IDENTIFICATION OF RICE (*Oryza* spp.) LANDRACES WITH NITROGEN USE EFFICIENCY IN GHANA

BY

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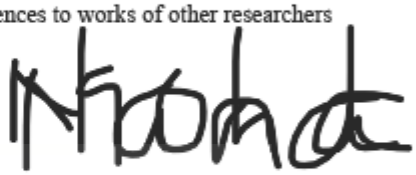
DECLARATION

I hereby declare that this thesis is unique, and that no part of this thesis has been presented for another degree in this University or elsewhere except for references to works of other researchers which have duly been acknowledged.



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DEDICATION

I dedicate this work to Almighty God for granting mankind a beautiful environment full of diversity, the bedrock of an extraordinary world; who makes all things possible and beautiful in His time.

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ABSTRACT

Sustainability in rice cultivation requires increasing yield while protecting the environment against pollution from the over-use of fertilizers through the utilization of varieties that are nitrogen use efficient. Hence, the goals of the study were to: 1) characterize the assembled landraces of rice 2) describe the growth response of rice landraces under different nitrogen levels 3) assess the relationship between yield related traits and NUE components and 4) evaluate the extent of genotypic variations of NUE components and yield related traits among the landraces. Two experiments were conducted in pots and on the field which involved 20 rice landraces tested under two nitrogen levels; (no nitrogen fertilizer application) and low nitrogen (i.e. 50 kg/ha of nitrogen fertilizer). The experiments were conducted in the University of Ghana farm from February 2019 to June 2019. The experimental design for the pot experiment was completely randomized design in a factorial arrangement with three replicates. A split plot design was used for the field experiment with three replicates. Average diversity index was 0.70 and 0.33 for quantitative and qualitative traits respectively. Five clusters were created at a similarity index of 67 % among the landraces. Principal component analysis showed four independent principal components accounted for 71.9 % of the total variation. Root dry weight, leaf length, leaf width, shoot dry weight, number of leaves, plant height, chlorophyll content, culm number and grain yield significantly increased at 50 kg/ha of nitrogen (N) fertilizer. Root length increased by 8.79 % in the absence of N fertilizer. There was a reduction in yield related traits under 0 kg/ha of nitrogen fertilizer compared to 50 kg/ha of nitrogen fertilizer. A significant increase in NUE by 31.72% and 5.73 % in pot and field experiments respectively was observed under no N conditions compared to low N. NUE correlated significantly with filled spikelet, grain yield, panicle length, 1000 - grain weight and Nitrogen Uptake efficiency (NUtE). Genotypic coefficient of variation was lower than

its corresponding estimates for phenotypic coefficient of variation in all yield related traits and NUE. It can be concluded that GH1550, GH1801, GH1822 and GH2145 were nitrogen efficient and may be used for cultivation and/or used in future breeding programmes to decipher loci involved in NUE for developing superior varieties.

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LIST OF ABBREVIATIONS

%	Percentage
€	Euros
ADP	Adenosine triphospahate
ANR	Apparent nitrogen recovery rate
Asn	Asparagine
ATP	Adenosine triphospahate
AUE	Agronomic use efficiency
BCE	Before the Common/Current/Christian era
CD	Culm diameter
CE	Common Era
CL	Culm length
cm	centimeters
CN	Culm number
CPHY	Chlorophyll
EC	Electrical conductivity
FLL	Flag leaf length
FLW	Flag leaf width
g	grams
GDH	Glutamate dehydrogenase
GDP	Gross domestic profit
Gln	Glutamate dehydrogenase
GNC	Grain nitrogen concentration

GOGAT	Glutamine -2-oxoglutarate aminotransferase
GY	Grain yield
H	Heritability
H´	Shannon-Weaver diversity index
HGW	100-grain weight
Kg/ha	Kilogram per hectare
LL	Ligule length
LN	Low nitrogen
LW	Leaf width
m^3	meters cube
mg/l	milligram per liter
mm	millimeters
MMT	Million metric tons
MoFA	Ministry of Food and Agriculture
N	Nitrogen
N	North
NADH	Nicotinamide adenine dinucleotide
NDM	Number of days to maturity
NDMH	Number of days to main heading
NL	Number of leaves
NR	Nirate reductase
NT	Number of tillers
NUE	Nitrogen Use Efficiency

NUEg	Nitrogen use efficiency in grain
NUpE	Nitrogen Uptake Efficiency
NUtE	Nitrogen Utilization Efficiency
^{o}C	Degree celcius
^{o}F	Farenhaeit
PL	Panicle length
PLTH	Plant height
PN	Panicle number
RL	Root length
RW	Root weight
SNC	Straw nitrogen concentration
SW	Straw weight
US$	United States Dollars
USDA	United Sates Department of Agriculture
W	West

CHAPTER ONE

1 INTRODUCTION

Nitrogen (N) is an element and a key driver in living systems and in agriculture because all important processes in plants are associated with the activities of proteins which have this element as a major building block. In addition, it aids the absorption and utilization of other nutrients such as potassium and phosphorus and regulates the growth of plants which ultimately increases crop yields (Leghari *et al*., 2016). Although 78 % of the atmosphere constitutes nitrogen, plants are unable to utilize it unless it is converted to ammonium or nitrate (Leghari *et al*., 2016). Naturally, N can be fixed by lightening or biological nitrogen fixing organisms such as cyanobacteria and diazotropic bacteria but contributes only a fraction of N necessary for the cultivation of plants (Choudhury & Kennedy, 2005; Miller & Cramer, 2005).

Thus, to meet with the pace of population increase and challenges faced with malnutrition and food production especially in developing countries, after World War II, the term Green Revolution was born which led to the adoption of industrial fertilizers (Hirel *et al*., 2011). This was a brilliant initiative as high input varieties were bred which required colossal amount of synthetic fertilizer for increased crop yield. Though this innovation solved the issues of food security, consequently, it led to environmental pollution (Erisman *et al*., 2008). This became a serious challenge especially for Africa where millions of people still live in abject poverty and are malnourished. More so, some areas are prone to lack of clean drinking water, which is buttressed by the fact that N leaches easily into water bodies (Pan Africa Chemistry Network –PACN, 2012). Balancing agricultural yield and maintaining environmental health especially in paddy fields that require high doses of fertilizer is challenging (Sharma & Bali, 2017). This is because, urea is the most popular nitrogen

fertilizer source for rice but its efficiency in rice production is meager (around 30-40%), in some cases even lesser. Volatilization, denitrification, leaching and runoff of N fertilizer has led to a decline in N use efficiency (Choudhury & Kennedy, 2005). Conversely, excessive use of nitrogen does not even boost grain yield rather it leads to worsening climate change, biodiversity loss, environmental and health concerns and also increases prevalence of foliar pathogens and plant lodging (Samonte *et al*., 2006; Hirel *et al*., 2011).

Rice, being rich in nutrients and contains a number of vitamins and minerals, is an excellent source of complex carbohydrates, one of the best source of energy making rice to occupy an enviable position among the cereal crops globally (Olembo *et al*., 2010), but the afore mentioned challenges limits its production and would hinder attaining food security if not addressed especially when the populace is envisioned to hit 9 billion by 2050 (Selvaraj *et al*., 2017). More so, the dilemma is not in the ability to cater for 9 billion people in 2050, but if it can be done sustainably, equitably and on time in the face of challenges such as the changing climatic conditions and poor soil fertility (Spiertz, 2010). For example, in the 1960s, cultivation of crops was carried out on a hectare of land which was able to feed two people without experiencing cost and pollution, by 2050, the same nutrient depleted soil will be required to feed more than triple the number of persons fed on a hectare. This will stir up farmers over applying fertilizer to increase yield especially for countries that import rice to meet up with the demand (Pan Africa Chemistry Network –PACN, 2012) which is already the current trend in today's agriculture especially for Africa. For instance, Ghana has not yet reached self-sufficiency in terms of rice production because billions of dollars is spent on importation of rice annually, which is a tremendous loss to foreign exchange (Angelucci *et al*., 2013). The major limiting factor faced by Ghanaian farmers is the high levels of N deficiency in

paddy fields. Farmers thus have to apply vast quantities of fertilizer which is not very much available and affordable as they are not even manufactured in Ghana (Fianko *et al*., 2011).

Application of fertilizers in high doses does not guarantee high yield because a critical analyses of the consumption of synthetic fertilizer globally indicate that for the past 4 decades, the amount of fertilizer agricultural crops have utilized is 7.4 fold as against the yield of 2.4 fold. This implies that nitrogen use efficiency (NUE) has declined (Hirel *et al*., 2011). The decline in NUE can be as a result of deficiency in two main NUE mechanisms: the absorption of N, also referred to as nitrogen uptake efficiency (NUpE) and the assimilation of the absorbed N necessary for grain production termed N utilization efficiency (NUtE) (Han *et al*., 2015). Thus, to improve NUE, several studies have been conducted through agronomic management (Yadav *et al*., 2017; Dubois *et al*., 2017). For the purposes of overcoming setbacks associated with agronomic management in terms of nitrogen fertilization, breeding rice varieties that are less dependent on the heavy application of N fertilizers and responsive to limited N fertilizers is essential. Varieties able to utilize limited nitrogen fertilizer with high grain yield would contribute towards the goal of achieving long term sustainable production system.

Sustainability in agriculture implies that improving resource-use efficiencies should center on higher yield with limited N fertilizer. It is based on the theory that the necessities of contemporary times are met without negotiating that of the future (Spiertz, 2010). Thus, improved NUE is a fundamental segment of a sustainable agriculture that fulfills human necessities, reduce production costs and safeguards biological diversity including the environment and health of the people.

Prior to breeding for NUE rice genotypes, it is important to identify genotypes with NUE through recognizing plant phenotypes that correlate with high yield as well as high NUE to enable breeders utilize these traits in breeding programmes (Naveen *et al*., 2016). To identify nitrogen efficient genotypes, existing hybrids might not be the best genetic material because they are mostly bred for high usage of synthetic fertilizer, (Ali *et al*., 2018). In view of this, utilizing landraces in terms of identifying NUE lines is very useful. This is due to their past evolutionary history as they are better adapted to environmental stress under low input conditions and therefore constitute a unique germplasm for ascertaining NUE lines (Ali *et al*., 2018).

Though NUE lines have been identified in various rice genotypes in Asia and some parts of Africa (Fageria *et al*., 2010; Segda *et al*., 2014; Rao *et al*., 2014; Lakew, 2015; Rao *et al*., 2018), there is a dearth of information on the identification of NUE lines in rice germplasm collections in West Africa such as Ghana (Segda *et al*., 2014). Thus, identification of NUE lines in Ghanaian rice landraces will aid in their usefulness as plant genetic resources.

Hence, the objectives of the study were to:

1. characterize rice landraces for morphological traits;
2. determine growth of the landraces as affected by different levels of nitrogen;
3. identify NUE lines and assess the relationship between NUE components and yield related traits;
4. assess genotypic variations of NUE components and yield related traits among the landraces.

CHAPTER TWO

2 LITERATURE REVIEW

2.1 Taxonomy, geographic origin, Botany, growth and life cycle of the rice plant

2.1.1 Taxonomy of the rice plant

Rice has been classified to the family, Gramineae or Poaceae and genus, *Oryza*. The rice genus (*Oryza*) is made up of about 25 annual and perennial grass species distributed across diverse climatic zones in Africa, Asia, Australia and Southern America. It may be typically considered as an annual grass adapted to both temperate and tropical climates under a variety of water regimes such as lowland and upland conditions. Two out of these rice species are cultivated, namely; *Oryza sativa* and *Oryza glaberrima*, the remaining are undomesticated (Global Rice Science Partnership, 2013).

2.1.2 Geographic origin

Rice history dates back to over a century ago when the seven continents, which were originally merged, progressively started drifting apart. Since then, rice has been growing everywhere apart from Antarctica (Shamar, 1991). Interestingly, rice in Latin is, "*Oryza*" in English it is "Rice" and these names have been coined out from the ancient Tamil word "Arisi". Merchants from Arabia procured arisi and named it 'Al-ruz" in Arabic. It is also called "Arroz", "Oriza", "Rizo", "Riz" and "Reis" in Spanish, Greek, Italian, French and in German respectively (Shamar, 1991).

Historically, Alexander the Great (400 BCE) was the first to describe rice in Africa. He imported rice into Egypt from India which led to the beginning of rice farming in 639 CE. The Greek philosopher and historian, Strabo, earlier noticed the presence of rice in Cyrinaica (Libya) around

12 CE (Nayar, 2012). The first description of rice farming in West Africa along the Niger River was by the Islamic scholar, Al-Bakri. Ibn Batuta, a well-known Moroccan traveler also gave account of the abundance of rice in the Inland Niger Delta. In the early 15th century, several early travelers also noticed the cultivation of rice in West Africa, before the arrival of the first Europeans (Nayar, 2012).

The main center of diversification of the African rice (*O. glaberrima*) is the marshy basin of the upper Niger river in West Africa, apparently created around 3000 BC (Linares, 2002). Poteres (1970, as stated by Sweeney and McCouch, 2007) re-counted that *O. glaberrima* was originally planted in flood waters. Rice cultivation later extended to the saline waters using non-floating cultivars and later cultivars were chosen and planted on upland fields which were watered by rainfall. Two secondary centers were created 5 centuries later in the South West close to the Guinean Coast. The initial account was on the coast of Gambia, Casamance and Guinea Bissau, followed by that of Guinea forest amid Sierra Leone and Western Ivory coast about 100 BC ago (Agnoun *et al*., 2012). The major rice growing countries outside West Africa are Congo DR, Egypt, Madagascar, Mozambique and Tanzania (Nayar, 2012).

Until the 1920s, most of the rice was produced in the Volta and Western regions of Ghana by traditional farmers. It was not until the 1960s that rice became an important crop in Ghana and now, the bulk of Ghana's rice comes from the Northern region of the country. In the Volta region, rice cultivation is frequently carried out by women while the men mostly cultivate coffee, cocoa and rubber. These rice cultivars have been passed on from generations to generations (Kranjac-

Berisavljevic *et al*., 2003). *O. glaberrima* is usually cultivated in upland conditions while some *O. glaberrima* varieties and *O. sativa* varieties are cultivated under lowland conditions (Kranjac-Berisavljevic *et al*., 2003) .

2.1.3 Botany of the rice plant

2.1.3.1 Root

The rice plant has a relatively shallow and dense root system as compared to other upland cereal crops like maize and wheat probably because it grows under flooded conditions (Morita & Nemoto, 1995). Its root architecture is in two main categories; crown roots and nodal roots which sprout from the nodes. The nodes beneath the soil surface is where the crown roots grow from while the nodal roots are formed from the nodes above the soil surface at a water level of above 80 cm deep. In water-logged soils, rice roots occasionally surpass a depth of 40 cm because inadequate oxygen diffuses through the aerenchyma cells of roots to sustain the developing root tips (Maclean *et al*., 2002).

2.1.3.2 Culm

The culm, which is also called a group of linked stems of rice consists of the nodes and internodes. As the rice plant develops, tillers sprout from the bottom of the main culm and these further gives rise to other tillers called the ancillary tillers. The ancillary tillers further gives rise to more tillers (Chang, 1965).

2.1.3.3 Leaves

The leaves bear the sheath and blade and can be found on the culm in dual positions, a single leaf at each node. Connected to the sheath is the blade which encircles the jointed stem above the node in different sizes, shape and rigidity (Chang, 1965). The blades are usually attached at the base of the culm and are uniform. Rice varieties differ in blade size, girth, area, form, color, position and presence/absence of hair. The leaf below the panicle is called the flag leaf which differs in form, size and position. (Chang, 1965).

Where the blade and the sheath intersect is a pair of claw-like attachments, known as the auricles. Rough hairs shields the external part of the auricles (Maclean *et al*., 2002). In the middle of the leaf sheath and the blade is a membranous, glabrous or ciliate ligule. It varies in length, color and form among varieties (Chang, 1965). The intersection between the sheath and blade is known as the collar or juncture. The collar appears as a raised region found at the back of the leaf (Chang, 1965).

2.1.3.4 Reproductive organs

The reproductive structures of rice are modified shoots. They consist of the panicle, spikelet and the flowers.

