.20*	-1.31	-0.29	-4.24	0.82	-0.18	-0.10
ZPMV 24802 x NL_0C-C_4	0.01	-0.01	1.35	0.27	19.32**	-0.02	-0.16	-0.87
ZPMV 24802 x TUSO	-0.01	0.02	-0.64	-0.29	-3.04	2.46**	-0.08	-0.27
ZPMV 24802 x ZPMV 28402	0.03	-0.04	0.36	0.15	4.43	-1.10	0.33*	0.75
ZPMV 24802 x KAUFELA	-0.01	-0.18	0.24	0.16	-16.46*	-2.16*	0.09	0.49
OKOA x KUOMBOKA	0.02	-0.05	-1.17	-0.69	2.68	-1.82	-0.03	-0.62
OKOA x NL_0C-C_4	-0.02	0.14	-0.18	0.54	-5.43	2.18*	-0.09	1.12*
OKOA x TUSO	0.03	-0.15	-1.18	-0.36	-0.96	-1.84	0.45**	-0.72
OKOA x ZPMV 28402	-0.01	-0.09	0.82	0.09	8.34	0.60	-0.09	0.27
OKOA x KAUFELA	-0.01	0.15	1.71	0.42	-4.63	0.88	-0.24	-0.06
SE	**0.02**	**0.10**	**0.93**	**0.51**	**6.79**	**0.96**	**0.16**	**0.53**

KEY: **Se:** Grain Se concentration, **GY**: Grain yield, **DF:** Days to 50% flowering, **M:** Days to Maturity, **PH**: Plant height, **PL**: Panicle length, **NT**: No. of productive tillers/plant, **TSW**: 1000 seed weight; **SE: Standard Error**
*, **,*** significant at 0.05, 0.01, 0.001 probability levels respectively.

For panicle length, only NL_0C-C_4 showed significant but negative GCA effects as female source. ZPMV 28402 and KAUFELA, both as female source, had shown significant positive and negative GCA effects (0.11 and -0.11), respectively, for number of productive tillers per plant. For thousand seed weight, significant positive GCA effects (0.48) were observed only in ICMV-IS-9031 while highly significant but negative GCA effects (-0.51 and -0.46) were recorded in TUSO and ZPMV 24802 as male and female sources, respectively.

Results further showed that none of the crosses had significant SCA effects for Se accumulation and days to maturity. For grain yield, only one cross ZPMV 24802 x KUOMBOKA displayed significant ($P<0.05$) positive SCA effects (0.20). ICMV-IS-9031 x KAUFELA showed significant ($P<0.05$) negative SCA effects (-1.96) for days to 50% flowering while ICMV-IS-9031 x KUOMBOKA and ICMV-IS-9031 x TUSO recorded significant ($P<0.05$) positive SCA effects for the same trait.

Significant positive SCA effects (4.35, 2.46 and 2.18) for panicle length were observed in ICMV-IS-9031 x ZPMV 24802, ZPMV 24802 x TUSO and OKOA x NL_0C-C_4 while significant negative SCA effect (-2.17 and -2.16) were observed in ICMV-IS-9031 x NL_0C-C_4 and ZPMV 24802 x KAUFELA. OKOA x TUSO and ZPMV 24802 x ZPMV 28402 had significant positive SCA effects (1.12 and 0.33) for number of tillers while ICMV-IS-9031 x TUSO showed significant negative SCA effect (-0.37) for the same trait. For thousand seed weight only OKOA x NL_0C-C_4 (1.12) showed significant positive SCA effects among all crosses.

4.3.1 Determination of gene action conditioning Se accumulation in pearl millet

Estimates of additive and non-additive variance components were made for the Se accumulation trait in pearl millet crosses. The ANOVA for the three sets are shown in tables 10, 12 and 14 for Set I, Set II and Set III, respectively. Of importance to note is that ANOVA results show that only Set II (Table 12) showed significant differences among crosses for Se accumulation. Therefore, estimation of variance components and heritability was only focused on Set II where significant difference for Se accumulation manifested.

