एमऐएस-व्युत्पन्न *lpa1* और *lpa2* आधारित कम फाइटिक एसिड मक्का जीनोटाइप का रूपात्मक और आणविक लक्षण वर्णन

Morphological and molecular characterization of MAS derived *lpa1* and *lpa2* based low phytic acid genotypes in maize

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Morphological and molecular characterization of MAS derived *lpa1* and *lpa2* based low phytic acid genotypes in maize

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SHRIDHAR RAGI

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CERTIFICATE

This is to certify that the thesis entitled **“Morphological and molecular characterization of MAS derived *lpa1* and *lpa2* based low phytic acid genotypes in maize”** submitted to the Faculty of Post Graduate School, Indian Agricultural Research Institute, New Delhi, in partial fulfillment of requirement of degree of **Master of Science** in **GENETICS AND PLANT BREEDING,** embodies the result of a bonafide research work carried out by **Mr. SHRIDHAR RAGI** (Roll No. 60035) under my guidance and supervision. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that any help or source of information, as has been availed of in this work, has been duly acknowledged.

Date : 26.09.2020
Place: New Delhi - 12

(VIGNESH, M)
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To my beloved parents

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"Success is a point where God's grace and a man's hard work will meet up"

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ABBREVIATIONS

µg	:	Microgram
µl	:	Microliter
AICMIP	:	All India Coordinated Maize Improvement Project
BHT	:	β-hydroxytoluene
CGIAR	:	Consultative Group on International Agricultural Research
CTAB	:	Cetyl Trimethyl Ammonium Bromide
DBT	:	Department of Biotechnology
DNA	:	Deoxyribo Nucleic Acid
dNTP	:	deoxynucleotide 5' triphosphate
DST	:	Department of Science and Technology
EDTA	:	Ethylene Diamine Tetra-acetic Acid
EtBr	:	Ethidium Bromide
FAO	:	Food and Agriculture Organization
HCl	:	Hydrochloric acid
IARI	:	Indian Agricultural Research Institute
ICAR	:	Indian Council of Agricultural Research
IIMR	:	Indian Institute of Maize Research
InDel	:	Insertions and Deletions
iP	:	Inorganic phosphorous
TP	:	Total Phosphorous
lpa	:	low phytic acid
MABB	:	Marker-Assisted Backcross Breeding
MAS	:	Marker-Assisted Selection
mg	:	Milligram
ml	:	Millilitre
Mm	:	Millimolar
NaOH	:	Sodium Hydroxide
PA	:	Phytic acid
PCR	:	Polymerase Chain Reaction
PIC	:	Polymorphism Information Content
QPM	:	Quality Protein Maize
Rpm	:	rounds per minute
SDS	:	Sodium dodecyl sulphate
SERB	:	Science and Engineering Research Board
SSR	:	Simple Sequence Repeats
TBME	:	tert-butyl-methyl ether
TE	:	Transposable Element
UHPLC	:	Ultra High Performance Liquid Chromatography
USDA	:	United States Department of Agriculture
WHO	:	World Health Organization

1 INTRODUCTION

Significant progress in agricultural research and development resulted in manifold enhancement in food-grain productions worldwide, however, more than half the world's population is affected by micronutrient malnutrition which is also know as as "hidden hunger" (Prasanna et el. 2020). Micronutrient malnutrition is a nutritional problem for two billions people of the world, and is particularly prevalent in developing countries such as Asia and Africa, where populations are dependent on cereal food grains that are deficient in micronutrients (Pfeiffer and McClafferty 2007). Micronutrients are beneficial and required in low amount but play critical function in the growth and development of human beings. Since human body cannot synthesize micronutrients and therefore has to rely on balanced diet (Dutta et al. 2020).

Micronutrients play vital roles in cellular functions, immunity reproductive healthand cognitive functions (Guerrant et al. 2000). Suboptimal consumption of micronutrients affects biological functions leading to poor health condition, impaired growth, under developed cognitive response, reproductive performance and work productivity, besides socio-economic losses (Bouis 2002 ; Welch and Graham 2004). Among the different micronutrients, the effects of iron (Fe)- and zinc (Zn)- deficiency have been quite prominent (Abhijith et al. 2020); and over 60% of the world's population are Fe deficient, and it is 30 % for Zn (White and Broadley 2009)

These deficiencies results in poor health of people irrespective of age, but their effects prominent in pregnant women and children, particulary infants. Hence efforts were made to increase dietary Fe and Zn by utilizing fortification, supplementation and diversification of diets. These strategies had limited success in resource-limited environments and poor countries where health care and food processing facilities are not well organized. Hence, genetic improvement also known as 'crop biofortification' of staple crops for the target micronutrient is a viable alternative approach, as delivery of Fe and Zn rich staple product is achieved through the development and promotion of new plant varieties that are aimed to alleviate the deficiencies (Tako et al. 2013).