2.1.3.4.1 Panicle

The culm bears the panicle which are arranged in a racemose pattern on the topmost internodes. Around the panicle axle is the inflorescence which extends from the bottom of the panicle to its apex. The nodes on the panicle develops into a main twig which gives rise to ancillary twigs (Chang, 1965).

2.1.3.4.2 Spikelet

The spikelet bears the pedicel which is a small stalk that is an extension of the panicle axle and the main or ancillary twig. At the upper end of the pedicel are two short rudimentary glumes: a duo of sterile lemmas and rachilla positioned amid the rudimentary glume and the spikelet (Yoshida, 1981). The flower is surrounded by the lemma and palea, which may perhaps be either awned or awnless (a fibrous bristle present in some cultivars, formed as an extension of the midrib of the lemma) (Yoshida, 1981).

2.1.3.4.3 Flower

The flower consists of six pollen-bearing organs which contains 2-celled anthers found on the filaments while the pistil bears a single ovule. At the base of the flower lies two variegated, pristine, plump-like appendages attached to the palea (Chang, 1965). The rice fruit is also known as the caryopsis. It has only one seed joined to the edge of the mature ovary (pericarp). The grain is the ripened ovary with the lemma, palea, rachilla, sterile lemmas and perhaps the awn. The husk or hull bears the lemma, palea, sterile lemmas, rachilla and the awn (Chang, 1965).

2.1.4 Growth and development of the rice plant

The life cycle of rice spans from 80 to over 200 days (depending on the species) (Verhey, 2010). There are three agronomic phases associated with the growth and development of rice, namely: vegetative phase, reproductive phase and ripening phase.

2.1.4.1 Vegetative phase

The seed imbibes water and becomes flexible. A sheath-like structure covering the radicle (coleorhiza) protrudes out of the seed coat, enabling the radicle to break forth from the coleorhiza and firmly attaches itself in the soil. This takes place within two days when temperatures ranges from 70 to 90 ^{o}F / 21 to 32 ^{o}C (Moldenhauer & Slaton, 2013). Germination will require longer time if temperatures are higher or lower than the above range. It involves rapid vegetative growth such as proliferation of tillers, boost in plant height and leaf development at steady rates. The main leaf acts as a shield for the emergence of new leaves. As the seedling grows, the emerging leaves protrudes and differentiates into three different fragments; the sheath, collar and blade (Olembo *et al*., 2010). Pre-tillering phase occurs during the growth of the main and ancillary leaves while tillering takes place when a sprout emerges from the crown just beneath the soil or the axle of the leaf base. This takes place three weeks after emergence (Verhey, 2010). Prior to the inception of the reproductive phase, the formation of tillers declines which is termed the vegetative lag phase. Subsequently, as the number of tillers reduces, plant height and stem girth progressively increases (Verhey, 2010).

2.1.4.2 Reproductive phase

Before the reproductive phase commences, there is a reduction in the formation of tillers, culm begins to increase in length, booting is observed, the flag leaf appears, heading takes place and finally flowering begins (Moldenhauer & Slaton,2013). This stage differs based on cultivar and weather conditions. The opening and closing of the spikelet is peculiar to the flowering/ anthesis stage for the purposes of pollination and may last for an hour or two, after which fertilization occurs within 6 hours (Moldenhauer & Slaton, 2013).

2.1.4.3 Ripening phase

According to Olembo *et al*. (2010), fertilization precedes ripening and may be divided into three phases, viz:

1) Milk stage – The starch in the kernels begin to form and a whitish fluid can be found in the center of the kernel.
2) Soft dough stage – The starch in the grain begins to thicken.
3) Hard dough stage – The spikelet becomes hard as it matures with 20 to 22 % moisture content. Ripening is attributed to leaf senescence and grain growth (Maclean *et al*., 2002).

2.2 Rice production systems in sub-Saharan Africa

Rice is a semi-aquatic grass and grows in varied soil types and water regimes (Verhey, 2010). It is also described by its plasticity which aids its growth in different environments of West and Central Africa (Defoer *et al*., 2004). Rice is cultivated in various agro-ecological zones from humid forests

to Sahel within which five major rice ecologies are noted based on the availability of water and topography in sub-Saharan Africa (Dofoer *et al*., 2004) viz:

1) Rainfed upland rice on plateaus and slopes;
2) Lowland rainfed rice in valleys and flood plains with varying degrees of water control;
3) Irrigated rice with relatively good water control in deltas and flood plains;
4) Deep water floating rice along river beds/ banks;
5) Mangrove swamp rice in lagoons and deltas in coastal areas.

Three out of these are the chief ecologies in West and Central Africa. They are: rainfed uplands, rainfed lowlands and irrigated systems (Dofoer *et al*., 2004).

1) Rainfed upland rice on plateaus and slopes - It covers the largest area of about 44 % of which the coastal areas in the humid and sub-humid agro-ecological zone constitute the largest part. Main water supply is rainfall but the water recedes after sometime (Dofoer *et al*., 2004). This ecology is characterized by unpredictable rainfall patterns, hence, early maturing and drought tolerant varieties adapt to this ecology. (MoFA, 2009). Rice varieties adapted to this ecology are grown in soils that have greater percentage of sand. Few areas within West Africa, experience two rice growing seasons within a year due to the bimodal rainy season. On the other hand,with an estimated rainfall of 1600 mm a year, ratooning is practiced in some areas (Kranjac-Berisavljevic *et al*., 2003).

2) Lowland rainfed rice in valley bottoms and flood plains with different water regimes - It covers an area of about 31% of the rice cultivation area (Dofoer *et al*., 2004). This ecology

has water management problems due to regular flooding from ground water and precipitation (MoFA, 2009).

3) Irrigated rice with relatively good water control in deltas and floodplains - It covers 12% of the rice cultivation area. This involves the use of irrigation schemes such as dams, water can also be channeled from rivers to cultivated lands (Dofoer *et al*., 2004). It may also be appropriate for rice-fish culture (MoFA, 2009).

2.3 Socio-economic importance of rice

Rice occupies a leading position among food crops in the world, along with wheat and maize. An area of 214 million hectares of wheat is harvested yearly followed by rice with 154 million hectares and maize with 140 million hectares, making wheat the most cultivated cereal. However, based on their consumption, rice ranks higher with about 85 % as compared to wheat (72 %) and maize (18 %) (Maclean *et al*., 2002). In fact, in some parts of the world like Bangladesh, Cambodia, Lao PDR, Myanmar and Vietnam, the consumption rate is greater than 150 kg/capita/year (Hay *et al*., 2013), while in Ghana rice consumption accounts for more than 28 kg/capital/year with urban areas accounting for the highest rice consumption. This is because in the cities, rice is favored over other staples, the reason being that it is simple and convenient to cook and it allows for a wide variety of dishes. (Angelucci *et al*., 2013; MoFA, 2009). Other factors responsible for its high consumption rates are its nutritional and medicinal values - rice lowers the problem of bowel disorder and protects the body against constipation. This is possible because it is rich in insoluble fiber. It is also rich in carbohydrates, low in fat, with some amount of proteins and plenty of vitamin B (Olembo *et al*., 2010). Rice also provides the global human population with 21% per capita

energy and 15% per capita protein (Maclean *et al*., 2002). Furthermore, it possesses socio-cultural values because it forms part of religious rites, festivals and ceremonies in some countries like Ghana (Norman & Kebe, 2006). It also has a paramount role in the ritual life of farming communities and it is also recognized as being of deep cultural significance among some Africans in the diaspora in South America (Teeken *et al*., 2012).

Rice is also used in the fight against poverty due to its wide range of uses as outlined below:

1) Rice starch - is an important constituent used for ice cream, custard powder, puddings, gel and alcohol among others (Olembo *et al*., 2010);
2) Broken rice - is used for baby foods, soups and for brewing purposes (Olembo *et al*., 2010);
3) Rice bran- can be utilized as feed for livestock and fish. Its oil is used for pharmaceutical products and for human consumption (used for pastries like bread, snacks, cookies and biscuits) (Olembo *et al*., 2010; Verhey, 2010);
4) Defatted rice bran- is useful as cattle feed, organic fertilizers, medicinal purposes and in making wax (Olembo *et al*., 2010);
5) Rice straw - can be transformed into paper and also a source of cellulose for ruminant livestock. It is also used as manure, making building materials, as an ultra-pure source of silica and for making footwear and headwear. Rice marbles are used to add decorative effects on book covers, which is a unique use of rice. (Verhey, 2010);
6) Rice husk- can be converted into fuel, manufacture board and paper, used to make building materials and insulators. It is also useful in the making of compost and as a chemical by-product (Olembo *et al*., 2010).

The leading producers of rice in the world are China and India. Thailand, India and Vietnam are the major rice exporting countries (Kam, 2011). According to FAOSTAT (2014), with respect to global cereal production, rice production accounted for 34.1% in 2011/2012 (about 485.9 million tonnes), 35.7% in 2012/2013 (about 490.1 million tonnes) and 36.3% in 2013/2014 (about 496.6 million tonnes). This shows that there is an increase in demand as a result of increase in population.

In Africa, rice consumption is third after maize and sorghum, yet Africa produces only about 3.6% of the world's paddy rice. Rice production ranks fifth with respect to area cultivated (alongside wheat) after millet (*Pennisetum glacum*; 21% area), sorghum (*Sorghum bicolor* (L.) Monenchafg; 19% area), maize (*Zea mays* L.; 12% area) and cassava (*Manihot esculenta* Cranz; 9% area) (Nayar, 2012). Rice production in Africa is relatively low; hence, African countries depend on large imports of rice. In fact, Africa is next to Asia in terms of rice importation (Kam, 2011). According to Samado *et al*. (2008) the cost of rice imports into sub-Sahara Africa, accounts for over US$1billion annually. This is a tremendous loss of foreign exchange especially for countries that are already in debt. For example, in Ghana rice import bills account for US$ 450 million annually (Angelucci *et al*., 2013).

In Ghana, rice ranks second after maize and its consumption keeps increasing due to increase in population, urbanization and modification in consumer eating habits (MoFA, 2009). It accounts for approximately 15 % of the gross domestic product (GDP) making it important to the economy and agriculture. An area of about 45 % is allocated to rice production and it is a source of

employment to the rural communities (Kranjac-Berisavljevic, 2000). Although rice production takes place in all the ten regions of the country, the chief rice producers are the Northern, Volta and Upper East regions, which together produce between 45000 – 60000 tonnes per year each. Rice production is mostly done by small holder farmers, whose farms are smaller than a hectare of land (Angelucci *et al.,* 2013). Local rice production, therefore, falls far below consumption resulting in a high dependence on imported rice which accounts for 400,000 tonnes yearly (MoFA, 2009).

2.4 Constraints in rice production

Rice as important as it is faces lots of challenges in its production pipeline. Its yield is controlled by a variety of environmental factors. In a study carried out in Vietnam, India and Burkina Faso problems which includes drought, problems related to minerals (such as acidity, alkalinity, phosphorus deficiency and iron toxicity), unavailability of suitable and improved varieties for diverse environments, weeds, pests and diseases, salinity, poor soil fertility and high cost of input conditions were observed (Thanh & Singh, 2006; Kam, 2011). In Ghana similar challenges are faced in rice production (MoFA, 2009).

Poor soil fertility as affected by high input conditions will be one out of the many challenges discussed in the present study. In view of this, the major causes of poor soil fertility may be associated with prolonged cultivation of rice on the same piece of land without crop rotation and heavy withdrawal of nutrients as a result of cultivation of high yielding varieties and excessive and imbalanced application of fertilizer has added to the poor quality of soil for crop cultivation (Talpur *et al*., 2013).

2.5 Nitrogen fertilizer: facts and effects

Important biochemical and physiological processes in plants are linked with proteins which contains nitrogen (Yadav *et al*., 2017). N promotes the development of root systems, which have a fundamental importance in the uptake of water and nutrients. It also aids in the transfer of energy such as adenosine diphosphate(ADP) and adenosine triphosphate (ATP) which plays a crucial part in the various metabolic processes of plants (Yadav *et al*., 2017). N not only supports physiological processes, but enhances food grain and nutritional qualities in plants (Leghari *et al*., 2016; Maheswari *et al*., 2017). Furthermore, dry matter production rises with the aid of leaf N associated with chloroplast, N also increases growth and tillering which regulates panicle number. (Samonte *et al*., 2006).

2.5.1 Consumption of N globally

Due to population increase, from the 1960's to date, fertilizer inputs are associated with yields from cultivated crops especially N fertilizers. The utilization of fertilizer surged from 70,000 tonnes in 1950 - 1951 to above 28 million tonnes in 2012, which has recorded 65 % being N fertilizer. (Rao *et al*., 2018). Fertilizer application trebled from a mean of 23 kg/ha to 109 kg/ha within 1961 to 2008 respectively (FAO, 2010). Since an estimated amount of 90 million metric tons (MMt) of N fertilizers are added to the global soil for agricultural purposes annually (Frink *et al*., 1999), it has been predicted to rise to 240 MMt by 2050. Despite the increase in N use, the overall increase in yield has been recorded to be only 2.4 fold (Tilman *et al*., 1999). In view of this, the affirmation from the World summit on food security demands a mean yearly surge in crop production of 44 million metric tons to cater for an estimated 9 billion people by 2050 (FAO, 2009). This has to correlate with N fertilizer application projected to rise in the next 4 decades.

Hence, unless NUE is significantly improved, grave consequences of N fertilizers will continue to occur (Good *et al*., 2004). Therefore a second green revolution is required which does not rely on intensive fertilization but would aim at boosting yield in soils with reduced fertilizer application.

2.5.2 Loss of N fertilizer

As important as nitrogen is to plants, the three most important cereals namely: rice, wheat and maize consumes about 60 % of only nitrogen fertilizer. Rice production utilizes about 20 % of the global N consumption, within which only 30 – 40 % of the N reaches the plant, the rest is lost to the environment (Rao *et al*., 2014). This is because the rice plant utilizes some amounts of N for its growth and development such as grain production. It has been predicted that 16 – 17 kg of N is used to produce one ton of rough rice with straw inclusive. In the process of increasing N fertilizer demand which would serve as insurance against poor soil fertility as the natural processes of nitrogen fixation contributes only a fraction to what is needed, excessive N amount is used (Rao *et al*., 2018). This act does not improve productivity because the efficiency of N fertilization is low due to ammonia volatilization, denitrification, leaching and runoff losses. To add with, the level at which N is lost depends on the environmental conditions and other agronomic management practices (Baldani *et al*., 2000).

2.5.3 Consequences of N over use

Although, the importance of N cannot be overemphasized, it is associated with various consequences such as economic loss to farmers, health risk, energy as well as environmental cost. With respect to energy cost, a joint report by the International Fertilizer Industry Association (http://www.fertilizer.org), and United Nations Environmental Programmes, established that 1

metric ton of fertilizer N manufactured through the Haber-Bosch process utilizes 873 m^3 of natural gas. For crops such as rice, N fertilizer is associated with colossal expenses and this will intensify as resources becomes even more rarer (Xu *et al*., 2012). N loss to the environment is currently costing the European Union €70 to €320 billion annually. Not only is the consequence of N evident in Europe, in China, 67 main lakes were polluted as a result of high nitrate concentrations (Peng *et al*., 2011). In Ghana, the Tatafo stream within the Mampong-Ashanti Municipality suffers from eutrophication. This is because the levels of nitrate analyzed were above the WHO limits of (5.0 mg/l) (Wiafe, 2013). This polluted water is not just harmful to aquatic organisms (as it reduces oxygen percolation for their respiration and may lead to the release of toxic substances harmful to them) but is also indirectly harmful to livestock and humans. Reports from earlier studies indicate that ingesting nitrate N in drinking water causes methemoglobinemia in infants (Peng *et al*., 2011). Furthermore, N deposition which is as a result of ammonia released to the atmosphere via volatilization from agricultural fields can return back to the atmosphere as co-deposition with sulphur oxide which tampers with biological diversity leading to interference with ecosystems functions and services. Lastly, nitrous oxide formed through denitrification is an essential N based greenhouse gas which contributes to about 5% of the total climate change (Yadav, *et al*., 2017).