4.3.2 Proportion of GCA and SCA to total variance for Se accumulation and agronomic traits in pearl millet

Proportional contribution (%) of GCA and SCA to total variation and Baker's ratio for Se accumulation and some agronomic traits are presented in Table 25. GCA effects for male and female parental genotypes and their interaction (SCA) effects were highly significant ($P<0.01$) for Se accumulation and other traits except for number of tillers and thousand seed weight (Table 12). Results showed that SCA contributed 64% to total variance while GCA contributed 36%. Baker's ratio of 0.36 for Se accumulation indicated that SCA was more important than GCA in conditioning the Se accumulation trait. Results further indicated that GCA contributed more than SCA to total variance for grain yield, days to 50% flowering, days to maturity, plant height and panicle length (85%, 86%, 82%, 88% and 82%), respectively. This indicated that there was a preponderance of GCA over SCA in conferring those traits. Baker's ratio (0.85, 0.86, 0.82, 0.88, 0.82), indicated that GCA was more important than SCA in conferring these traits.

Table 25: Proportional contribution (%) of GCA and SCA variances to the total genetic variation for Se accumulation and agronomic traits in pearl millet crosses

Trait	Total GCA	SCA	Baker's ratio
Se accumulation (µg g^{-1})	36	64	0.36
Grain yield (t ha^{-1})	85	15	0.85
Flowering (days)	86	14	0.86
Maturity (days)	82	18	0.82
Plant height (cm)	88	12	0.88
Panicle length (cm)	82	18	0.82

Total GCA = sum of male and female GCA sum of squares

4.3.3 Variances and Heritability Estimates

The parameter estimates conditioning the Se accumulation trait in pearl millet crosses are presented in Table 26. Variation due to genotype ($\sigma^2_{G)}$ was high and contributed 72% of the total phenotypic variation (σ^2_P). Additive effects (σ^2_A) contributed 28% to total variation (σ^2_P) while dominance effects contributed 44% and environmental variation contributed 28% to total variation. The degree of dominance (1.6) indicated that there is over-dominance of genes in the selenium accumulation trait.

Narrow sense heritability (h^2) was found to be 0.28 for the Se accumulation trait (Table 26). It was found to be very low and, therefore, suggesting the recurrent selection method to be employed in the improvement of this trait.

Table 26: Estimates of variances and genetic parameters conditioning Se accumulation trait in pearl millet

Variance component	Se accumulation
σ^2_P	0.0018
σ^2_G	0.0013
σ^2_A	0.0005
σ^2_D	0.0008
σ^2_E	0.0005
σ^2_D/σ^2_A	1.6
h^2_{ns}	0.28

KEY: $\boldsymbol{\sigma^2_P}$ **=** Total variation, $\boldsymbol{\sigma^2_G}$ **=** Genotypic variation
$\boldsymbol{\sigma^2_A}$ = variance due to additive effects, $\boldsymbol{\sigma^2_D}$ = Variance due to dominance
$\boldsymbol{\sigma^2_E}$ = variance due to environment**,** $\boldsymbol{h^2_{ns}}$ = narrow sense heritability

CHAPTER FIVE

5.0 DISCUSSION

The importance of Se in human nutrition and health has been well documented (Schwarz and Foltz, 1957; Rayman, 2000; Lyons *et al.*, 2004) and provision of this nutrient element has been found to be best through crop biofortification. In order, therefore, to ensure Se intake among human populations in countries, where pearl millet [*Pennisetum glaucum* (L.) R. Br] is a dominant staple crop, the goal should be, development of pearl millet varieties that accumulate Se in good amounts. This study aimed at determining the genetics of Se accumulation for the purpose of developing pearl millet varieties that accumulate Se. In doing this, the evaluation of the magnitude of variation in the Se accumulation among the selected pearl millet genotypes and the determination of the gene action conditioning the Se accumulation trait were done.

Selenium analysis for the parental genotypes showed that pearl millet has the potential of accumulating Se and wide significant genetic variation in grain Se accumulation exists in pearl millet genotypes. The high coefficient of variation (CV =135%) from non-sprayed genotypes and CV =156% from sprayed genotypes (Table 2) indicated that there was a wide inherent genetic variability in Se accumulation among pearl millet genotypes used in this study. These findings are consistent with those of Rai *et al.* (2012) who found in their studies on genetic enhancement of grain iron (Fe) and zinc (Zn) in pearl millet that high levels and large variability for both Fe and Zn contents in pearl millet grains were present. This finding, therefore, indicated the potential of genetic enhancement for grain Se content in pearl millet as well.

Graham *et al.* (2001) also found substantial genotypic variation in cereals for Zn, Fe and vitamin A, which means that varieties high in these nutrients can be bred. In wheat, Lyons *et al*. (2005) found that genotypic variation in Se accumulation exists among wheat genotypes. Equally, Kopsell and Randle (2001) also found that variation in Se accumulation exists in rapid-cycling *Brassica oleracea* populations.