Maize is the third most important cereal crop in the country next wheat and rice. Owing to its highest genetic yield potential among the cereals, maize is known as the 'queen of cereals'. Maize is the staple food for 310 million people over 20 countries, contributing 20-30% of total calories in human diets (Shiferaw et al. 2011). Maize occupies an important place in Indian agriculture with cultivated area of 9.6 m ha and annual production of 27.14 million metric tonnes (USDA 2019). About 20% of maize in the country is consumed as food and processed products, while 60% as feed and 20% as industrial products. (Yadav et al. 2016). Significant genetic variation has been reported for Znand Fein Indian maize germplasm(Gupta et al. 2015), but considerable achievement in terms of cultivars could not be made as the trait is governed by many genes with minor effects and is highly influenced by environment (Gupta et al. 2015).

Bioavailability is defined as the amount of nutrients isreadily available for human bodyabsorption and utilization in metabolic processes. The supply of nutrients to the human body not only depends on the amount of the nutrient in a food, but also on its bioavailability (Gupta et al.2014). The bioavailability of the nutrients in the human body is influenced by many different factors. Anti-nutritional factors like phytic acid, tannins, oxalates and dietary fibres play a key role in reducing the bioavailability of micronutrients to the human and animals. Among them, phytates play a significantpart in lowering the bioavailability of Zn and Fe in humans (Adams 2000). Hence, bringing down the phytic acid levelin maize would be an important approachfor biofortification (Gupta et al. 2015).

Phytic acid is myo-inositol hexakisphosphate, Ins(1,2,3,4,5,6)P6, or InsP6, is the primary storage compound of phosphorus in seeds contributing about 80% of the total phosphorus in seed and amounting as much as 1.5% of seed dry weight (Raboy 2000). The level of inorganic phosphorous in the seeds and seedlings are also regulated by the phytic acid (Strother 1980). The negatively charged phosphate in the phytic acid make the metallic cations insoluble by binding to them, leading to their non-availability in the human gut. When released during food digestion, phytic acid binds to minerals and makes them unavailable and therefore phytic acid is considered as an anti-nutritional factor (Zhou and Erdman 1995). Besides, phytic acid is also poorly digested in the monogastric animals limiting their growth. Moreover, excreta of monogastric animals possessed undigested phytic acid that increases the phosphorous in the environment and through eutrophicationphenomenon leads to water pollution through (Cromwell and Coffey 1991).

Reduction of phytic acid increases bioavailability of micronutrients leading to improvement of nutritional value of meal. Further, reduction of phytic acid through genetic methods can be considered as a feasible alternative as it offers a sustainable solution to alleviate malnutrition. Low phytic acid (*lpa*) mutants have been discovered in many crops, however they were isolated in maize for first time (Raboy 2000). These *lpa* mutations affectdifferent levels in the phytic acid biosynthesis pathway thereby lowering the levels of phytic acid (Raboy et al. 2000). Levels of total phosphorous remains same except for the decreased phytic acid in the seeds. The phosphorous uptake and its subsequent transport to maturing seeds is not affected in these mutations, but there is blockage in themetabolization of phosphorous into phytic acid in the seed (Pilu et al. 2002). Several *lpa* mutants have been isolated in maize viz. *lpa1*, *lpa2*, *lpa3*and *lpa241*. Out of which, *lpa1-1* mutation leads to 55-65% reduction of phytic acid in maize seed and is due to mutation in trans-membrane transporter protein (MRP). The *lpa2-1* due to mutation in inositol phosphate kinase (IPK) enzyme,which leads to 50% reduction(Raboy et al. 2000). These mutants can be used as a best source in the breeding programme for development of *lpa*-based elite maize lines.