2.6 NUE as a concept

The complexity of the term 'Nitrogen use efficiency (NUE)', may be due to the interplay between the environment and genetic factors. It may be described as the total biomass or grain yield produced per unit available N fertilizer. It could also possess different meaning in different context. Such as: Nitrogen uptake efficiency (NUpE): the absorption of N via the roots, Nitrogen utilization

efficiency (NUtE): the remobilization of N for grain production (Xu *et al*., 2012; Haung *et al*., 2004). Apparent nitrogen recovery rate (ANR): the proportion of net increased total N absorbed by the plant with or without N fertilization to total amount of fertilizer, Agronomic use efficiency (AUE): the fraction of grain weight in the presence or absence of N fertilization to the total N fertilizer applied, Nitrogen physiological use efficiency (NpUE): the ratio of net increased grain weight to net increased N uptake with and without application of fertilizer N. Nitrogen use efficiency in grain (NUEg): grain production per unit available N, Harvest Index (HI): grain production of the total plant biomass (Xu *et al*., 2012; Han *et al*., 2015). Though a crop plant could produce huge amounts of biomass per unit N, without changing the acquired N to seed production and therefore have a low NUEg and HI. In a nut shell, two plant physiological components—NUpE and NUtE makes up plant NUE (Xu *et al*., 2012).

2.6.1 Mechanism of N assimilation

In summary, NUE constitute two key parts, they are: NUpE and NUtE. Understanding the mechanisms regulating these processes is crucial for improving crop NUE.

2.6.2 Nitrogen uptake/ assimilation in plants

Nitrogen uptake involves the absorption of N for the synthesis of proteins. Plants absorb nitrogen preferably in the form of nitrate. Therefore nitrogen, whatever the form it is present, should be changed into the available nitrate form which would then be converted or reduced into nitrite. Nitrite is also converted to ammonium which is used in the tissues to create various organic compounds (Yadav *et al*., 2017). Nitrate has to be broken down to ammonium which is further disintegrated into amino acids. The process of reducing nitrate into nitrite is catalysed in the cytosol

by the enzyme nitrate reductase (NR) (Cassman *et al*., 2002). After which, nitrite is translocated to the chloroplast where it is broken down to ammonium by an enzyme called nitrite reductase (Berntsen *et al*., 2003). Ammonium from nitrate reduction and photorespiration or amino acid recycling is taken up into the plastid/chloroplast via the GS/glutamine-2-oxoglutarate aminotransferase (GOGAT) cycle (Yadav *et al*., 2017).

2.6.3 Nitrogen utilization/ remobilization

Nitrogen remobilization is the ability of crops to utilize the acquired N for grain production. The processes are outlined as follows: In the course of the active growth stage, the leaves absorb N; subsequently, at maturity, the N stored in the leaves is channeled to the developing grains mainly as amino acids. For example, *Arabidopsis* and oilseed rape, from earlier studies have been shown to remobilize N from mature leaves to younger leaves at the vegetative phase and from mature leaves to seeds at the reproductive phase (Malagoli *et al*., 2005; Diaz *et al*., 2008; Lemaıˆtre *et al*., 2008). An estimated 95% of proteins in the grains is obtained from amino acids that are transferred to the grains after the proteins in leaves have been degraded, and the remaining is added from the soil and late top-dressed fertilizers. Glutamate (Gln) and asparagine (Asn) are chief classes of total amino acids in phloem and xylem sap of rice plants (Masclaux-Daubresse *et al*., 2010). A rise in both Asn and Gln concentrations during senescence in the phloem sap indicate their key role in representing N available for remobilization from the mature leaves. Some isoforms of NADH-glutamate dehydrogenase (GDH), and asparagine synthetase (AS) are strongly stimulated during N remobilization (Masclaux-Daubresse *et al*., 2010).

2.7 Improving NUE

2.7.1 Agronomic practices

Improvement in agriculture will depend on increase in crop yields in soils with low fertilizer application (Kabir, 2014). Two basic approaches can be followed in order to improve crop productivity in a sustainable fashion. Firstly, effectual fertilizer management and innovative agronomic practices may improve NUE. Several reports have indicated agronomic practices that can contribute to NUE (Altom *et al.*, 1996; Solie *et al.*, 1996; Hirel, 2011; Dubios *et al.*, 2017; Yadav *et al.*, 2017). Secondly, varieties need to be responsive, to reduce adopting costly or labour intensive practices by the farmers. This can be achieved through initially screening genotypes for the desired traits before breeding can be initiated.

2.7.2 Genotype selection

Characterization of germplasm may aid in identifying superior lines that may be used to breed cultivars with high NUE and of course high yields. NUE is hindered by poor characterization of the phenotype and genotype for crop N response and NUE. It is therefore important to first validate the variations in the genotypes through agromorphological characterization to obtain a clear- cut distinction among the varieties to be harnessed.

"Characterization" means to describe a character or quality of an individual. The word "characterize" also means to "distinguish", that is to differentiate or to separate into kinds, classes or categories. Therefore, characterization of genetic resources involves the differentiation of accessions (De Vincente *et al.*, 2005). Morphological characterization of genotypes is necessary for documenting identical varieties, detection of exceptional traits and also the type of the

population to be conserved (De Vincente *et al*., 2005). Morphological diversity is evaluated by taking note of variations in traits such as growth cycle, color of leaves, among others (Machoene, 2009). Morphological markers are also referred to as 'traditional markers'. The level of analysis of these markers is phenotypic. There are certain demerits of morphological markers, namely: they are controlled by environmental conditions, they are labor demanding and require large populations of plants in performing breeding experiments. They also require large plots of land and/or green house for their growth, but they are still very useful and are of high recommendation before more in-depth biochemical or molecular studies are carried out (Smith & Smith, 1992). The main advantage of conducting morphological characterization is that published descriptors are readily available for most major crop species. Therefore, characterization of the agro-morphological characters of the rice landraces collected in this study and subsequent comparison of their diversity or relatedness, using the rice descriptors suggested by Bioversity *et al*., (2007), will provide useful information for future breeding purposes.

2.8 The relationship between genetic variability and Nitrogen use Efficiency

Genetic variability in crops may be described as the heritable character of a species that portrays variability in growth in contrast to other species under favorable or unfavorable conditions. These conditions could be absorption, translocation and utilization of mineral elements (Fageria & Baligar, 2003). Due to the environmental and ecological challenges faced with nitrogen fertilizer input, an optimum fertilization level in paddy fields where NUE is of utmost priority is the need of the hour. Thus, screening for genotypic variability for NUE in breeders' germplasm, coupled with genotype x N interactions as well as the precision in selection with varied levels of N is

essential in discovering the right breeding strategies (Garnett *et al*., 2015). Understanding the mechanism associated with NUE in terms of the relationship with morphological and physiological traits would aid in simplifying and improving NUE's selection efficiency as it is a complex trait (Hirel *et al*., 2007). Therefore, the heritability of traits related to NUE and its components (NUpE & NUtE) under different N levels is important. Indeed, this has been investigated in several studies in irrigated or rainfed low land rice in Asia (Inthapaya *et al*., 2000; Koutroubas & Ntanos, 2003; Haefele *et al*., 2008; Wu *et al*., 2016).

However, previous studies revealed that different levels of nitrogen affects growth, yield and NUE components. Haque & Haque (2016) investigated the growth, yield and NUE of a new rice variety under six levels of nitrogen (0, 20, 40, 60, 80 & 100 kg/ha). Number of tillers, grain per panicle, panicle number and yield increased with increasing N but was higher at 60 kg/ha of N. In Ethiopia, twelve upland rice varieties under 0 and 64 kg/ha of nitrogen had higher grain yield, number of field spikelets, harvest index and grain N concentration as nitrogen increased. However, NUE declined at 64 kg/ha of nitrogen implying poor nitrogen absorption by the genotypes. Heritability estimates were also high indicating high variations among the varieties (Lakew, 2015). Interactions between N and genotypes were not significant except for flowering, grain N concentration, harvest index, NUE and NUtE in a study carried out by Rakotoson *et al*. (2017) in thirteen varieties under two contrasting levels of N fertilizer (0 kg/ha and 90 – 120 kg/ha). The traits that where influenced by N fertilizer where associated with N uptake and biomass production. Furthermore, panicle number increased when N was applied.

Studies carried out on the function of roots in nitrogen assimilation indicates its importance to the growth of rice plants. To buttress the preceding point, studies show that root growth of super hybrid cultivars tends to decrease when subjected to high N treatment (Hu *et al*., 2017; Liu *et al*., 2018). Gallias & Coque (2006) observed that maize cultivated under either high or low N, had root architecture being a controlling factor on grain yield. This illustrates the underpinning significance of root traits in NUE. Hamoakoa *et al*., (2013) studied six rice cultivars under standard and low N conditions in laboratory conditions. Total dry weigh of straw were lighter under low N (LN), conversely, root dry weight and NUE increased under LN as compared to the standard N condition.

Highlight from several studies showed positive or negative significant correlation between NUE and some morphological traits. A negative association among NUtE, grain and straw N concentrations has been described (Inthapanya *et al*., 2000; Koutroubas and Ntanos, 2003; Samonte *et al*., 2006; Wu *et al*., 2016). With respect to grain yield, an earlier study indicated a strong association between grain yields of rice and NUE (Haefele *et al*., 2008). Kumar (2016) recorded high heritability for plant height, number of productive tillers, panicle length, number of spikelets per panicle, grain yield per plant and NUE at low N conditions (50 % of the recommended level of N in irrigated conditions. Ju *et al*. (2015) found a significant genotype x nitrogen interaction for grain yield and N uptake in irrigated conditions. They associated the high NUE of two lowland japonica varieties with greater root biomass, deeper root distribution, longer root length and greater root oxidation activity. It is evident that nitrogen assimilation plays a central part in NUE for rice, as it accounts for 70 – 90 % of the total N in the grain (Tabuchi *et al*., 2007). Rao *et al*. (2018) studied NUE on rice landraces in Asia and they were able to identify donors for high N uptake and N translocation into grain and grain yields under low N. Numerous spikelets on

the secondary branches, increase in N content in grain and yield seems to be associated low N. Identification of NUE has been carried out in Ethiopia and Burkina Faso (Lakew, 2015; Segda *et al*., 2014). Through selection and plant breeding techniques, resilient rice varieties against some biotic and abiotic stress are in the production pipeline. Similar achievement can be done through firstly screening for nitrogen efficient rice crops in Ghanaian rice landraces.

CHAPTER THREE

3 MATERIALS AND METHODS

3.1 Description of the study area

The experiment was carried out in the research farm of the Crop Science Department in the University of Ghana, Legon. The site has geographical co-ordinates of 5° 39 N, 0° 539 W in the Greater Accra region of Ghana and a gentle topography of 0.30.

The region experiences a bi-modal seasonal rainfall pattern with an annual average precipitation which ranges between 700-1000 mm. The major and minor rainfall seasons start from April to July and September to December, respectively. The average annual temperature recorded at the site during the period is about 26.9 °C, with a maximum temperature of 33.3 ^{o}C and minimum temperature of 22.1 ^{o}C. The relative humidity for the night ranges from 60 to 90 % while in the day its ranges from 20 to 55 % throughout the year.

The soil type is savanna ochrosol locally called Toje series which has been classified by Eze, (2008) as a Rhodustalf and Rhodic Lixisol according to USDA (1999, 2003). Toje series is among the most widely cultivated soils of the Accra Plains. Toje is developed on Quartzite schist (Fiagbedzi, 1989).

3.2 Plant material

This comprised a set of twenty (20) landraces which were obtained from the Plant Genetic Resources Research Institute, Bonsu (Appendix 1). These genotypes varied in origin, cultivar type, plant height, and average growth duration.

3.3 Soil sampling and analysis

A soil auger was used to collect soils at a depth of 0-15 cm. The following soil properties were analyzed before the experiment; pH, electrical conductivity (EC), soil texture, nitrogen (N), potassium (K), phosphorus (P) and carbon (C) (Table 3.1).

Table 3.1: Physical and chemical properties of soil in the experimental site

Soil properties	**Value**
pH	6.5
Electrical conductivity(ds/m)	0.04
Phosphorus (mg/kg)	30.1
Potassium (cmol/kg)	0.79
Carbon (%)	2.99
Nitrogen (%)	0.13
Soil particle size	**Value**
Sand (%)	56
Clay (%)	25
Silt (%)	19
Soil textural class	Sandy clay loam

3.4 Pot experiment

Pot experiment was conducted under natural temperature and sunlight in order to mimic the field experiment and compare the outcome under both conditions. Soil bags of 23 cm in width and 20 cm in length, were used for the experiment. The total number of pots used was 120.

The soil was garnered from an uncultivated field at a depth of 0 – 15 cm. Roots and other plant debris were removed from the soil. The soil was subsequently crushed and sieved through 2 mm size mesh to obtain fine earth fraction. Five kilogram (5 kg) of the soil was weighed into each pot.

The site for the pot experiment was cleared using cutlass and a hoe. Plastic sheets were used to cover the area to avoid soil micro infestation and reduce weeds proliferation. The trial was carried out from February 2019 to June 2019. The experimental design was a completely randomized design arranged in a factorial manner and replicated three times. Forty combinations of treatments were compared (20 landraces × 2 levels of fertilization) with three seeds sown in each pot. Two increasing doses of N namely; available N (no N) and low N (50 kg/ha) were applied. Nitrogen fertilizer was applied in two split doses: 50 % at tillering (25 kg/ha) and 50 % at panicle initiation (25 kg/ha). Other major nutrients such as P and K, were applied to all pots at 90 kg/ha. P in the form of triple superphosphate and K in the form of potassium chloride were applied in two split applications; tillering (45 kg/ha) and panicle initiation (45 kg/ha). Plants were watered on a daily basis to maintain moisture in the soil.

3.5 Field experiment

The field experiment was conducted using a split plot design with three replicates. This was carried out from February to June 2019. Plot size was 2 m by 1.7 m. Beds were raised and the spacing between each bed and row was 70 cm with an alley of 1m between blocks. Two doses of N (0 and 50 kg/ha) constituted the main plot treatments while the subplots were represented by the 20 rice landraces. The seeds were sown directly into the soil and were maintained at 4 to 6 seeds per bed. Nitrogen fertilizer was applied in two split doses: 50 % at tillering (25 kg/ha) and 50 % (25 kg/ha) at panicle initiation. Other major nutrients such as P and K, were applied to all pots at 90 kg/ha. P in the form of triple superphosphate and K in the form of potassium chloride were applied in two split applications; tillering and panicle initiation stage. Plants were watered on a daily basis to maintain moisture in the soil. The trial was conducted in an upland condition during the rainy season. The plants were watered on rainless days. For the control of weeds, manual weeding of the alleys and hand weeding of the beds was done when necessary.

3.6 Phenotypic data scored

In both pot and field experiments, data collection was conducted 3 times during the entire growth period at the following stages: active tillering (when the plant attained the first 3 tillers), heading (when panicle primordia starts protruding) and harvesting (when about 80 % of the spikelets were straw-coloured) *sensu* Getachew & Nabiyu (2018). For each sampling, three representative plants (in pot experiments) or hills (in field trials) for each landrace were garnered.

3.6.1 Agro-morphological characterization

Agro-morphological characterization was implemented to distinguish among the landraces and to record vegetative data to facilitate comparison among the landraces. Agro-morphological evaluation was monitored in the pot experiment using 13 quantitative and 17 qualitative rice descriptors in accordance with the method described by Bioversity International *et al*. (2007) (Table 3.2).