Graham *et al.* (2001) further stated that the existence of large genetic variation in grain micronutrients is essential for a successful breeding programme aimed at the development of new micronutrient-rich plant genotypes and progress in any crop improvement venture depends mainly on the magnitude of genetic variability in the source material. This, therefore, means that the Namibian and Zambian breeding programme on Se crop enrichment can benefit from genotypes used in this study.

The findings of this study indicated that the grain Se concentration among the non-sprayed genotypes ranged from 0.00 µg g^{-1} to 0.09 µg g^{-1} with the overall mean of 0.01 µg g^{-1} while the concentrations among sprayed genotypes ranged from 0.01 µg g^{-1} to 0.63 µg g^{-1} with the overall mean of 0.08 µg g^{-1}. Since little is known about Se accumulation in pearl millet, these findings could not be compared to any other findings. However, since pearl millet falls under non-accumulators (grains and grasses) which do not accumulate more than 50 mg Se kg^{-1} under field conditions, these findings could be regarded as moderate for most genotypes though few had high Se concentration. Therefore, considering that the Se requirements of humans are between 50 and 300 µg Se kg^{-1} (0.05 and 0.3 µg g^{-1}) dry matter, depending on physiological stage (Gissel-Nielsen *et al*., 1984), for people who consume pearl millet two times a day (200 g), the medium accumulators did not meet the daily dietary requirement for Se especially for the poorest of the poor who depend entirely on pearl millet as their sole source of Se.

Conversely, the results on natural Se accumulation through the roots might not reflect the true capability of the crop, because the availability of soil Se and its uptake by the plant depends upon various factors which include, among others, the soil reaction (pH), texture and redox potential of the soil, cation exchange capacity (Banuelos and Schrale, 1989), calcium carbonate levels, aluminium (Al) and iron (Fe) oxides levels, sulphur and phosphate levels in the soil (Oldfield, (1999).

The highest accumulating genotype before spraying was SDMV 59009 from SADC with 0.09 µg g^{-1} followed by LCIC 9702 from Zambia with 0.08 µg g^{-1} while after spraying the highest accumulator was NLC-C_3 from Zambia with 0.63 µg g^{-1} followed by SDMV 59009 with 0.36 µg g^{-1} (Table 6). This indicated that these genotypes have good genetic accumulating ability. However, further research should

be carried out in a different environment to ascertain whether similar results would be obtained.

Results further revealed that spraying the crop with 2 mg L^{-1} solution of Se (Na_2SeO_4) increased grain Se content by 6.19% on average. The paired t-test that was performed on this data showed that there was strong evidence (P=0.004) of the positive effect spraying Se on enhancing Se accumulation. This could be due to the fact that foliar spraying enables plants to accumulate Se and retain it, hence, this makes it a good screening strategy for plant Se accumulation. These results are consistent with findings reported by Curtin *et al.* (2006), who, in their study done on wheat, found that foliar Se applied at growth stage 31 produced the highest grain Se levels.

It was also observed that genotypes used in this study showed inconsistency in Se accumulation from the soil and through leaves. This means that genotypes with high remobilisation efficiency were not the ones with high ability to absorb and retain (AAR) Se. This implies that, the genotypes' Se accumulation ability from the soil before spraying cannot be used to predict the genotype's response to Se foliar application. However, this finding is in conflict with the results on correlation which showed a significant ($P<0.05$) positive correlation (r=0.37) between sprayed and non-sprayed treatments, demonstrating the relationship between the two treatments. Indicating that the high accumulators naturally, from the soil, were the high accumulators after spraying, but that was not the case in this study.

Though the focus of this study was on Se accumulation, important agronomic traits were also put into consideration during evaluation of crosses. This was done in order to understand the potential impact Se accumulation efforts might have on other important traits. Analysis of variance indicated that pearl millet crosses evaluated in this study performed differently for different traits, with some combining the high Se accumulation and good agronomic traits. The range for Se accumulation in set I was 0.19 – 0.62 μg g^{-1}; whereas for set II was 0.08 – 0.19 μg g^{-1} and set III was 0.16 – 0.27 μg g^{-1}. The overall highest accumulator was ZPMDC x NEC-C_3 with 0.62 μg g^{-1} from set I whereas the overall lowest accumulator was NLBC-C_3 x TARAM with 0.08 μg g^{-1} from set II.