Very few *lpa*-based maize genotypes in subtropically adapted background are available,whichis serious concern in maize improvement programme. Efforts are being made at ICAR-Indian Agricultural Research Institute, New Delhi to develop *lpa*-based maize inbreds and hybrids through marker-assisted selection (Bhatt et al. 2018). Functional markers have been developed and reported for *lpa1-1* and *lpa2-1* genes, and several *lpa*-based inbreds have been developed from the F_2 segregants between normal and *lpa*-donor inbreds (Abhijith et al. 2020). These *lpa*-based inbreds are required to be characterized for their agronomic, yield performance and yield contributing traits. Further, molecular characterization of these inbreds using molecular markers would lead to their enhanced utility in the maize hybrid breeding programme. Keeping this in view, the study has been aimed:

1. To evaluate the *lpa1* and *lpa2*-based maize inbreds for, agronomic, yield and yield-related traits
2. To analyse the genetic diversity among *lpa1* and *lpa2* -based low phytic acid maize inbred using microsatellite markers

2

REVIEW OF LITERATURE

2.1 Importance of maize

Maize is a crop of global importance owing to its multiple purposes such as human food and livestock feed; and serves as raw material for industrial products (Hossain et al. 2019a). In addition, maize acts as a model organism for biological research around the world (Hossain et al. 2019b). A major portion of maize produced worldwide is used for animal consumption due to high nutritional value of maize (Muthusamy et al. 2016). It also serves as a vital source of proteins and calorie to billions of people in developing countries, particularly in Africa, Mesoamerica and Asia (Shiferaw et al. 2011). Forecasts suggest that the demand for maize will be doubled by the middle of this millennium due to increasing demand both in the poultry industry and human food chain (Prasanna et al. 2020; Rosegrant et al. 2009).

Globally, 1108.62 million metric tonnes (MMT) of maize grains produced by over 165 countries from an area of 192 million hectare (m ha) with an average productivity of 5.77 tonnes per ha (USDA 2019). After rice and wheat, maize is most important food grain crop in India ranking in third among cereals (Gupta et al. 2019). It is cultivated in 9.6 m ha area and having production of 27.14 MMT with productivity of 3.05 tonnes per ha in the country (USDA 2019). Maize is a major staple food with very high per capita consumption in various developing nations (Prasanna et al. 2020). By 2050, maize consumption at global level is expected to be increased from 32 to 52 kg/person/year.

Maize grain constitutes endosperm (82%), germ (12%) and pericarp (6%). The main portion of the endosperm is starch as in other cereals, constituting an average grain size of 70%, while 10% of the grain is protein (Prasanna et al. 2001). It also contains oil (4%), crude fibre (2.3%), albuminoides (10.4%) and ash (1.4%) constituents. Apart from these, it also has more provitamin A, vitamin B2 & B3, and vitamin E) compared to other cereals (Nuss and Tanumihardjo 2010). Maize is also dubbed as a “Poor Man’s Nutri-cereal” as it contains starch which constitutes 72 to 73% of kernel weight (Nuss and Tanumihardjo 2010). Other carbohydrate fractions like glucose, sucrose and fructose are present in 1 to 3 % of the kernel weight. Protein content varies from 8 to 11% of the kernel weight and major portion of the protein is located in the endosperm. Oil content varies from 3 to 18%, located mostly in the embryo. The embryo has comparatively high content of minerals compared to endosperm i.e. about 78% of the total minerals localized in the embryo itself. Phosphorous is the most dominant mineral in the kernel where it is found as phytate salts of Mg, Fe, Ca, and Zn. Micronutrients are present in very less amount in maize grain as in the case of other cereals (Gupta et al. 2015a & b).

2.2 Micronutrient malnutrition

Micronutrients are one of the essential players in the metabolic activities of the human body (Bendik et al. 2014); and these have to be met from foods through the diet. However, less than the recommended levels of micronutrients in staple food have resulted in malnutrition in large population. Even though considerable advances have been made in agricultural research and technological developments, malnutrition remains a widespread problem (Neeraja et al. 2017). Micronutrient malnutrition is a serious global health problem more prevalent in underdeveloped and developing countries; it is mostly known as “hidden hunger” (Bouis and Saltzman 2017). A total of two billion people is affected, and of this, nearly 820 million-people are under-nourished. From among the total, 667 million-children under the five year of age, 155 million are stunted and 52 million possessed abnormal height-weight ratio (Global Nutrition Report 2018). 45% death of children under the five year of age is associated with micronutrient deficiency (Black et al. 2013). 11% loss in the annual gross domestic product (GDP) is reported in Asia & Africa. It also causes loss to annual GDP in some African countries to an extent of as high as 16.5%. As per the estimate, every $1 investment in nutrition programme offers benefits of $16 (Global Nutrition Report 2017). Thus, the efforts attempted towards the balanced and nutritious food development and dissemination assume great importance.