Table 3.2: List of agro morphological traits scored

Qualitative trait	Quantitative trait
Awn presence	Awn length
Awn color	Culm diameter
Auricle color	Culm number
Culm habit	Days to flowering
Culm kneeing ability	Days to maturity
Culm diameter	Flag leaf length
Culm lodging resistance	Flag leaf width
Culm strength	Ligule length
Caryopsis color	Leaf blade length
Caryopsis shape	Leaf blade width
Leaf blade pubescence	Hundred grain weight
Leaf blade width type	Panicle length
Leaf blade length type	Panicle number
Lemma and palea color	
Panicle attitude	
Panicle exertion	
Stigma color	

3.6.2 Growth, yield related traits and NUE components

Data was accrued from the pot experiment for only growth parameters to avoid bias. This is because root destruction was only carried out in the pot experiment. Conversely, both pot and field data was taken for yield related traits and NUE components. During active tillering, chlorophyll content was recorded using a chlorophyll meter, SPAD-502 Plus (Konica Minolta, INC). Chlorophyll was recorded on five leaves and averaged. Leaf length was recorded from the base of the leaf sheath to the tip and leaf width was measured within the center of the leaf using a 30 cm meter rule. At maturity, number of tillers were counted and plant height was measured using a 30 cm meter rule. Furthermore, roots from potted plants marked for destructive sampling were gently removed, thoroughly washed and separated from shoot portion with knife. The root length was measured using a 30 cm meter rule from the base of the stem to the tip of the root. The roots were dried in an oven at 60˚C for 72 h until constant weight was obtained and was expressed in grams.

Plants were reaped and disjointed into straw and panicle at maturity. Filled grains (spikelets) were separated from empty grains, oven dried at 60˚C for 72 h and weighed. Filled grains were used to evaluate grain yield (GY) and grain N concentration (GNC). 250 filled grains and 250 empty grains were weighed for the estimation of the total number of filled and empty grains. Straw samples were oven dried at 60 °C for 72 h and weighed to measure straw yield (SY). The harvest index (HI) was enumerated from GY and SY (Table 3). NUE, NUpE, NUtE were calculated *sensu* Moll *et al*. (1982) (Table 3).

Table 3.3: Description of the 15 measured and calculated yield and NUE related traits

Code	Trait	Formula	Unit
FG	Filled grain	100 × FG / total number of spikelets	%
GNC	Grain N concentration	Grain N concentration of 3 hills at maturity	%
GY	Grain yield	PN × SPIPAN × FG × TGW	kg/ha
HI	Harvest Index	GY/(GY + SY)	-
NUE	Nitrogen use efficiency	GY/N supply	kg grain kg/N
NUpE	Nitrogen uptake efficiency	TNUP/N supply	kg N kg/N
NUtE	Nitrogen utilization efficiency	GY/TNUP	kg grain kg/N
PN	Number of panicles	Mean of panicle number of 3 hills	-
SNC	Straw N concentration	SNC of 3 hills at maturity	%
SPIPAN	Number of spikelets per panicle	Mean of number of spikelets of 3 hills	-
SY	Straw Yield	Biomass of 9 hill	kg/ha
TGW	1000-grain weight	Weight of 250 filled spikelets × 4	g
TNUP	Total plant N uptake	GNC × GY + SNC × SY	kg/ha
RL	Root length	Measured from base of stem to tip of roots	cm
RW	Root weight	Weight of oven dried roots	g

3.6.3 Tissue nitrogen concentration

N concentration in the leaf blades, sheaths plus stems and panicles were analyzed by the Kjeldahl procedure using the standard protocol (Piper, 1966). The straw and grain samples were oven dried at 65°C for 72 h and pulverized separately into fine powder. 0.1g of the samples was used for the analysis. The percent N was calculated using the formula below:

$$\% \text{ TN} = \frac{(Titre\ value - Blank) * 1400}{weight * 5 * 1000}$$

3.7 Statistical Analysis

3.7.1 Germplasm characterization

Phenotypic diversity of the landraces was estimated using Shannon-Weaver diversity index (H′) (Sotto & Rabara, 2007). Based on an arbitrary scale adapted from Jamago & Cortes (2012), indices were divided into maximum (H′= 1.00), high (H′ = 0.76 – 0.99), moderate (H′ = 0.46 – 0.75) and low diversity (0.01– 0.45). This was calculated based on the method used by Lexerød & Eid (2006):

$$H' = pi\ (\log_2 pi)/\log_2 N$$

pi = frequency proportion of the descriptor state

N = number of states

The standardized Shannon-Weaver provided a constrained index between zero and one with the highest value indicating maximum abundance (Jamago & Cortes 2012). Correlation, cluster and principal component analysis were carried out using Minitab® 19 statistical analysis package.

3.7.2 Growth parameters, yield and NUE components

Analysis of variance was carried out using GENSTAT statistical analysis package (version 12) Sources of variation such as genotype, N level, replication and the interaction of genotype × N level were used in the statistical model. These were considered as fixed effects (genotype, N level, replication, genotype × N). A significant level of $p \leq 0.05$ was computed and a Post Hoc test for values with $p \leq 0.05$ using Turkey's test was carried out.

Pearson phenotypic correlation coefficients based on means of varieties over replicates were calculated for all traits using Minitab® 19 statistical analysis package. Correlation coefficients were classified as weak ($r = 0.35$), moderate ($r = 0.36$) and strong ($r > 0.68$) (Taylor, 1990).

3.7.3 Estimates of variance components

The population's variability were estimated using the mean, phenotypic and genotypic variance and coefficient of variation. To estimate the phenotypic and genotypic variance, phenotypic and genotypic coefficients of variation were calculated *sensu* Rosmania *et al.* (2016) as follows:

$$\sigma^2 G = [(MSG) - (MSE)] / r$$

$$\sigma^2 P = [\sigma^2 G + (\sigma^2 E/r)],$$

Where: $\sigma^2 G$ = Genotypic variance; $\sigma^2 P$ = Phenotypic variance; $\sigma^2 E$ = environmental variance (error mean square from the analysis of variance); MSG = mean square of genotypes; MSE = error mean square; r = number of replications. Genotypic coefficient of variation (GCV) = $(\sigma^2 G)^{1/2}/\underline{X}) \times 100$; Phenotypic coefficient of variation (PCV) = $(\sigma^2 P)^{1/2}/\underline{X}) \times 100$, where: $\sigma^2 G$ = Genotypic variance; $\sigma^2 P$ = Phenotypic variance; $\underline{X}$ is grand mean of a character. Broad-sense heritability (H^2) for all traits at each level of N was calculated from variance components using the formula:

$$H = \sigma^2_G/\sigma^2_P,$$

CHAPTER FOUR

4 RESULTS

4.1 Characterization of landraces

4.1.1 Diversity in qualitative traits in pot experiment

Based on the qualitative traits recorded, ligule shape and culm kneeing ability were invariables. This implies that all the landraces characterized had a 2-cleft ligule shape and the culms had no kneeing ability (Table 4.1). Eight of the traits scored were predominated by one character in each trait with a distribution ranging between 78 % - 95 %. These included awn related characters (awn presence and color). Most of the landraces had dense culms and upright culm habit.

Moderately diverse traits were observed for six descriptors with indices ranging between 0.46 - 0.70. Most of these traits were inflorescence related such as panicle and spikelet characters. Diversity in seed coat color was evident. These were white seed coat (60 %), red (10 %), light brown (20 %) and black (10 %). Two out of the 19 traits had high diversity with a mean index of 0.82. These traits were culm-related which measured rice hardiness during maturity and harvest. Though, the principal character was intermediate lodging resistance, 35 % of the landraces had strong to very strong lodging resistance at maturity (Table 4.1).

Table 4.1: Qualitative traits showing the predominant state observed, distribution (%) and the calculated Shannon diversity indices (H′) for each descriptor scored in pot experiment.

Descriptor	Predominant State	%	H′ index
Invariant			
Ligule shape	2-cleft	100.00	0.00
Culm kneeing ability	Strong	100.00	0.00
Low diversity			
Awn presence	Awnless	94.03	0.11
Awn color (late observation)	Awnless	94.04	0.12
Leaf blade pubescence	Intermediate	95.20	0.12
Culm diameter type	Thick	91.10	0.12
Culm habit	Erect (< 15˚)	84.08	0.26
Auricle color	Whitish	89.05	0.31
Panicle attitude	Drooping	84.11	0.32
Stigma color	White	78.12	0.45
Moderate diversity			
Lemma and Palea color	Straw	57.09	0.46
Leaf blade width type	Intermediate (~ 50 cm)	61.12	0.55
Leaf blade length type	Intermediate	60.01	0.54
Panicle exertion	Well exerted	54.13	0.67
Caryopsis pericarp color (seed coat color)	White	50.18	0.68
Caryopsis shape	Long spindled shape	43.03	0.70
High diversity			
Culm lodging resistance	Intermediate	39.03	0.80
Culm strength	Intermediate	42.11	0.83
	Average diversity		0.33

4.1.2 Diversity in quantitative traits in pot experiment

Awn length and Ligule length were the only traits that portrayed low diversity (Table 4.2). Only five landraces exhibited awns in their grains (GH 1531, GH 1552, GH 2145, GH1599 and GH 1514). GH2145 had the shortest awn length (0.6 cm) while GH1552 and GH1514 had the longest awn length (3.5cm). Ligule length was observed to be the longest (3.5 cm) in two out of 20 landraces (GH 1538; GH 1574). The shortest ligule length was demonstrated by GH1574 which had a length of (0.5 cm).

Culm diameter was the only trait which exhibited moderate diversity (0.52). Ten out of the 13 quantitative traits had high diversity with a diversity index ranging from 0.72 – 0.90 (Table 4.2). It was observed that GH2145 had the shortest flag leaf length (16.00 cm) while GH1538 had the longest flag leaf length (26.90 cm). Maturity of the characterized germplasm ranged from 55 – 105 days. GH1514 had the shortest days to maturity while GH 1519 had the longest days to maturity. Grain weight diversity was observed in the germplasm with GH 1550 having the lightest grain weight (1.00 g) and GH2145 having the heaviest grain weight (2.20 g). Two varieties had equal number of panicle and culm number with GH1550 recording the lowest number (8) and GH2145 recording the highest number (33).

In a nut shell, the mean index was 0.70. Almost all the traits measured exhibited moderate to high diversity.

Table 4.2 Quantitative descriptors and calculated Shannon-Weiner index (H′) of evaluated rice landraces in pot experiment

Descriptors	H′	Min. Trait Value	Variety	Max. Trait Value	Variety	Mean (± Standard deviation)
			Low diversity			
Ligule length (cm)	0.11	0.50	GH1574	1.10	GH1552	0.89 ± 0.23
Awn length (cm)	0.12	0.60	GH2145	3.50	GH1514; GH1552	0.43 ± 1.05
			Moderate diversity			
Culm diameter (cm)	0.52	0.40	Aunty Jane	1.20	GH1515	0.54 ± 0.06
			High diversity			
Flag leaf length (cm)	0.72	16.00	GH2145	26.90	GH1538	20.67 ± 3.26
Leaf blade length(cm)	0.78	23.50	GH1514	40.10	GH1583	32.14 ± 5.01
Flag leaf width (cm)	0.82	0.80	GH1801	1.80	GH1552	1.09 ± 0.23
Flowering (days)	0.85	40.00	GH1514	79.00	GH1519	54.70 ± 9.88
Leaf blade width (cm)	0.85	0.50	AUNTY JANE	1.10	GH1549	0.85 ± 0.18
Maturity (days)	0.87	55.00	GH1514	105.00	GH1519	85.00 ± 13.88
Culm number	0.88	8.00	GH1550	33.00	GH2145	18.05 ± 6.96
100 Grain weight(g)	0.88	1.00	GH1552	2.00	GH2145	1.44 ± 0.30
Panicle length	0.89	18.00	GH1822	26.50	GH2145	20.91 ± 2.42
Panicle number	0.90	8.00	GH1550	33.00	GH2145	18.05 ± 6.96
Average diversity	0.70					

4.1.3 Correlation among traits in pot experiment

76 % of the trait combinations had weak correlations while 14 % had moderate correlations. Several traits showed significant correlations ($p \leq 0.05, \leq 0.01$) among each other. Two of these trait combinations had strong correlation; panicle number per plant with culm number (r = 0.97) and number of days to main heading with number of days to maturity (r = 0.89), while 7 trait combinations had moderate correlations. These were: flag leaf width with leaf blade length (r = 0.53), number of days to maturity with culm number (r = 0.53), culm length with leaf blade length (r = 0.52), number of days to maturity with ligule length (r = 0.48), number of days to maturity with panicle number (r = 0.47), number of days to main heading with leaf blade length (r = 0.46) and panicle length with flag leaf length (r = 0.46) (Table 4.3).

Table 4.3: Correlation matrices of the 20 quantitative variables in pot experiment

	LL	LBL	LBW	FLL	FLW	CL	CN	CD	PN	PL	NDMH	NDM
LBL	0.26^{ns}											
LBW	-0.19^{n}	0.18^{ns}										
FLL	0.22^{ns}	0.36^{ns}	0.24^{ns}									
FLW	0.43^{ns}	0.53*	0.36^{ns}	0.38^{ns}								
CL	0.15^{ns}	0.52*	0.28^{ns}	0.31^{ns}	0.35^{ns}							
CN	0.33^{ns}	0.10^{ns}	-0.47^{ns}	0.33^{ns}	0.03^{ns}	0.07^{ns}						
CD	-0.06^{ns}	0.23^{ns}	0.15^{ns}	0.06^{ns}	0.06^{ns}	0.28^{ns}	0.18^{ns}					
PN	0.29^{ns}	0.02^{ns}	-0.45^{ns}	0.32^{ns}	0.06^{ns}	0.07^{ns}	0.97**	0.14^{ns}				
PL	0.08^{ns}	0.36^{ns}	0.20^{ns}	0.46*	0.05^{ns}	0.31^{ns}	0.14^{ns}	0.09^{ns}	0.13^{ns}			
NDMH	0.44^{ns}	0.46*	-0.10^{ns}	0.03^{ns}	0.34^{ns}	0.02^{ns}	0.41^{ns}	0.16^{ns}	0.36^{ns}	0.19^{ns}		
NDM	0.48*	0.30^{ns}	-0.15^{ns}	0.21^{ns}	0.25^{ns}	0.19^{ns}	0.53*	0.13^{ns}	0.47*	0.32^{ns}	0.89**	
HGW	-0.48^{ns}	0.31^{ns}	0.20^{ns}	0.02^{ns}	0.42^{ns}	0.14^{ns}	0.20^{ns}	0.06^{ns}	0.13^{ns}	0.41^{ns}	-0.77^{ns}	-0.77^{ns}

*, **, ^{ns} are significant at the 5 and 1% probability level and non- significant, respectively

LL: Ligule length
LBL: Leaf blade length
LBW: Leaf blade width
FLL: Flag leaf length
FLW: Flag leaf width
CD: Culm diameter
PL: Panicle length
NDMH: Number of days to main heading
NDM: Number of days to maturity
HGW: 100-grain weight
CN: Culm number
CL: Culm length
PN: Panicle number

4.1.4 Cluster and principal component analysis among rice landraces in pot experiment

A dendogram was generated for the 20 rice landraces based on their observable characters. At a similarity index of 67%, the landraces were grouped into 5 clusters (Figure 4.4). Cluster 1 had the largest number of landraces. These landraces were peculiar for their light grain weight. Cluster 2 and 1 were similar in ligule length and culm diameter except that cluster 2 had longer leaf length and flag leaf length. Cluster 3 were early maturing, had heavier grain weight and highest panicle number. The fourth cluster had 2 genotypes which had the longest days to maturity and longest culms which implies that they were tall plants. Cluster 5 were shorter due to their culm length and had longer panicle length.