Results showed that though ZPMDC x NEC-C_3 was the highest accumulator for Se, it was not the one that recorded high grain yield, it had 1.43 t ha^{-1} while the lowest accumulator NLBC-C_3 x TARAM had 1.50 t ha^{-1}, given the range for grain yield as from 0.69 – 2.63 t ha^{-1} in this study. On the other hand, GWAGWA x LCIC 9702 from set II which recorded highest grain yield of 2.63 t ha^{-1} , had 0.11 μg g^{-1} of Se while OKOA x KUOMBOKA from set III had the lowest grain yield of 0.69 t ha^{-1} and 0.21 μg g^{-1} of Se. This indicated that there seems to be a tendency that genotypes which had high Se concentration were not the ones which had high grain yield. Similar findings were also reported by Curtin *et al.* (2006), who stated that the yield of the crop could influence grain Se concentration through dilution effects, i.e. a high yielding crop may have lower grain Se than a crop with low yield. This could be the case in this study and might be the reason why grain yield showed a negative but non-significant correlation with Se concentration in Set I and II.

Results further demonstrated that crosses evaluated in this study showed greater preponderance for early flowering and maturity across all sets. Pearl millet usually flowers from 40 to 55 days (Rai *et al*., 2012). Days to flowering ratings across sets in this study ranged from 44 – 65 days while the days to maturity ranged from 86 – 98 days. Cross ICMV 155 Br x LCIC 9702, was the earliest, flowering at 44 days and matured at 86 days; whereas NLBC – C_3 x NEC-C_3 was the latest, flowered at 65 days and matured late at 98 days.

These results, therefore, showed that, despite the differences in days to flowering and maturity, the Se accumulation for these two crosses was similar, 0.12 μg g^{-1}. This finding introduces conflict as correlation results show that Se accumulation is significantly strongly positive correlated to days to flowering and negatively correlated to days to maturity. This could have been attributed to the fact that since foliar application was done during vegetative stage, there was no enough time for Se to be assimilated and partitioned to grains in the case of early flowering genotypes, resulting in low grain Se concentration. This could explain why most of the crosses had low Se in grains, because most of the genotypes used in this study flowered early.

The analysis of combining ability indicated significant ($P<0.05$) differences among males, females and male x female interaction. Of importance to note is that only crosses in set II showed significant differences for Se accumulation although individual genotypes and crosses showed significant GCA and SCA, respectively. This could have been due to the wide genotypic variation in Se accumulation for parents used in this set. Notable was that the lowest accumulator 570028 R1w parental genotype was one of the parents used in this set.

For Se accumulation, positive GCA effects are desirable for breeding for high Se accumulation levels. In this study, the highly significant ($P=0.01$) positive GCA effects (0.02) of parent ZPMV 28402 among all parents suggest that this parent is best general combiner and can be used as a source of Se accumulation trait in hybrid combinations. On the other hand, parents KUOMBOKA and NLBC-C_3 had highly significant ($P<0.01$) negative GCA effects (-0.05, -0.02), respectively, suggesting that they had a stable contribution towards low Se accumulation in crosses from the set where they were used as parents. Therefore, crosses involving these three parents could be suitable for mapping Se accumulation quantitative trait loci (QTL) in pearl millet.

Positive SCA effects are also desirable for Se accumulation. The cross SOSANK x NL_0C-C_4 had the highest significant positive SCA effects (0.16), followed by ZPMV 24801 x KUOMBOKA (0.15) and SEPO x OKASHANA 1 (0.15). These particular crosses were regarded as good specific combiners and their inclusion in breeding programmes would be useful for Se accumulation trait improvement. Crosses which combined Se accumulation with grain yield, earliness and reduced plant height were 570028 R1w x NEC-C_3, SOSANK x LAGRAP and SEPO x OKASHANA 1. All these can be used in the breeding program for Se enrichment without compromising on the yield and earliness.