The effects of zinc (Zn) andiron (Fe) deficiency are quite prominent among the different micronutrients (Dalmiya and Schultink 2003). More than 30% of the world's seven billion people are deficient in Zn, while Fe accounts for 30% (White and Broadley 2009). These deficiencies affect irrespective of age, but their effects are prominent in pregnant women and children. Micronutrients mainly play important role in cellular and immunity, cell signalling and function, reproductive health, work capacity, learning and cognitive functions (Guerrant et al. 2000; Kapil and Bhavna 2008). The balanced food with micronutrients needs to be consumed as human body cannot synthesize micronutrients. Lack of micronutrients in food leads to adverse metabolic conditions resulting in poor physical and mental health (Bouis 2002; Welch and Graham 2004).

2.3 Role of iron and its deficiency symptoms

The human body needed iron (Fe) for healthy development of the muscle, blood cells especially RBCs and brain (Roeser 1986). It is oxygen carrier to the cells, electrons transport medium and major part of enzymes (Brock et al. 1994; Hallberg 1982). Fe is mostly integral part of haemoglobin in the myoglobin and erythrocytes, Fe-containing oxygen storage protein. These enzymes are required for energy transfer during the oxidative metabolism in the cells (Mascotti et al. 1995). Fe-deficiency induces anaemia among the people and it is important symptom of Fe-deficiency in body (DeMaeyer and AdielsTegman

1985). The Fe-deficiency is found mostly in children, infants, pregnant women and adolescents (Lozoff et al. 1991).

2.4 Role of zinc (Zn) and its deficiency symptoms

Zn is an essential micronutrient for plants, animals and humans for many biological functions; and is part of >300 enzymes in humans. These enzymes required for the metabolism of proteins, lipids, nucleic acids and carbohydrates (Sandstorm 1997). It is important for maintenance of cell- and organ- integrity in the human body. Furthermore, in the process of transcription and gene expression, Zn is required (Sandstorm 1997). It also required for proper immune response, cellular and humoral immunity (Hambidge 1987; Shankar and Prasad 1998).

As per the World Health Organization (WHO) estimates, Zn-deficiency poses serious issue to 17% of the global population particularly prevalent in Africa, the eastern Mediterranean and South and Southeast Asia (Caufield et al. 2004). The risk of, pneumonia, diarrhoeal disease and malaria in young children is associated with Zn-deficiency. Major Zn-deficiency is accompanied with skin disorders, impaired immunity, short stature, hypogonadism, anorexia cognitive dysfunction (Brown et al. 1998; Goldenberg et al. 1995). Besides, severe depression and mental illness, and altered reproductive biology are also associated with Zn-deficiency (Solomons 2003).

2.5 Development of maize enriched with Fe and Zn

The development of maize enriched with Fe and Zn would improve the human health as maize is being grown and consumed across the countries. Biofortification of maize with Fe and Zn by plant breeding promises sustainable cost-effective solution to micronutrient malnutrition (Bouis 2018; Mayer et al. 2008). Significant variation has been observed for both nutrients in maize grain that can be used in biofortification. Genetics analysis of their accumulation in endosperm have also been established with identification respective QTL/genes. In addition, the concentration of the same in maize endosperm can be increased by gene introgression imparting their uptake and transport. The recommended level of Fe on dry weight basis (dwb) (with 5% bioavailability and 90% retention after processing) is 60 µg/g by estimated average requirement (EAR) of 1,460 µg/day of Fe for non-pregnant and non-lactating women (Bouis and Welch 2010) while with EAR of 1,860 µg/day and with25% bioavailability and 90% retention, the same has been fixed at 38 µg/g for Zn (Gupta et al.2015b).

2.6 Indirect approaches to develop maize enriched with Fe and Zn

Indirect approach is related to increase the bioavailability of these essential minerals. Even though, we can increase the concentration of these minerals in the maize grain, but bioavailability of these minerals is very less in the human gut when consumed. “Bioavailability is defined as the amount of a micronutrient that is available for absorption in the gastrointestinal tract and in turn utilized for normal metabolic function (Welch and Graham 2004)”. The bioavailability of Fe is only 5% and that for Zn is 25% in the gut of the human (Bouis et al.2011). Different factors such as status of nutrients in the body, food type, state of health, competition for the common transporters among nutrients, gut-pH level, food timing in gut, and different anti-nutritional and promoting factors that determine the levels of bioavailability of Fe and Zn (Bohn et al. 2004; Frossard et al. 2000; Lopez et al. 2002; Welch and Graham 2004).

The anti-nutritional substances which reduces the bioavailability of nutrients includes oxalic acids, haem-agglutinins, goitrogen, heavy metals, tannins and polyphenols; and fibres act as anti-nutrients and lowers the Fe- and Zn-bioavailability (Ortiz-Monasterio et al. 2007; Welch and Graham 2004). Among various anti-nutritional components, phytic acid/phytate is responsible for lowering the Fe- and Zn- bioavailability.