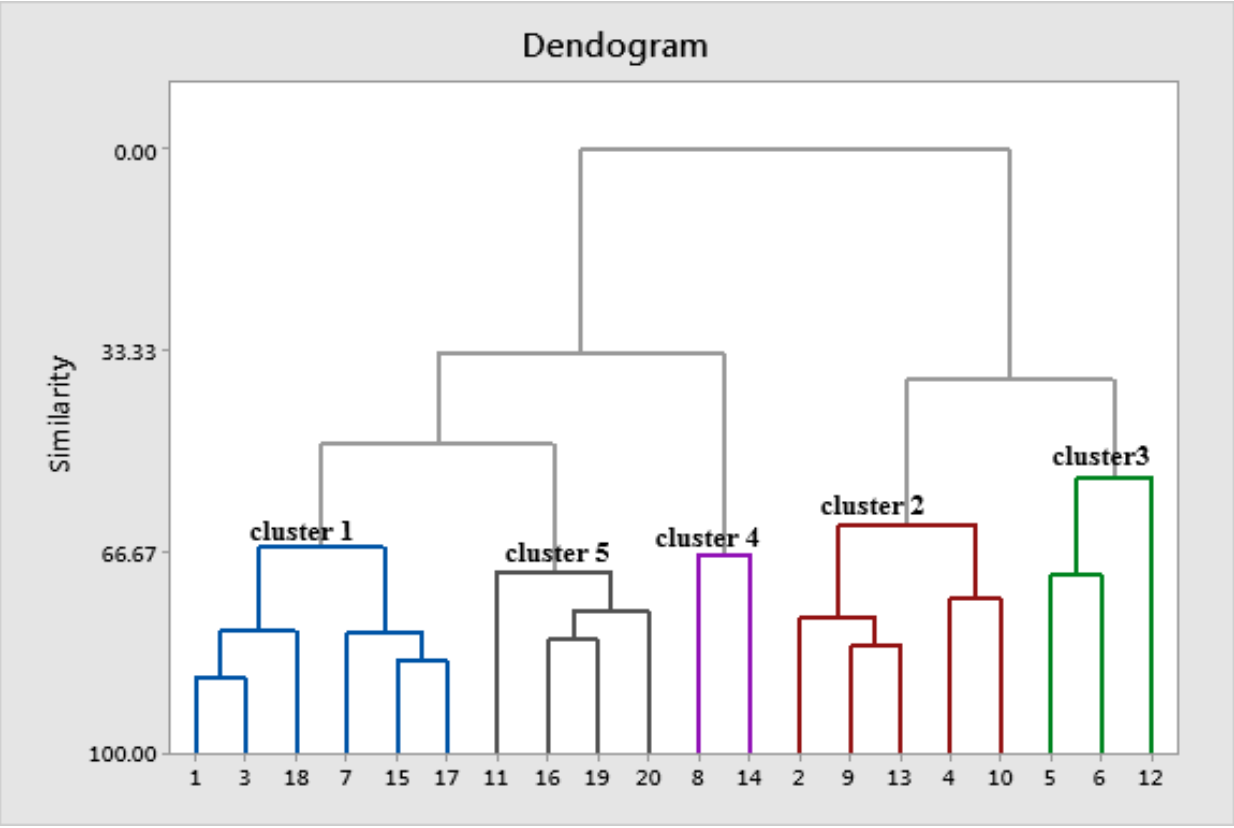

Figure 4.1: Dendogram generated by cluster analysis based of morphological characters in pot experiment

1: GH1549	6: GH1550	11: GH1531	16: GH1590
2: GH1587	7: GH1515	12: GH2145	17: GH1583
3:GH1574	8: GH1552	13: GH 1801	18: GH1599
4: GH1822	9. GH1535	14: GH1519	19: GH 1516
5:GH1514	10. GH1538	15: GH 1599	20: Aunty Jane

4.1.5 Principal component analysis based on the 14 quantitative traits

Principal component analysis was carried out to describe the relative contribution of the different traits to the total variation in the landraces. Four significant principal components were identified and accounted for 71.9 % of the total variation. The first component accounted for 28.9 % of the total variation while the second, third and fourth components contributed 28.9 %, 21.8 %, 11.6 % and 9.6 % respectively (Table 4.4). Quantitative traits such as ligule length, culm number, panicle number, number of days to main heading and number of days to maturity contributed positively to the variations in PC1 (Table 4.4). Leaf blade length, flag leaf length, flag leaf width and culm number, positively correlated with PC2. PC3 was associated with culm diameter and awn length. In the fourth component, culm diameter had a high loading of 0.31.

Table 4.4: Principal components analysis based on the 14 quantitative trait

Variable	PC1	PC2	PC3	PC4
Ligule Length	0.306	0.190	0.211	0.014
Leaf Blade Length	0.177	0.395	-0.122	-0.258
Leaf Blade Width	-0.180	0.291	-0.290	0.079
Flag Leaf Length	-0.086	0.411	0.052	-0.147
Flag Leaf Width	0.161	0.424	-0.004	0.113
Culm Length	-0.019	0.381	0.244	-0.228
Culm Number	0.364	-0.196	0.270	-0.336
Culm Diameter	-0.087	0.173	0.303	0.307
Panicle Number	0.328	-0.223	0.302	-0.333
Awn Length	-0.064	0.217	0.612	0.178
Panicle Length	-0.160	0.179	-0.207	-0.587
Number of Days to Main heading	0.426	0.120	-0.243	0.013
Number of Days to Maturity	0.453	-0.000	-0.200	0.114
Grain weight	-0.381	-0.129	0.147	-0.365
Eigenvalue	4.05	3.06	1.61	1.34
Proportion	0.29	0.22	0.12	0.09
Cumulative	0.29	0.51	0.62	0.72

4.2 Response of different accessions to different nitrogen levels in pot experiment

Observations from the twenty genotypes indicated that there were significant differences ($p \leq 0.05$)for all the growth parameters under two levels of nitrogen application (Tables 4.5, 4.6, 4.7). The genotype by nitrogen interaction (G × N) was significant for all the traits.

Root length was influenced significantly by the two nitrogen levels among the genotypes. However, the longest root length was recorded for Aunty Jane variety (60.00 cm) when no nitrogen was applied while the shortest root length was recorded for variety GH1531 at 50 kg/ha of nitrogen application (Table 4.5).

Significant differences in root dry weight was observed under different levels of nitrogen among the genotypes. The heaviest root dry weight was recorded for GH1552 (95.00 g) when nitrogen was applied while the lightest was noted in variety GH1514 (6.00 g) at 0kg/ha of nitrogen (Table 4.5).

Different levels of nitrogen affected the leaf length of the genotypes significantly. Variety GH1549 had the longest leaf length (49.00 cm) when 50 kg/ha of nitrogen was applied while variety GH1514 had the shortest leaf length (23.17 cm) when nitrogen was not applied. However GH1515 remained constant (32.00 cm) across the two levels of nitrogen (Table 4.5).

Leaf width was affected noticeably with the addition of nitrogen fertilizer. The maximum leaf width was recorded in variety GH1587 (1.70 cm) when 50 kg/h of nitrogen was applied while the minimum was variety GH1531 (0.60 cm) with no nitrogen application (Table 4.5).

Significant differences were observed for number of leaves. Number of leaves increased as nitrogen level increased. GH2145 had the highest leaf number (60.00) when N was applied while GH1549 and GH1589 had the lowest number of leaves (7.00) (Table 4.6)

Under different levels of nitrogen, plant height differed significantly with variety GH1552 having the highest plant height of 144.50 cm at 50 kg/ha of nitrogen while the shortest observed plant height was 78.00 cm from varieties GH1574 and GH1599 in the absence of nitrogen application (Table 4.6).

Due to variations in nitrogen levels, chlorophyll concentrations differed significantly. It increased (44.53) when N was 50 kg/ha in variety GH1549 while the minimum was from GH1531 at 0 kg/ha of nitrogen (Table 4.6).

Number of tillers significantly increased (30.00) when nitrogen was applied in variety GH2145 while variety GH1549 recorded the minimum tiller number of 7.00 (Table 4.7).

Different levels of nitrogen fertilizer affected shoot dry weight significantly. Variety GH1587 had the maximum shoot dry weight of 35.00 g when nitrogen was applied while variety GH1514 had the minimum shoot dry weight of 6.00 g without nitrogen fertilizer (Table 4.7).

Grain yield significantly increased when nitrogen was applied at 50 kg/ha. This was observed in variety GH2145 with 8035 kg/ha of yield having the highest grain yield while GH1549 had the lowest grain yield of 357.00 kg/ha when nitrogen was not applied (Table 4.7).

Table 4.5: Effect of two nitrogen levels on root length and dry weight, leaf length and width of twenty rice landraces in pot experiment

	Root length (cm)			Root dry weight (g pot^{-1})			Leaf length (cm)			Leaf width (cm)		
Genotype	**No N**	**Low N**	**Mean**	**No N**	**Low N**	**Mean**	**No N**	**Low N**	**Mean**	**No N**	**Low N**	**Mean**
GH1549	40.00cd	42.00gh	41.00	30.00e	45.00d	37.50	26.23b	49.00k	37.62	1.03cd	1.60ef	1.31
GH1587	38.00bc	35.00de	36.50	75.00l	81.00j	78.00	36.17gh	44.00hi	40.08	1.03cd	1.70f	1.36
GH1574	42.00de	46.00i	44.00	55.00j	83.00j	69.00	27.00bc	35.00de	31.00	0.90bcd	1.10c	1.00
GH1822	43.00de	39.00fg	41.00	25.00d	37.00c	31.00	33.00f	46.00ij	39.50	1.11d	1.40de	1.25
GH1514	44.00ef	30.00ab	37.00	6.00a	10.00a	8.00	23.17a	42.17fg	32.67	0.70ab	1.10c	0.90
GH1550	47.00f	38.00ef	42.50	11.00b	25.00b	18.00	29.00cd	29.17ab	29.08	0.70ab	1.40de	1.05
GH1515	32.00a	57.00k	44.50	66.67k	95.00k	80.84	32.00ef	32.00c	32.00	0.80abc	0.80ab	0.80
GH1552	42.00de	50.00j	46.00	85.33m	95.67k	90.50	39.00i	42.00fg	40.50	1.10d	1.00bc	1.06
GH1535	40.00cd	38.00ef	39.00	39.00g	57.00f	48.00	34.00fg	58.17l	46.08	0.9bcd	1.50ef	1.20
GH1538	35.00ab	29.00ab	32.00	15.00c	27.00b	21.00	36.53gh	31.00bc	33.77	1.00cd	1.50ef	1.25
GH1531	43.00de	27.00a	35.00	50.00hi	75.00i	62.50	30.17de	35.00de	32.58	0.66a	0.60a	0.60
GH2145	43.00de	33.00cd	38.00	52.00i	62.67g	57.34	26.00b	33.00cd	29.50	1.00cd	1.01c	1.06
GH1801	40.00cd	35.00cd	37.50	66.00k	85.00j	75.50	33.00f	41.00fg	37.00	0.60a	1.00bc	0.81
GH1519	43.00de	40.00fg	41.50	35.00f	40.00c	37.50	38.03hi	37.00e	37.50	0.80abc	0.80ab	0.80
GH1599	40.00cd	40.00fg	40.00	58.00j	63.00g	60.50	38.00hi	47.00jk	42.50	0.80abc	0.97bc	0.88
GH1590	33.00a	35.00de	34.00	36.00fg	49.00de	42.50	36.00gh	43.00gh	39.50	0.97bcd	1.20cd	1.08
GH1583	43.00de	30.00ab	36.50	30.00e	68.00h	49.00	36.03gh	40.50fg	38.27	0.80abc	1.50ef	1.15
GH1599	35.00ab	38.00ef	36.50	49.00hi	64.00gh	56.50	37.00hi	40.00f	38.50	0.80abc	1.20cd	1.00
GH1516	47.00f	32.00bc	39.50	29.00e	50.00e	39.50	27.00bc	35.00de	31.00	0.80abc	1.00bc	0.91
Aunty Jane	60.00g	43.00hi	51.50	48.00h	74.00i	61.00	29.00cd	27.00a	28.00	0.97bcd	1.00bc	1.00
Mean	41.5	37.85		45.20	59.32		32.35	39.35		0.87	1.18	
G												
N			**			**			**			**
G × N			**			**			**			**
CV (%)			**			**			**			**
			2.5			6.8			2.5			8.9

G: genotype, N: nitrogen, CV: coefficient of variation

Means followed by the same letter in the same column are not significantly different at the 5% probability level by Tukey's test. ** Significant at p = 0.001

Table 4.6: Effect of two nitrogen levels on number of leaves, plant height and chlorophyll of twenty rice landraces in pot experiment

Genotype	Number of leaves (No)			Plant height (cm)			Chlorophyll		
	No N	Low N	Mean	No N	Low N	Mean	No N	Low N	Mean
GH1549	15.00ab	45.00cde	28.50	82.50b	92.00c	87.25	30.50i	44.53j	37.52
GH1587	16.00bc	38.00bc	27.50	111.50j	138.5n	125.00	25.13g	46.00j	35.57
GH1574	17.00bcd	48.67cdef	32.50	78.00a	82.00a	80.00	20.20cd	35.63cde	27.92
GH1822	22.00efg	60.00hi	35.50	95.53e	116.00k	105.77	28.00h	34.63c	31.32
GH1514	16.00bc	26.67a	17.83	95.00e	110.5ij	102.77	19.63bcd	37.07ef	28.35
GH1550	25.00g	40.00cd	28.67	82.00b	100.50fg	91.25	23.13f	34.00c	28.57
GH1515	20.00def	51.00efgh	31.00	96.57e	102.0g	99.28	23.63fg	41.13hi	32.38
GH1552	24.00g	54.00efgh	36.00	124.50k	144.50m	124.50	34.13j	39.53gh	36.83
GH1535	20.00def	59.33ghi	38.67	104.50gh	120.00l	112.25	19.50bcd	35.23cd	27.37
GH1538	24.00g	57.67fghi	35.67	106.50hi	113.0j	109.75	27.30h	40.63hi	33.97
GH1531	20.00def	66.00i	38.17	88.53d	89.00b	88.77	17.03a	30.37a	23.70
GH2145	30.00h	67.00i	43.00	101.00f	105.00h	103.00	18.20ab	38.00fg	28.10
GH1801	25.00g	59.00fghi	39.50	103.60g	123.50m	113.55	18.63abc	41.50i	30.07
GH1519	18.00bcd	48.00cdef	30.00	107.00i	110.00i	108.5	18.00ab	29.60a	23.80
GH1599	12.00a	28.00ab	20.00	88.00d	92.00c	90.00	19.56bcd	38.00fg	28.78
GH1590	19.00cde	20.00a	14.50	81.33b	95.00d	88.17	25.10g	39.50gh	32.30
GH1583	15.00ab	25.00a	19.50	87.50d	98.00ef	92.75	22.53ef	37.07ef	29.80
GH1599	12.00a	28.00ab	20.00	78.00a	80.00a	79.00	24.10fg	32.27b	28.18
GH1516	19.00cde	27.00ab	21.00	85.00c	90.00bc	87.50	27.57h	36.63def	32.10
Aunty Jane	23.00fg	38.00bc	24.50	95.00e	97.00de	96.00	21.00de	41.07hi	31.03
Mean	13.88	44.32		94.58	103.93		23.15	37.62	
G			**			**			**
N			**			**			**
G × N			**			**			**
CV (%)			9.4			0.8			1.8

G: genotype, N: nitrogen, CV: coefficient of variation. Means followed by the same letter in the same column are not significantly different at the 5% probability level by Tukey's test. ** Significant at p = 0.001.