On the inheritance of the selenium accumulation trait, results showed significantly higher SCA (64%) than GCA (36%) contribution to total variation in selenium accumulation (Table 25) indicated that non-additive effects were more important than additive effects in conditioning the Se accumulation trait. The variance components using Baker's ratio (0.36) further supported that non-additive effects

were more important in conditioning the Se accumulation trait. On the other hand, results showed preponderance of GCA over SCA in conferring grain yield, days to 50% flowering, days to maturity, plant height and panicle length traits. Sprague and Tatum (1942) reported that SCA effects are due to non-additive genetic portion while GCA effects are due to additive gene action. The SCA effects are considered a reliable index for the identification of superior hybrids. This implies that improvement of this trait can be done through individual hybrid selection. This finding is in contrast with earlier studies done on pearl millet by Velu *et al*. (2011b) and in rice by Zhang *et al*. (2004), which reported largely additive gene action for Fe and Zn micronutrients. This indicates that improvement of this trait can be done using specific hybrid selection.

Narrow sense heritability (h^2_{ns}) of 0.28 for Se accumulation trait was found to be low according to Hallauer and Miranda (1981), who reported that the heritability estimates of > 70 % is considered very high; 50-70 % high; 30-50 % moderate and < 30 % low. This indicates that improvement of this trait can be done through recurrent selection method. The production of pearl millet hybrids will be a solution so that we take advantage of hybrid vigour and heterosis. Kopsell and Randle (2001) also found out that narrow-sense heritability estimates for Se accumulation in a rapid-cycling *Brassica oleracea* L. population were moderate (0.55) and gains from selection were successful. McQuinn *et al.* (1991) reported that progress from selection for Se content in tall fescue was possible and that the Se accumulation trait is heritable. Narrow sense heritability expresses the extent to which phenotypes are determined by the gene transmitted from the parents (Falconer and Mackay, 1996). Knowledge of heritability indicates to the breeder the possibility to which genetic improvement is possible through selection.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

The study was set out to evaluate the potential of selenium (Se) accumulation in pearl millet genotypes and assess the magnitude of genetic variation for Se accumulation, the key factor in crop improvement. Thirty seven pearl millet genotypes were analysed for Se, hybridised and crosses evaluated for Se accumulation, agronomic performances and the gene action and heritability (key factors in plant breeding) conferring the Se accumulation trait was studied.

The study established the presence of adequate genotypic variation among pearl millet genotypes on the Se accumulation. This suggests that it is feasible to develop appropriate pearl millet varieties which could accumulate high Se in the grains. Such varieties could contribute to the positive implications for ensuring adequate Se intake for improved nutrition through a pearl millet diet if adopted by more farmers.

Genotypes NLC-C_3 (0.63 µg g^{-1}) and SDMV 59009 (0.36 µg g^{-1}) had high grain Se concentration and were considered high accumulators. These two genotypes (NLC-C_3 and SDMV 59009) are available in the Southern Africa Development Community (SADC) Gene Bank and could be considered for selection as parental material to be used in breeding programme aimed at developing appropriate varieties for higher Se accumulation. The genotype 570028 R1w (0.01 µg g^{-1}) is a poor accumulator since it failed to uptake any Se from the soil when no foliar application of Se was done and even with foliar spraying of Se it had the lowest grain Se. Results further revealed that foliar application with Se in the form of sodium selenate at 2 mg L^{-1} solution was found to increase grain Se content.

In addition, on the classification of crosses based on Se accumulation, it was concluded that crosses used in this study were generally medium Se accumulators and only few manifested the ability of being higher Se accumulators. ZPMDC x NEC-C_3 (0.62 µg g^{-1}) emerged out as the overall highest Se accumulator cross in this study.

Those two parents can be considered in hybrid development for the improvement of the Se accumulation trait in pearl millet. The non-significant positive and negative correlation between Se concentration and grain yield obtained in all set could have been due to the suspected dilution effects.

Results revealed that the Se accumulation trait is conditioned by non-additive gene action type, therefore, improvement of this trait can be done through selection of individual hybrids or specific combiners. The parent ZPMV 28402 was the only one that had significant positive GCA effects for Se accumulation and can be used as a source of Se accumulation trait, without compromising on important agronomic traits. On the other hand, KUOMBOKA and NLBC-C_3 had significant negative GCA effects for the same trait, suggesting that crosses involving these three parents could be suitable for mapping Se accumulation quantitative trait loci (QTL) in pearl millet.