Table 4.7: Effect of two nitrogen levels on number of tillers, shoot dry weight and grain yield of twenty rice landraces in pot experiment

Genotype	Number of tillers (No)			Shoot dry weight (g pot^{-1})			Grain yield (kg/ha)		
	No N	Low N	Mean	No N	Low N	Mean	No N	Low N	Mean
GH1549	7.00a	15.00ab	11.00	11.00cd	20.00c	15.50	357.00a	2970.00m	1664.00
GH1587	9.00abcd	16.00b	12.33	30.00i	35.00g	32.50	1117.10m	1459.00d	1288.00
GH1574	7.00ab	17.00bcd	12.17	22.00efg	28.00ef	25.00	597.10e	1449.00d	1023.00
GH1822	12.00def	23.00efg	17.00	19.00e	30.00f	24.50	1676.10o	2841.00l	2259.00
GH1514	8.00abc	17.00bc	12.00	6.00a	11.00a	8.50	957.50j	1173.00b	1065.00
GH1550	25.00g	25.00g	25.00	25.00gh	28.00ef	26.50	1099.00l	4703.00p	2901.00
GH1515	11.00cdef	21.00def	15.5	10.00bcd	25.00de	17.50	1024.00k	3564.00o	2294.00
GH1552	12.00def	24.00g	18.00	23.00fg	30.00f	26.50	699.10g	2448.00j	1574.00
GH1535	8.00abc	21.00def	14.00	13.00d	24.00d	18.50	1160.10n	2995.00m	2078.00
GH1538	10.00abc	23.00g	17.00	10.00bcd	13.00ab	11.50	703.00h	1921.00i	1308.00
GH1531	10.00abc	20.00def	15.17	9.00abc	16.00b	12.50	679.00f	1792.00h	1236.00
GH2145	14.00f	30.00h	22.00	21.00ef	31.00f	26.00	2092.10r	8035.00q	5064.00
GH1801	13.00ef	25.00g	19.00	20.00ef	23.00cd	21.50	1970.00q	2800.00k	2385.00
GH1519	9.00abcd	18.00bcd	13.5	19.00e	28.00f	23.50	599.20e	1652.00f	1126.00
GH1599	8.00abc	14.00a	10.00	8.00abc	15.00b	11.50	450.00b	627.00a	539.00
GH1590	12.00def	19.00cde	15.5	28.00hi	35.00g	31.50	1785.00p	3420.00n	2602.00
GH1583	9.00abcd	15.00ab	12.00	13.00d	31.00f	22.00	579.20d	1741.00g	1160.00
GH1599	10.00abc	18.00bcd	14.00	7.00ab	10.00a	8.50	576.00c	1359.00c	968.00
GH1516	11.00cdef	19.00cde	15.00	13.00d	22.00cd	17.50	779.10i	1539.00e	1159.00
Aunty Jane	12.00def	24.00fg	17.5	11.00cd	16.00b	13.50	699.10g	3002.00m	1850.00
Mean	10.88	19.88		15.90	23.55		979.94	2574.08	
						**			
G			**			**			**
N			**			**			**
G × N			**			5.1			**
CV (%)			6.8						0.4

G:genotype, N: nitrogen, CV: coefficient of variation. Means followed by the same letter in the same column are not significantly different at the 5% probability level by Tukey's test. ** Significant at p = 0.001.

4.3 Correlation among the growth parameters in pot experiment

Significant positive correlation was observed for six trait combinations (Table 4.8). Four out of the seven trait combinations had strong positive correlation. These were: root weight with number of tillers (r = 1.00), grain yield with number of tillers (r = 0.74), grain yield with number of tillers (r = 0.74) and leaf width with chlorophyll (r = 0.64). Moderate correlations were observed in: plant height with number of leaves (r = 0.47), number of leaves with number of tillers (r = 0.46), number of leaves with grain yield (r = 0.46). 80 % of the trait combination had weak correlation coefficient.

Table 4.8: Phenotypic correlation coefficient of growth parameters among twenty rice landraces in pot experiment

	CPHY	NT	GY	LL	LW	NL	PLTH	RL	RW
NT	-0.04^{ns}								
GY	0.00^{ns}	0.74**							
LL	0.16^{ns}	-0.46^{ns}	-0.29^{ns}						
LW	0.64**	-0.08^{ns}	0.16^{ns}	0.36^{ns}					
NL	-0.09^{ns}	0.46*	0.46*	-0.06^{ns}	-0.00^{ns}				
PLTH	0.30^{ns}	0.19^{ns}	0.15^{ns}	0.34^{ns}	0.28^{ns}	0.47*			
RL	0.11^{ns}	0.17^{ns}	0.04^{ns}	-0.27^{ns}	-0.11^{ns}	0.07^{ns}	0.00^{ns}		
RW	-0.04^{ns}	1.00**	0.74**	-0.46^{ns}	-0.08^{ns}	0.46^{ns}	0.19^{ns}	0.16^{ns}	
SW	0.21^{ns}	0.32^{ns}	0.44^{ns}	0.19^{ns}	0.34^{ns}	0.18^{ns}	0.35^{ns}	0.06^{ns}	0.32^{ns}

*, **, ^{ns} are significant at the 5 and 1% probability level and non- significant, respectively

NT: Number of tillers
GY: Grain yield
LL: Leaf length
LW: Leaf width
PLTH: Plant height
RW: Root weight
SW: Straw weight
CPHY: Chlorophyll
NL: Number of leaves
RL: Root length

4.4 Yield related traits of rice landraces under no and low nitrogen levels in pot and field experiment

Rice landraces showed different nitrogen uptake ability attributing to yield and nitrogen use efficiency at different nitrogen levels. A wide range with a general trend of reduction for the eight yield related traits was observed in the twenty landraces under no N compared to low N across both pot and field evaluation (Table 4.9). Significant differences and interactions were observed for all the traits. However, unfilled spikelets was higher as nitrogen level increased which ranged from 70 – 750 and 86 – 781 in pot and field experiments respectively. Low unfilled spikelets was recorded at no N level which ranged from 10 – 90 and 31 – 71 in pot and field experiments, respectively. Among the rice genotypes, GH2145 had the highest grain yield (8,035 kg/ha) followed by GH1550 (6,025 kg/ha). GH2145 and GH1550 also had the highest number of filled spikelets per panicle in pot (150 and 138) and field (161 and 152) experiments (Tables 4.9). Genotypes showed significant differences for N harvest index with mean ranging from 38.36 – 50.06 % under no and low N conditions in pot experiment and 33.19 % - 46.78 % in field experiment. N harvest index of GH2145, GH1550, GH 1822, GH1801 and GH1590, GH1514 and Aunty Jane were higher than the overall mean.

4.5 Nitrogen use efficiency and its component traits in pot and field experiment

There were significant genotypic effects for N use efficiency and its component traits ($p < 0.05$) (Table 4.10). In this study, wide ranges of means were recorded with a general trend in increase for NUE, NUpE, and NUtE under no N conditions as compared to low N conditions in pot and field experiments. GNC and SNC increased slightly under low N conditions as compared to no N. The highest grain yield producing landraces had the highest NUE.

Table 4.9: Summary of ANOVA for yield related traits under no and low nitrogen levels of twenty rice landraces in pot and field experiment

Trait	Range				Mean						
	Pot		Field		Pot		Field				
	No N	Low N	No N	Low N	No N	Low N	No N	Low N	N	G	G × N
FS	300.00-1400.00	522.00 – 3360.00	311.00 – 1461.00	580.00 – 3407.00	683.40	1419.20	730.83	1482.25	**	**	**
GY	357.00-2092.00	627.13 – 8035.00	403.00 – 2121.00	636.00 – 8107.00	979.94	2574.08	1011.72	2633.33	****	****	****
PN	7.00 – 14.00	11.00 – 30.00	10.00 – 19.00	15.00 – 34.00	10.40	19.83	14.18	23.35	**	**	*
PL	16.00 – 26.50	20.00 – 30.00	18.00 – 29.00	22.00 – 33.00	20.80	25.31	22.27	26.83	**	**	**
TGW	10.00 – 20.00	10.00 – 25.00	9.00 – 24.00	17.00 – 32.00	14.45	17.75	16.41	20.77	**	**	**
UFS	10.00 – 90.00	70.00 – 750.00	31.00 – 71.00	86.00 – 781.00	32.72	381.91	50.65	415.70	**	**	**
SPN	50.00 -100.00	70.00 – 150.00	55.00 – 121.33	76.00 – 161.00	65.45	91.15	77.55	101.15	**	**	**
HI	21.36 – 61.65	29.43 – 65.25	21.23 – 50.31	26.14 – 69.25	38.36	50.06	33.19	46.78	**	**	**

*, **, ^{ns} are significant at the 5 and 1% probability level and non- significant, respectively

FS: Filled spikelet
GY: Grain yield
PN: Panicle number
TGW: 1000-grain weight
UFS: Unfilled spikelet
SPN: Spikelet per panicle
HI: Harvest Index

Table 4.10: Summary of ANOVA for Nitrogen use efficiency parameters under no and low nitrogen levels in pot and field experiments of twenty rice landraces.

Trait	Range				Mean						
	Pot		Field		Pot		Field				
	No N	Low N	No N	Low N	No N	Low N	No N	Low N	N	G	G × N
NUE	27.46 – 160.88	27.17 – 160.71	30.94 – 163.11	12.72 – 163.11	75.38	51.47	76.11	71.75	**	**	**
NUpE	79.77 – 609.20	34.15 – 567.85	102.80 – 566.71	41.89 – 546.57	255.98	159.10	262.19	159.44	****	****	****
NUtE	0.13 – 0.93	0.22 – 0.69	0.19 – 0.75	0.23 – 0.59	0.33	0.35	0.32	0.35	**	**	*
GNC	0.65 – 2.37	0.78 – 2.88	0.68 – 2.33	0.79 – 2.82	1.53	1.79	1.53	1.77	**	**	**
SNC	0.71 – 1.77	0.82 – 1.87	0.53 – 1.72	0.65 – 1.97	1.07	1.21	0.88	1.05	**	**	**

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

NUE: Nitrogen Use Efficiency
NUpE: Nitrogen Uptake Efficiency
NUtE: Nitrogen Utilization Efficiency
GNC: Grain nitrogen concentration
SNC: Straw nitrogen concentration

4.6 Genotypic and phenotypic coefficient of variations and heritability estimates for yield related traits and NUE components in pot and field experiments

Genotypic coefficient of variation (GCV) was less than its corresponding estimates of phenotypic coefficient of variation (PCV) for all yield related traits (Table 4.11). There were differences between PCV and GCV for NUE and NUpE. For NUtE, GNC and SNC, there was no difference between GCV and PCV (Table 4.11)

In the present study, broad-sense heritability estimates for the yield related traits ranged from 97.83 % to 100 % under pot and field conditions (Table 4.12). NUE components observed for both pot and field experiments had heritability estimates of 95.37% to 100.00% (Table 4.12).

Table 4.11: Estimation of genetic variability parameters for yield related traits in rice landraces under pot and field conditions.

Trait	Mean		$\sigma^2 G$		$\sigma^2 P$		GCV (%)		PCV (%)		H (%)	
	Pot	Field	Pot	Field	Pot	Field	Pot	Field	Pot	Field	Pot	Field
FG	1051.3	1106.54	11200.89	12246.05	11201.97	12246.05	10.00	9.74	10.00	9.75	99.00	100.00
GY	1777.01	1822.53	74874.29	80090.67	74874.99	80092.08	15.39	15.53	15.40	15.53	99.99	99.99
PN	15.11	18.76	0.69	0.90	0.70	0.91	5.50	5.05	5.54	5.08	98.57	98.90
PL	23.05	24.55	0.45	0.68	0.46	0.69	2.91	3.35	2.94	3.38	97.83	98.55
TGW	16.10	18.59	0.93	1.01	0.94	1.02	5.98	5.40	6.02	5.43	98.94	99.02
UFS	211.32	233.18	755.60	782.37	756.87	782.38	13.01	11.99	13.02	11.99	99.83	99.99
SPIPAN	78.30	89.35	23.75	26.59	23.76	26.59	6.22	5.77	6.23	5.77	99.96	100.00
HI	44.26	39.98	7.61	6.24	7.64	6.26	6.23	6.25	6.24	6.26	99.61	99.68

FG: Filled spikelet
GY: Grain yield
HI: Harvest Index
PN: Panicle number
PL: Panicle length
TGW: 1000- grain weight
SPIPAN: Spikelet per panicle
UFS: Unfilled spikelet

Table 4.12: Estimation of genetic variability parameters for nitrogen use efficiency in rice landraces under pot and field under conditions.

Trait	Mean		$\sigma^2 G$		$\sigma^2 P$		GCV (%)		PCV (%)		H (%)	
	Pot	Field	Pot	Field	Pot	Field	Pot	Field	Pot	Field	Pot	Field
NUE	63.43	73.93	76.98	170.44	80.72	171.54	13.83	17.65	14.16	17.72	95.37	99.34
NUpE	207.54	210.81	1220.39	1187.08	1221.31	1187.38	16.83	16.34	16.84	16.35	99.92	99.97
NUtE	0.34	0.33	0.01	0.01	0.01	0.01	29.41	30.30	29.41	30.30	100.00	100.00
GNC	1.65	1.65	0.01	0.01	0.01	0.01	6.06	6.06	6.06	6.06	100.00	100.00
SNC	1.14	0.96	0.01	0.01	0.01	0.01	8.68	9.43	8.73	9.43	100.00	100.00

NUE: Nitrogen Use Efficiency
NUpE: Nitrogen Uptake Efficiency
NUtE: Nitrogen Utilization Efficiency
GNC: Grain nitrogen concentration
SNC: Straw nitrogen concentration

4.6 Correlations among yield related traits and NUE components in pot and field experiments

Several traits showed significant positive correlation and were observed for 21 % of trait combinations (Table 4.13). Significant correlations were observed between grain yield and field spikelet (r = 0.91), panicle length with grain yield (r = 0.81). Similarly, 1000 - grain weight showed significant correlation with grain yield (r = 0.81) and panicle length (r =1.00) (Table 4.13). Spikelet per panicle showed positive correlation with field spikelet (r = 0.63) and unfilled spikelet (r = 0.75). NUE also had significant correlation with filled spikelet (0.96), grain yield (0.96), panicle length (0.76), and one thousand grain weight(r = 0.76). NUpE had significant correlation with filled spikelet (r = 0.90) and grain yield (r = 0.93). HI also correlated with grain yield (r = 0.56), panicle length (r = 0.69), 1000 - grain weight (r = 0.69), NUE(r = 0.55). On the other hand, 59% of the trait combinations had weak correlations.

Positive correlations were observed among yield related traits and NUE components in the field experiment. (Table 4.14). About 59 % of the trait combinations had weak correlations. Strong positive correlation were observed for: straw nitrogen concentration and nitrogen uptake efficiency (r = 0.98), NUpE with grain yield (r = 0.95) and filled spikelet (r = 0.90), grain yield with harvest index (r = 0.70), filled spikelet with harvest index (r = 0.61) and grain yield (r = 0.90) and panicle number with grain yield (r = 0.74),

In both pot and field experiments, strong correlations were observed between NUE with filled spikelet, grain yield, panicle length and one thousand grain weight. Also, NUpE had strong correlations with filled spikelet, grain yield harvest index, panicle length, one thousand grain weight and NUE. Furthermore, NUtE had weak correlations with all the yield parameters, NUE and NUtE.

Table 4.13: Correlation for yield related traits and nitrogen use efficiency in pot experiment

	FS	GY	PN	PL	UFS	TGW	SPIPAN	NUE	NUpE	NUtE	GNC	SNC
GY	0.91**											
PN	0.06^{ns}	-0.01^{ns}										
PL	0.59*	0.81*	0.07^{ns}									
UFS	0.11^{ns}	-0.14^{ns}	0.07^{ns}	-0.33^{ns}								
TGW	0.59*	0.81*	0.07^{ns}	1.00**	-0.33^{ns}							
SPIPAN	0.63**	0.41^{ns}	0.23^{ns}	0.15^{ns}	0.75**	0.15^{ns}						
NUE	0.96**	0.96**	0.09^{ns}	0.76**	-0.03^{ns}	0.76**	0.50*					
NUpE	0.90**	0.93**	0.10^{ns}	0.63**	-0.08^{ns}	0.63**	0.44^{ns}	0.92**				
NUtE	-0.17^{ns}	-0.21^{ns}	-0.01^{ns}	0.16^{ns}	-0.02^{ns}	0.16**	-0.04^{ns}	-0.14^{ns}	-0.43^{ns}			
GNC	0.54*	0.52*	-0.19^{ns}	0.03^{ns}	-0.06^{ns}	0.03^{ns}	0.19^{ns}	0.48^{ns}	0.65**	-0.71^{ns}		
SNC	0.48*	0.64*	0.15^{ns}	0.60**	-0.38^{ns}	0.60**	0.05^{ns}	0.57*	0.71**	-0.43^{ns}	0.38^{ns}	
HI	0.48*	0.56*	-0.14^{ns}	0.69**	-0.32^{ns}	0.69**	0.06^{ns}	0.56*	0.31^{ns}	0.47^{ns}	0.03^{ns}	0.35^{ns}

*, **, ^{ns} are significant at the 5 and 1% probability level and non- significant, respectively

FG: Filled spikelet
GY: Grain yield
PN: Panicle number
TGW: 1000- grain weight
NUpE: Nitrogen uptake efficiency
SNC: Straw N concentration
UFS: Unfilled spikelet
SPN: Spikelet per panicle
HI: Harvest Index
NUE: Nitrogen use efficiency
GNC: Grain N concentration
NUtE: Nitrogen utilization efficiency

Table 4.14: Correlation for yield related traits and nitrogen use efficiency in field experiment

	FS	GY	PN	PL	UFS	TGW	SPIPAN	NUE	NUpE	NUtE	GNC	SNC
GY	0.91**											
PN	0.70**	0.74**										
PL	0.08^{ns}	0.03^{ns}	-0.18^{ns}									
UFS	0.12^{ns}	-0.14^{ns}	-0.37^{ns}	0.09^{ns}								
TGW	0.42^{ns}	0.62**	0.50**	0.03^{ns}	-0.37^{ns}							
SPIPAN	0.65**	0.43^{ns}	0.02^{ns}	0.25^{ns}	0.73**	0.03^{ns}						
NUE	0.66**	0.76**	0.45*	0.30^{ns}	-0.21^{ns}	0.56**	0.38^{ns}					
NUpE	0.90**	0.95**	0.74**	0.08^{ns}	-0.09^{ns}	0.56**	0.49*	0.76**				
NUtE	-0.13^{ns}	-0.19^{ns}	-0.29^{ns}	0.17^{ns}	0.07^{ns}	0.06^{ns}	-0.01^{ns}	-0.18^{ns}	-0.39^{ns}			
GNC	0.53*	0.59**	0.61**	-0.26^{ns}	-0.28^{ns}	0.39^{ns}	0.07^{ns}	0.47*	0.67**	-0.66^{ns}		
SNC	0.87**	0.97**	0.72**	0.01^{ns}	-0.17^{ns}	0.58**	0.42^{ns}	0.76**	0.98**	-0.38^{ns}	0.70**	
HI	0.61**	0.70**	0.53**	0.01^{ns}	-0.30^{ns}	0.65**	0.17^{ns}	0.68**	0.56**	0.31^{ns}	0.33^{ns}	0.58**

*, **, ^{ns} are significant at the 5 and 1% probability level and non- significant, respectively

FG:Filled spikelet
GY: Grain yield
PN: Panicle number
TGW: 1000-grain weight
UFS: Unfilled spikelet
SPN: Spikelet per panicle
HI: Harvest Index
NUE: Nitrogen use efficiency
NUpE: Nitrogen uptake efficiency
GNC: Grain N concentration
SNC: Straw N concentration
NUtE: Nitrogen utilization efficiency

CHAPTER FIVE

5 DISCUSSION

5.1 Characterization of germplasm

5.1.1 Diversity among morphological traits in pot experiment

Sturdy culm was an important trait with high diversity in the qualitative trait. Most of the landraces characterized (98 %) had dense culms. Conversely, when the landraces were evaluated for lodging resistance, only 37 % indicated strong or very strong lodging resistance. High culm strength, tightly wrapped leaf sheath and plant height (< 150 cm) may contribute to the varieties being resistant to lodging (Hitaka, 1969; Mahbub *et al*., 2007). This is an important trait needed to improve yield as lodging leads to low yield in rice production. On the average, the diversity index for the qualitative traits scored was low (0.33). Low qualitative diversity may be as result of similar geographic locations where landraces used for the study were collected. This result resonates with a study carried out in the Philippines, were 307 varieties used had low qualitative diversity (Rabara *et al*., 2014). Future collection trips should focus on these traits to enhance their diversity in our gene bank.

Awns were present in five of the landraces which is considered as a valuable trait in rice domestication. Awn is an important trait which aids the dispersal of seeds as seeds attach themselves to animal fur and protects them from animal predators. Furthermore, in barley, it aids in photosynthesis during grain filling. Conversely, the awns in rice lack chlorenchyma and cannot aid in photosynthesis (Bullard, 1985; Furata *et al*., 2015). The bulu or *Javanica* collection in the tropical *Japonica* varieties possess awned grains together with limited tillers and elongated

panicles (Vaughan, 2008). Farmers prefer varieties with no to short awns because it allows for easier harvesting and reduces yield loss (Hu *et al*., 2011; Frank *et al*., 2015).

5.1.2 Correlation among traits in pot experiment

Panicle number per plant and culm number was the only trait combination that was strong, indicated that almost all tillers were productive tillers and able to bear inflorescence. Flag leaf length correlated with most of the traits. Previous studies indicate that flag leaves are the principal source of phloem-delivered photoassimilates during the grain-filling stage in rice (Yu *et al*., 2006; Narayanan *et al*., 2007). Earlier studies have shown that when flag leaves are removed, about 45 % grain yield loss is observed (Abou-Khalifa *et al*., 2008). They are also useful in grain filling, because 80 % of the entire carbohydrate reserved in the grains are produced by flag leaves in rice (Gladun & Karpov, 1993). Similar findings were found in a study carried out by Rabara *et al*. (2014). Number of days to maturity correlated with panicle number indicating that the higher the panicle number the longer the days to maturity (Lakew, 2015).

5.1.3 Cluster and principal component analyses among landraces in pot experiment

The overall cluster analysis showed the diversity in the accessions collected. It has also aided in grouping the accessions with similar traits (Wijayawardhana *et al*., 2015; Baloch *et al*., 2016). Sohrabi *et al*. (2012), clustered 50 accessions of upland rice into six groups based on 12 quantitative traits. Ahmadikhah *et al*. (2008) clustered 58 rice varieties into four groups based on 18 morphological traits. In this study, 5 clusters were created at a similarity index of 68 %. Principal component analysis indicated the most important traits that contributed to the cluster

analysis such as ligule length, culm number, panicle number, number of days to main heading and number of days to maturity, leaf blade length, flag leaf length, flag leaf width and culm number and culm diameter. Caldo *et al*. (1996) recorded the first 10 principal components accounting for 67 % of the total variation. Lasalita-Zapico *et al.* (2010) also computed 82.7 % of the total variation among 32 upland rice varieties.

5.2 Growth response of the rice genotypes in different nitrogen treatments in pot experiment

Findings from the pot experiment indicated that root dry weight, shoot dry weight, plant height, number of leaves, number of tillers, leaf length, leaf width, chlorophyll and grain yield increased as a result of nitrogen application except for root length which declined as nitrogen was applied.

5.2.1 Effect of nitrogen on root morphology

In the present study, sub-optimal N fertilization reduced root length as compared to no N levels. N stress might have caused an increase in root length density to explore a larger soil volume or increase N uptake (Mi *et al*., 2008). Technically, increase in nitrate supply causes a decline in auxin concentration in the phloem, suggesting that shoot-root auxin transport may be inhibited by high N supply (Coenen & Lomax, 1997). Considering the antagonism between auxin and cytokinin, increase in cytokinin levels may lead to a decrease in auxin level which may have negative influence on root apex activity. This causes weakening in root apical dominance, leading to a reduction in root elongation and an increase in lateral root growth (Mi *et al*., 2008). Similar studies supports this finding (Evans *et al*., 1994; Chen *et al*., 2008). This finding was contrary to López-Bucio et al. (2003) who found out that fertilizer application led to root elongation.

5.2.2 Effect of nitrogen on number of tillers, leaf length, leaf width and leaf number

The number of tillers, leaf length, leaf width and leaf number increased due to an increase in the size and number of meristematic cells which results in new shoots (Lawlor, 2002). N fertilizer has been identified as a promoter of cytokinin levels which affects the expansion of cell wall (Arnold *et al*., 2006). Hence, it is reasonable to suspect that N was involved directly or in directly in the expansion and division of new cells and in the production of tissues which were accountable for the increase in growth characteristics. The reduction in tiller number, leaf length and width observed at 0kg/ha of nitrogen may be as a result of intra plant competition for nitrogen. This is in agreement with Mesquita & Pinto (2000) and Pathan, (2010).

5.2.3 Effect of nitrogen on grain yield

Nitrogen promotes carbohydrate build up in culms and leaf sheaths throughout the pre-heading phase and in the grain during the ripening phase of rice (Bahmanyar & Ranjbar, 2007). Furthermore, high grain yield may be as a result of nitrogen fertilizer encouraging dry matter production, improving rice growth rate, promoting elongation of internodes and the activity of growth hormones like gibberellins. Singh *et al*. (2000) also observed similar findings.

5.2.4 Effect of nitrogen on chlorophyll content

Chlorophyll concentration increased due to fertilizer application. Chlorophyll depends on nitrogen nutrition which stimulates the production of active photosynthetic pigments by increasing the quantity of stromal and thylakoid proteins in leaves (Cooke *et al*., 2005; Filho *et al*., 2011 & Li *et al*., 2012). This increases the formation of chloroplast throughout the period of leaf growth.

Furthermore, optimum availability of N is involved in cell division and the formation of active photosynthetic pigments such as chlorophyll. This result is in agreement with Razaq *et al*. (2017).

5.2.5 Effect of nitrogen on Plant height

Application of N increased plant height, due to an increase in protein formation from manufactured carbohydrates, less carbohydrate is deposited in the vegetative portion of the plant which leads to increase in plant height. On the contrary, when N is deficient, carbohydrates are deposited in the vegetative cells causing them to thicken (Tisdale *et al*., 2003). Similar findings were observed a study carried out by Fageria *et al*. (2010).

5.2.6 Effect of nitrogen on shoot dry weight

Shoot dry weight among the genotypes was heavier when nitrogen was applied. This may be due to N the decrease in spikelet sterility. Similar findings was observed in a study carried out by Fageria & Baligar (1999). Furthermore, a decline in the number of sterile spikelet trebles the number of grain and eventually increases grain yield performance. An increase in yield will lead to an increase in grain number and heavier shoot dry weight.

5.2.7 Correlation among growth parameters in pot experiment

Root dry weight showed a strong association with number of tillers. It is logical to speculate that the root surface is in close contact with the soil which led to high absorption of nutrients. This indicates that resource uptake increases with root surface area. Increase in nutrient assimilation may have aided the crop to generate more tillers (Casen & Barber, 1976). A strong relationship was also observed between root weight and grain yield. This may be due to the nutrient absorption

achieved through increased root mass. This might have led to the increase in grain yield. Similar findings were observed in studies carried by Narayanan *et al*. (2014). Leaf width and chlorophyll content had positive correlation. This may be due to better penetration of sunlight for the formation of active photosynthetic pigment.

5.3 Yield related traits in pot experiment

Genetic variations were observed among the landraces due to significant differences indicated by the analysis of variance. The varied range of responses to no N in the present study affirms the differences in N metabolism proficiencies of the landraces. The reduction in panicle number under no N could be due to competition for assimilates among young panicles and tillers in the course of panicle development. This leads to slow growth among many young tillers which may senesce without producing panicle (Fageria & Baligar, 2001). Analogous observations were described by other authors (Mendhe *et al*., 2002; Uddin *et al*., 2011). Spikelet per panicle increased as nitrogen fertilizer was applied. Increase in number of grains per panicle at higher nitrogen rate might be due to increase in nitrogen uptake which promoted formation of higher number of twigs per panicle (Rahman *et al*., 2007). Previous studies observed similar findings (Lakew, 2015; Rao *et al*., 2018).

The high yielders had more filled spikelet per panicle. This indicates that number of filled spikelet promotes high grain yield due to adequate supply of N fertilizer. These findings resonates with that of Lawal & Lawal (2002). On the contrary, unfilled spikelet were increased when nitrogen was applied. This could be because nitrogen increases the number of spikelet as a result of increase in panicle number which may reduce the production of carbohydrate from sink to support the growth of all spikelet leading to a reduction in filled spikelet. This might happen especially when

solar radiation is low leading to less assimilation of photosynthates. Similar result was observed in a study carried out by Yuan *et al*. (2013). Conversely, Lakew (2015) experienced reduction in filled spikelet when nitrogen was applied.

In the case of 1000 - grain weight, the difference was relatively low between the two levels of nitrogen because it has be reported to be a genetically controlled character. Comparable results were established by other scientists and they concluded that there is little opportunity to improve grain size through agronomic management (Maske *et al*., 1997; Ahmed *et al*., 2005). High yielding genotypes had high harvest index indicating the importance of harvest index as a yield component. Rao *et al*. (2018) also found similar results.

5.4 Nitrogen use efficiency and its component traits

Although significant differences were observed for NUE, NUpE and NUtE. The variations between no and low nitrogen level was small. The assessment of NUE in crop plants is significantly required to measure the fate of applied nitrogen and their role in improving maximum economic yield through efficient absorption or utilization by the plant. The relatively similar trend of NUE at no and low N levels indicate that the rice genotypes in the current study are able to absorb or utilize N at no N levels. This may be due to the fact that they are landraces with the ablility to grow mostly under low or minimal input conditions and are therefore likely to harbour the trait of resource use efficiency such as NUE. Perhaps, higher levels of N (levels above sub-optimal level used in the present study) may have shown lower NUE as observed in previous studies (Lakew, 2015; Haque & Haque, 2016).

Interestingly, some of the high yielders such as GH 2145, GH1822 and GH1801 produced high grain under both no and low nitrogen levels. This may be buttressed by the fact that nitrogen efficient varieties may be able to produce high grain yields under no, low and high N fertility conditions (Beatty *et al.*, 2010). Use of such better N use efficient varieties could improve the success of farmers as much of applied N can be absorbed and utilized for higher yield.

5.5 Genotypic and phenotypic coefficient of variation

Genotypic coefficient of variation (GCV) was lower than its equivalent evaluations of phenotypic coefficient of variation (PCV) for all traits signifying the vital role of the environment in the expression of these traits. The difference between PCV and GCV for the yield related parameters indicates that these traits were influenced by the environment as compared to the genotypic effect. For NUE and its component traits as well as grain and straw nitrogen concentrations both the environment and genetic components played nearly equal roles as little to no difference was observed. Lakew, (2015) also observed similar results in his study.

5.6 Heritability

Broad-sense heritability estimates for traits such as FS, GY, PN, PL, TGW, SPIPAN and HI as well as NUE and its components had high to very high heritability estimates. High heritability estimates can be used as a baseline for selection according to the morphological traits. Earlier studies by Woldeyesus *et al*. (2004) on barely genotypes and by Alemayehu *et al*. (2006) on tef genotypes indicated that broad-sense heritability estimates were high for yield related traits and NUE and its component traits.

5.7 Correlation among traits in pot and field experiment

In order to improve a trait of interest, selecting traits that show strong association is pivotal. Grain yield correlated significantly with filled spikelet, panicle length and total grain weight. This was in accordance with results obtained by Rao *et al*. (2018). Furthermore, grain yield also had strong positive correlation with NUE and NUpE. This indicates that the ability of plants to become high yielders is as a result of their ability to efficiently absorb limited amount of nitrogen and channel the photoassimilates gained for grain production. Grain and straw nitrogen concentration as well as harvest index correlated with grain yield which indicates possible improvement of these traits. This is similar to earlier studies (Lakew, 2015).

In this study, NUpE had strong association with NUE than the comparison between NUtE and NUE. Hence, NUpE seems more important in determining NUE. Earlier studies indicated by Van Sanford & Mackown (1986) and Lakew (2015) showed that strong positive correlation was observed between NUE and NUpE while weak correlations were observed between NUE and NUtE. Also, Muurinen *et al*. (2006) on rice and Woldeyesus *et al*. (2004) on barely genotypes found out that NUpE was more significant than NUtE in influencing NUE.

Variations between pot and field experiments and their correlations may be ascribed to dissimilarity in the light intensity, nutrient absorption and water availability. Pot experiment appears to be more vulnerable to no N conditions for filled spikelet, grain yield, panicle number,

panicle length, 1000-grain weight, spikelet per panicle and harvest index, whereas, unfilled spikelet seem vulnerable in field experiment at low N.

CHAPTER SIX

6 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Twenty rice landraces were characterized to evaluate their phenotypic diversity. Generally, the rice landraces exhibited moderate diversity based on quantitative characters (average index of 0.70) while qualitative characters had low diversity (average index of 0.33). Cluster analysis identified landraces with similar characteristics and principal component analysis indicated 71.9% of the total variation. Hence, in order to bridge the gap in terms of traits identified as low diversity, germplasm collection mission can be embarked upon and particularly, landraces that have been passed on from generation to generation and still cultivated in communities may be garnered. Furthermore, important traits such as early maturing and high tiller number identified can be utilized in breeding programs.