The cross 570028 R1w x NEC-C_3 registered desirable SCA effects for Se accumulation and other four important economic traits viz, grain yield, days to 50% flowering, days to maturity and plant height. Other crosses SOSANK x LAGRAP and SEPO x OKASHANA 1 recorded significantly SCA effects for Se accumulation and one important trait. These were also identified as superior crosses and can be effectively employed in hybrid breeding programmes, hence suggesting that their full utilisation could lead to the improvement of the Se accumulation trait in pearl millet. The heritability of the Se accumulation trait was found to be low and this would imply that the trait can be improved through recurrent selection method. Overall, it can be concluded that it is feasible to breed for Se accumulation in pearl millet using the genotypes used in the study.

It is recommended that, since this was a once off study, further experiments should be carried out in different environments, in order to ascertain whether the same Se accumulation results would be obtained. In addition, further research is required in order to confirm on the findings of the dilution effects between grain yield and grain Se concentration, in order to caution plant breeders in their selection of breeding materials.

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APPENDICES

SET I

Appendix I: ANOVA for Grain Se Concentration

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	38.1184	19.0592	35.57	
Male	3	0.366	0.122	0.23	0.877
Female	5	6.0189	1.2038	2.25	0.065
Male x Female	15	11.3614	0.7574	1.41	0.181
Residual	46	24.6497	0.5359		
Total	71	80.5144			

Appendix II: ANOVA for Grain Yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	1.0407	0.5203	1.96	
Male	3	0.1205	0.0402	0.15	0.929
Female	5	1.9613	0.3923	1.47	0.217
Male x Female	15	2.657	0.1771	0.67	0.803
Residual	46	12.2389	0.2661		
Total	71	18.0184			

Appendix III: ANOVA for Days to 50% Flowering

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	190.86	95.43	4.72	
Male	3	194.26	64.75	3.2	0.032
Female	5	853.74	170.75	8.44	<.001
Male x Female	15	381.99	25.47	1.26	0.266
Residual	46	930.47	20.23		
Total	71	2551.32			

Appendix IV: ANOVA for Days to Maturity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	100.333	50.167	9.03	
Male	3	24.153	8.051	1.45	0.241
Female	5	208.458	41.692	7.5	<.001
Male x Female	15	80.264	5.351	0.96	0.507
Residual	46	255.667	5.558		
Total	71	668.875			

Appendix V: ANOVA for Plant Height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	1067.1	533.6	2.17	
Male	3	472.6	157.5	0.64	0.593
Female	5	4084.2	816.8	3.32	0.012
Male x Female	15	3866.2	257.7	1.05	0.427
Residual	46	11309.2	245.9		
Total	71	20799.2			

Appendix VI: ANOVA for Panicle Length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	2.724	1.362	0.3	
Male	3	17.051	5.684	1.24	0.305
Female	5	183.404	36.681	8.03	<.001
Male x Female	15	272.287	18.152	3.97	<.001
Residual	46	210.151	4.569		
Total	71	685.617			

Appendix VII: ANOVA for Number of Productive Tillers per Plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	2.9777	1.4889	4.5	
Male	3	1.6709	0.557	1.68	0.183
Female	5	5.6358	1.1272	3.41	0.011
Male x Female	15	3.6828	0.2455	0.74	0.73
Residual	46	15.2115	0.3307		
Total	71	29.1788			

Appendix VIII: ANOVA for Thousand Seed Weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	4.412	2.206	0.71	
Female	5	134.444	26.889	8.64	<.001
Male	3	10.405	3.468	1.11	0.353
Female x Male	15	36.798	2.453	0.79	0.684
Residual	46	143.188	3.113		
Total	71	329.247			

SET II

Appendix IX: ANOVA for Grain Se Concentration

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP	2	0.0003036	0.0001518	0.28	
Female	2	0.0001009	0.0000504	0.09	0.912
Male	4	0.0133005	0.0033251	6.06	0.001
Female x Male	8	0.0143773	0.0017972	3.28	0.009
Residual	28	0.0153605	0.0005486		
Total	44	0.0434427			

Appendix X: ANOVA for Grain Yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP	2	1.1378	0.5689	6.02	
Female	2	1.10876	0.55438	5.86	0.007
Male	4	3.56385	0.89096	9.42	<.001
Female x Male	8	1.59962	0.19995	2.11	0.068
Residual	28	2.64748	0.09455		
Total	44	10.05752			

Appendix XI: ANOVA for Days to 50% Flowering

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP	2	124.98	62.49	3.62	
Female	2	438.71	219.36	12.72	<.001
Male	4	472.58	118.14	6.85	<.001
Female x Male	8	350.62	43.83	2.54	0.032
Residual	28	483.02	17.25		
Total	44	1869.91			