The rice landraces responded differently to nitrogen levels. Panicle length, plant height, number of leaves, leaf length, leaf width, chlorophyll, grain yield, root dry weight and shoot dry weight increased as nitrogen was applied. Hence, 50 kg/ha of nitrogen can improve yield in landraces such as GH2145 and GH1550.

NUE correlated with filled spikelet, grain yield, panicle length, 1000-grain weight and NUpE in the present study. Negative correlation was observed for NUE and NUtE. True to the study's premise, landraces with promising yield under no N with efficient utilization of absorbed N were identified. They include; GH2145, GH1550, GH1822 and GH1801. Based on results of this study, it can be concluded that the identified landraces can be used in breeding programmes.

Correlation coefficient provide a measure of association between the assessed characters which aids in the identification of significant as well as insignificant traits. This promotes the importance of these traits during selection for breeding purposes.

Phenotypic coefficient of variation, genotypic coefficient of variation and heritability were high for yield related traits and NUE components. Selection of landraces with high heritability may aid breeding during crop improvement.

6.2 Recommendations

1. GH 1550, GH 2145 and GH1514 can be used to produce superior lines in terms of early maturity and high panicle number.
2. GH2145, GH1550, GH1822 and GH1801 are nitrogen use efficient lines, hence can be used in breeding programmes.
3. Filled spikelets, grain yield, panicle length, 1000-grain weight and nitrogen uptake efficiency are associated with nitrogen use efficiency and should be considered in breeding as well as elicit the essentialities of these characters during plant selection for increased yield.

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7 APPENDICES

7.1 Appendix A: Accessions of rice and their collection site

S/N	Accession number	Gene bank
1.	GH1514	PGRRI/Bonsu
2.	GH1515	PGRRI/Bonsu
3.	GH1516	PGRRI/Bonsu
4.	GH1519	PGRRI/Bonsu
5.	GH1531	PGRRI/Bonsu
6.	GH1535	PGRRI/Bonsu
7.	GH1538	PGRRI/Bonsu
8.	GH1549	PGRRI/Bonsu
9.	GH1550	PGRRI/Bonsu
10.	GH1552	PGRRI/Bonsu
11.	GH1574	PGRRI/Bonsu
12.	GH1583	PGRRI/Bonsu
13.	GH1587	PGRRI/Bonsu
14.	GH1590	PGRRI/Bonsu
15.	GH1599	PGRRI/Bonsu
16.	GH1599	PGRRI/Bonsu
17.	GH1801	PGRRI/Bonsu
18.	GH1822	PGRRI/Bonsu
19.	GH2145	PGRRI/Bonsu
20.	AUNTY JANE	CRI/Kumasi

7.2: Appendix B: ANOVA for growth parameters in pot experiment

Appendix 1b: Analysis of variance for chlorophyll

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
GeNOtype	19	1606.7349	84.5650	288.95	<.001
N	1	6285.7688	6285.7688	21477.57	<.001
Genotyp.N	19	665.9362	35.0493	119.76	<.001
Residual	80	23.4133	0.2927		
Total	119	8581.8532			

Appendix 2b: Analysis of variance for culm number

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotyp	19	1585.367	83.440	75.85	<.001
N	1	2430.000	2430.000	2209.09	<.001
Genotyp.N	19	353.000	18.579	16.89	<.001
Residual	80	88.000	1.100		
Total	119	4456.367			

Appendix 3b: Analysis of variance for grain yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	19	1.138E+08	5.990E+06	1.063E+05	<.001
N	1	7.624E+07	7.624E+07	1.352E+06	<.001
Genotyp.N	19	5.172E+07	2.722E+06	48284.70	<.001
Residual	80	4.510E+03	5.637E+01		
Total	119	2.418E+08			

Appendix 4b: Analysis of variance for leaf length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	19	2819.4016	148.3896	190.43	<.001
N	1	1484.7367	1484.7367	1905.34	<.001
Genotype.N	19	1757.7649	92.5139	118.72	<.001
Residual	80	62.3400	0.7792		
Total	119	6124.2433			

Appendix 5b: Analysis of variance for Leaf width

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	19	4.388250	0.230961	27.72	<.001
N	1	2.852083	2.852083	342.25	<.001
Genotype.N	19	1.862917	0.098048	11.77	<.001
Residual	80	0.666667	0.008333		
Total	119	9.769917			

Appendix 6b: Analysis of variance for number of leaves

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	19	7863.133	413.849	54.88	<.001
N	1	27785.633	27785.633	3684.28	<.001
Genotype.N	19	5498.700	289.405	38.37	<.001
Residual	80	603.333	7.542		
Total	119	41750.800			

Appendix 7b: Analysis of variance for plant height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	19	20619.7676	1085.2509	1554.43	<.001
N	1	2621.7401	2621.7401	3755.18	<.001
Genotype.N	19	1753.6782	92.2989	132.20	<.001
Residual	80	55.8533	0.6982		
Total	119	25051.0393			

Appendix 8b: Analysis of variance for root length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	19	2370.825	124.780	124.78	<.001
N	1	399.675	399.675	399.68	<.001
Genotype.N	19	2805.825	147.675	147.67	<.001
Residual	80	80.000	1.000		
Total	119	5656.325			

Appendix 9b: Analysis of variance for root weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	19	63125.825	3322.412	1916.78	<.001
N	1	5978.408	5978.408	3449.08	<.001
Genotype .N	19	3508.092	184.636	106.52	<.001
Residual	80	138.667	1.733		
Total	119	72750.992			

Appendix 10b: Analysis of variance for straw weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	19	6082.425	320.128	320.13	<.001
N	1	1755.675	1755.675	1755.68	<.001
Genotype.N	19	459.825	24.201	24.20	<.001
Residual	80	80.000	1.000		
Total	119	8377.925			

7.3 Appendix C: ANOVA for yield related traits and NUE components in pot experiment

Appendix 1c: Analysis of variance for filled spikelet

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	19	17028534.4	896238.7	5366.70	<.001
N	1	16244256.7	16244256.7	97271.00	<.001
Genotype .N	19	7342232.8	386433.3	2313.97	<.001
Residual	80	13360.0	167.0		
Total	119	40628383.9			

Appendix 2c: Analysis of variance for grain yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	19	1.138E+08	5.990E+06	1.063E+05	<.001
N	1	7.624E+07	7.624E+07	1.352E+06	<.001
Genotype .N	19	5.172E+07	2.722E+06	48284.70	<.001
Residual	80	4.510E+03	5.637E+01		
Total	119	2.418E+08			

Appendix 3c: Analysis of variance for Harvest index

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	19	11630.417	612.127	211.10	<.001
N	1	4037.284	4037.284	1392.32	<.001
Genotype .N	19	3916.322	206.122	71.08	<.001
Residual	80	231.975	2.900		
Total	119	19815.999			

Appendix 4c: Analysis of variance for NUE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	19	1.227E+05	6.458E+03	2.158E+05	<.001
N	1	1.715E+04	1.715E+04	5.730E+05	<.001
Genotype .N	19	2.730E+04	1.437E+03	48008.30	<.001
Residual	80	2.395E+00	2.993E-02		
Total	119	1.672E+05			

Appendix 5c: Analysis of variance for panicle length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	19	708.6029	37.2949	41.44	<.001
N	1	609.7521	609.7521	677.50	<.001
Genotype .N	19	29.2029	1.5370	1.71	0.052
Residual	80	72.0000	0.9000		
Total	119	1419.5579			

Appendix 6c: Analysis of variance for panicle number

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	19	1070.0333	56.3175	57.27	<.001
N	1	2669.6333	2669.6333	2714.88	<.001
Genotype .N	19	282.0333	14.8439	15.10	<.001
Residual	80	78.6667	0.9833		
Total	119	4100.3667			

Appendix 7c: Analysis of variance for spikelet/panicle

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	19	36130.200	1901.589	1768.92	<.001
N	1	19814.700	19814.700	18432.28	<.001
Genotype .N	19	13890.300	731.068	680.06	<.001
Residual	80	86.000	1.075		
Total	119	69921.200			

Appendix 8c: Analysis of variance for unfilled spikelets

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	19	1.150E+06	6.055E+04	59553.51	<.001
N	1	3.828E+06	3.828E+06	3.765E+06	<.001
Genotype .N	19	8.796E+05	4.630E+04	45538.09	<.001
Residual	80	8.133E+01	1.017E+00		
Total	119	5.858E+06			

Appendix 9c: Analysis of variance for 1000-grain weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	19	1426.800	75.095	75.09	<.001
N	1	326.700	326.700	326.70	<.001
Genotype .N	19	531.300	27.963	27.96	<.001
Residual	80	80.000	1.000		
Total	119	2364.800			

Appendix 10c: Analysis of variance for straw nitrogen concentration

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	19	15.271313	0.803753	95.31	<.001
N	1	0.604920	0.604920	71.73	<.001
Genotype .N	19	0.907247	0.047750	5.66	<.001
Residual	80	0.674667	0.008433		
Total	119	17.458147			

Appendix 11c: Analysis of variance for grain nitrogen concentration

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	19	15.806997	0.831947	617.40	<.001
N	1	1.986613	1.986613	1474.30	<.001
Genotype .N	19	2.055987	0.108210	80.30	<.001
Residual	80	0.107800	0.001348		
Total	119	19.957397			

Appendix 11c: Analysis of variance for nitrogen uptake efficiency

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	19	1.8119584	0.0953662	209.43	<.001
N	1	0.0080325	0.0080325	17.64	<.001
Genotype. N	19	0.2396036	0.0126107	27.69	<.001
Residual	80	0.0364296	0.0004554		
Total	119	2.0960241			

Appendix 12c: Analysis of variance for nitrogen utilization efficiency

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	19	1856390.75	97704.78	1331.04	<.001
N	1	281538.76	281538.76	3835.42	<.001
Genotype .N	19	199612.00	10505.89	143.12	<.001
Residual	80	5872.40	73.41		
Total	119	2343413.91			

7.4 Appendix D: Analysis of variance for yield related traits and NUE under field experiment

Appendix 1d: Analysis of variance for filled spikelet

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	6.672E+01	3.336E+01	571.86	
Rep.N stratum					
N	1	1.694E+07	1.694E+07	2.904E+08	<.001
Residual	2	1.167E-01	5.833E-02	0.37	
Rep.N.Genotype stratum					
Genotype	19	1.768E+07	9.307E+05	5.977E+06	<.001
N.Genotype	19	7.067E+06	3.719E+05	2.389E+06	<.001
Residual	76	1.183E+01	1.557E-01		
Total	119	4.169E+07			

Appendix 2d: Analysis of variance for grain yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	8.645E+01	4.322E+01	57.00	
Rep.N stratum					
N	1	7.889E+07	7.889E+07	1.040E+08	<.001
Residual	2	1.517E+00	7.583E-01	0.71	
Rep.N.Genotype stratum					
Genotype	19	1.156E+08	6.087E+06	5.685E+06	<.001
N. Genotype	19	5.238E+07	2.757E+06	2.575E+06	<.001
Residual	76	8.137E+01	1.071E+00		
Total	119	2.469E+08			

Appendix 3d: Analysis of variance for harvest index

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	52.734	26.367	7.23	
Rep.N stratum					
N	1	5546.849	5546.849	1521.16	<.001
Residual	2	7.293	3.646	2.57	
Rep.N.Genotype stratum					
Genotype	19	9044.173	476.009	335.67	<.001
N.Genotype	19	3031.348	159.545	112.51	<.001
Residual	76	107.775	1.418		
Total	119	17790.173			

Appendix 4d: Analysis of variance for NUE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	161.69	80.85	0.97	
Rep.N stratum					
N	1	571.86	571.86	6.87	0.120
Residual	2	166.53	83.27	1.00	
Rep.N.Genotype stratum					
Genotype	19	247705.08	13037.11	156.42	<.001
N.Genotype	19	129154.56	6797.61	81.56	<.001
Residual	76	6334.52	83.35		
Total	119	384094.25			

Appendix 5d: Analysis of variance for panicle length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	70.3500	35.1750	4221.00	
Rep.N stratum					
N	1	625.6333	625.6333	75076.00	<.001
Residual	2	0.0167	0.0083	0.04	
Rep.N.Genotype stratum					
Genotype	19	984.0333	51.7912	262.99	<.001
N.Genotype	19	42.7000	2.2474	11.41	<.001
Residual	76	14.9667	0.1969		
Total	119	1737.7000			

Appendix 6d: Analysis of variance for panicle number

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	66.6167	33.3083	3997.00	
Rep.N stratum					
N	1	2520.8333	2520.8333	3.025E+05	<.001
Residual	2	0.0167	0.0083	0.04	
Rep.N.Genotype stratum					
Genotype	19	1308.1333	68.8491	326.35	<.001
N.Genotype	19	357.8333	18.8333	89.27	<.001
Residual	76	16.0333	0.2110		
Total	119	4269.4667			

Appendix 7d: Analysis of variance for Spikelet/ panicle

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	78.0500	39.0250	520.33	
Rep.N stratum					
N	1	16708.8000	16708.8000	2.228E+05	<.001
Residual	2	0.1500	0.0750	0.43	
Rep.N.Genotype stratum					
Genotype	19	38403.6333	2021.2439	11696.54	<.001
N.Genotype	19	16381.5333	862.1860	4989.30	<.001
Residual	76	13.1333	0.1728		
Total	119	71585.3000			

Appendix 8d: Analysis of variance for unfilled spikelet

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	7.035E+01	3.517E+01	201.00	
Rep. N stratum					
N	1	3.998E+06	3.998E+06	2.284E+07	<.001
Residual	2	3.500E-01	1.750E-01	1.43	
Rep. N. Genotype stratum					
Genotype	19	1.130E+06	5.946E+04	4.859E+05	<.001
N. Genotype	19	9.181E+05	4.832E+04	3.949E+05	<.001
Residual	76	9.300E+00	1.224E-01		
Total	119	6.046E+06			

Appendix 9d: Analysis of variance for 1000-grain weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	19.2667	9.6333		
Rep.N stratum					
N	1	567.6750	567.6750		
Residual	2	0.0000	0.0000	0.00	
Rep.N.Genotype stratum					
Genotype	19	1472.4917	77.4996	83.27	<.001
N.Genotype	19	316.8250	16.6750	17.92	<.001
Residual	76	70.7333	0.9307		
Total	119	2446.9917			

Appendix 10d: Analysis of variance for grain nitrogen concentration

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	9.032E-03	4.516E-03	126.02	
Rep.N stratum					
N	1	1.697E+00	1.697E+00	47356.49	<.001
Residual	2	7.167E-05	3.583E-05	0.99	
Rep.N.Genotype stratum					
Genotype	19	1.713E+01	9.017E-01	24799.00	<.001
N.Genotype	19	8.821E-01	4.643E-02	1276.83	<.001
Residual	76	2.763E-03	3.636E-05		

Total	119	1.972E+01

Appendix 11d: Analysis of variance for nitrogen uptake efficiency

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.0048305	0.0024152	6.37	
Rep.N stratum					
N	1	0.0249399	0.0249399	65.78	0.015
Residual	2	0.0007582	0.0003791	3.27	
Rep. N. Genotype stratum					
Genotype	19	1.0152424	0.0534338	460.45	<.001
N.Genotype	19	0.2469330	0.0129965	111.99	<.001
Residual	76	0.0088196	0.0001160		
Total	119	1.3015237			

Appendix 12d: Analysis of variance for nitrogen utilization efficiency

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.

Rep stratum	2	1951.37	975.68	3.10	
Rep.N stratum					
N	1	316753.42	316753.42	1004.80	<.001
Residual	2	630.48	315.24	13.74	
Rep.N.Genotype stratum					
Genotype	19	1714581.92	90241.15	3933.13	<.001
N.Genotype	19	177521.76	9343.25	407.22	<.001
Residual	76	1743.73	22.94		
Total	119	2213182.69			

7.5 Appendix E: Diversity in grain colour of some landraces used