Appendix XII: ANOVA for Days to Maturity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP	2	21.511	10.756	3.08	
Female	2	123.511	61.756	17.68	<.001
Male	4	124.533	31.133	8.91	<.001
Female x Male	8	129.6	16.2	4.64	0.001
Residual	28	97.822	3.494		
Total	44	496.978			

Appendix XIII: ANOVA for Plant Height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP	2	3593.4	1796.7	11.62	
Female	2	3277.4	1638.7	10.6	<.001
Male	4	5776.2	1444.1	9.34	<.001
Female x Male	8	2923.7	365.5	2.36	0.044
Residual	28	4329.8	154.6		
Total	44	19900.5			

Appendix XIV: ANOVA for Panicle Length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP	2	18.919	9.46	2.05	
Female	2	93.344	46.672	10.13	<.001
Male	4	177.731	44.433	9.64	<.001
Female x Male	8	124.086	15.511	3.37	0.008
Residual	28	128.997	4.607		
Total	44	543.078			

Appendix XV: ANOVA for Number of Tillers per Plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP	2	0.5906	0.2953	0.86	
Female	2	0.3441	0.172	0.5	0.611
Male	4	0.9303	0.2326	0.68	0.614
Female x Male	8	2.6736	0.3342	0.97	0.477
Residual	28	9.6215	0.3436		
Total	44	14.16			

Appendix XVI: ANOVA for Thousand Seed Weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP	2	6.521	3.261	0.79	
Female	2	5.721	2.861	0.69	0.508
Male	4	6.03	1.508	0.37	0.831
Female x Male	8	28.37	3.546	0.86	0.56
Residual	28	115.365	4.12		
Total	44	162.008			

SET III

Appendix XVII: ANOVA for Grain Se Concentration

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP	2	0.001421	0.000711	0.32	
Female	4	0.009859	0.002465	1.11	0.373
Male	2	0.010209	0.005105	2.29	0.12
Female x Male	8	0.014663	0.001833	0.82	0.59
Residual	28	0.062383	0.002228		
Total	44	0.098535			

Appendix XVIII: ANOVA for Grain Yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP	2	1.57461	0.78731	8.37	
Female	4	1.03724	0.25931	2.76	0.047
Male	2	1.26485	0.63243	6.72	0.004
Female x Male	8	0.68862	0.08608	0.91	0.519
Residual	28	2.63417	0.09408		
Total	44	7.1995			

Appendix XIX: ANOVA for Days to 50% Flowering

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	68.133	34.067	4.41	
Female	4	94.089	23.522	3.04	0.034
Male	2	60.4	30.2	3.91	0.032
Female x Male	8	80.044	10.006	1.29	0.287
Residual	28	216.533	7.733		
Total	44	519.2			

Appendix XX: ANOVA for Days to Maturity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP	2	25.733	12.867	5.55	
Female	4	11.244	2.811	1.21	0.328
Male	2	3.6	1.8	0.78	0.470
Female x Male	8	11.289	1.411	0.61	0.763
Residual	28	64.933	2.319		
Total	44	116.8			

Appendix XXI: ANOVA for Plant Height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP	2	1740	870	2.09	
Female	4	818.7	204.7	0.49	0.741
Male	2	1568	784	1.89	0.170
Female x Male	8	4916.1	614.5	1.48	0.209
Residual	28	11628.3	415.3		
Total	44	20671.2			

Appendix XXII: ANOVA for Panicle Length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP	2	8.233	4.117	0.49	
Female	4	166.856	41.714	5.01	0.004
Male	2	50.925	25.463	3.06	0.063
Female x Male	8	99.311	12.414	1.49	0.206
Residual	28	233.35	8.334		
Total	44	558.675			

Appendix XXIII: ANOVA for Number of Tillers per Plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP	2	0.3152	0.1576	0.65	
Female	4	2.2269	0.5567	2.3	0.083
Male	2	1.1573	0.5786	2.39	0.110
Female x Male	8	2.3809	0.2976	1.23	0.318
Residual	28	6.7689	0.2417		
Total	44	12.8493			

Appendix XXIV: ANOVA for thousand seed weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP	2	8.779	4.39	1.72	
Female	4	32.593	8.148	3.19	0.028
Male	2	6.728	3.364	1.32	0.284
Female x Male	8	19.929	2.491	0.97	0.476
Residual	28	71.614	2.558		
Total	44	139.644			