The factors which promotes the bioavailability of micronutrients includes carotenoids; organic acids like ascorbic acid, fumarate, malate and citrate; certain amino acids like methionine, histidine, cysteine and lysine; and inulin and other non-digestible carbohydrates enhance the bioavailability of Fe and Zn (Bouis and Welch 2010; Graham et al. 2001; Ortiz-Monasterio et al. 2007; Welch and Graham 2004). Haemoglobin also increases the absorption of Fe while long-chain fatty acids like palmitic acids enhance the Zn-bioavailability (Bouis and Welch 2010).

Hence, the bioavailability of these minerals can be increased by breeding for enhanced promoting factors and reduced anti-nutritional factors (Gupta et al. 2015b). Several studies suggested the synergistic effect of *opaque2 (o2)* gene for improving the amount of zinc in maize (Arnold et al. 1977; Chakraborti et al. 2009; Welch et al. 1993). There is an increase in Zn-absorption from 64% to 69% with supplementation of lysine (House et al. 1996). Therefore, improvement of lysine in maize has capacity to improve kernel Zn-concentration and -absorption through digestion. In maize, 1.8-foldincrease of Fe absorption has been observed (Garcia-Casal et al. 2000) due to the presence of β-carotene. Also, lutein addition in diet prepared from maize increased the Fe-bioavailability by two-folds (Garcia-Casal 2006). The basic mechanism of reduction of micronutrient bioavailability mainly because of chelating or reducing the solubility of the nutrients.

2.7 Phytic acid

Phytic acid (myo-inositol-1,2,3,4,5,6-hexakisphosphate, InsP6) is present in plant/seed tissues and also in cells of eukaryotes (Sasakawa et al. 1995). Phytic acid is the most abundant source of phosphorus (P) in plants, it found in seeds with nearly 85% of total phosphorus and 1000-folds higher than those found in vegetative tissues. Total P concentration typically ranges 3.0-4.0 mg/gm with phytic acid P ranging 2.0-3.0 mg/gm in seed. When synthesized, most phytic acid P is deposited as salt of K, Mg, Fe and Zn, known as phytin (Lott 1984). In cereals, most of the phytates are localized in the germ and aleurone tissues (O'Dell et al. 1972). During germination, phytate salts are broken down by the action of phytases, releasing their P and myo-inositol for use by the growing seedling. Nearly 80% of phytic acid is contained in the aleurone and bran parts of barley, wheat and rice; only a small amount accumulates in the embryo. On the contrary, 80% of phytate distribution in maize grain deposited in scutellum and embryo.

Phytic acid readily precipitates in the form of phytate salts which is highly negatively charged and hence binds to essential mineral cations such as Fe, Zn, K, Ca and Mg. In the guts of monogastric animals including humans, lacks the enzyme phytases and therefore unable to digest seed phytate. Hence, undigested phytic acid reduces the nutritional value of seeds by decreasing P and micronutrient bioavailability (Boling et al. 2000; Maenz and Classen 1998; Pilu et al. 2003; Schroder et al. 1996; Singh et al. 2011; Wodzinski and Ullah 1996).

The major portion of P taken up by plant roots is ultimately packaged into seed phytic acid P. This single small molecule will act as a bottleneck in the movement of P in the world's agricultural ecology. It was recently estimated that the global annual production of seed phytic acid P by crops represents a sum equivalent to more than 50% of total elemental P applied in mineral fertilizers worldwide (Lott et al. 2000).

However, metabolism of phytic acid is crucial for cell signalling, growth &development and ribonucleic acid (RNA) export (Safraney et al. 1999; Sasakawa et al. 1995; York et al. 1999). It is also actively involved in plants' defence response against biotic and abiotic stresses (Graham et al. 2001; Welch and Graham 2004). In addition, it is also important for increased seedling-vigour and reduced-grain aflatoxin production (Morris 1995). Phytate is acts as scavenger for seed against oxidative stress damage (Doria et al. 2009). Therefore, the development of maize genotypes with low phytic acid and higher yield is a challenging task.

2.8 Phytic acid in maize

Phytate content is a serious concern in maize as around 80% seed phytate is located in the germ of the maize (O'Dell et al. 1972). This is an undesirable character in maize since phytate cannot be completely removed through physical processing such as milling or hulling. In other cereal crops, phytates are mainly found in the bran fraction hence it can be easily removed. In maize, concentration of phytic acid phosphorous varied from 2.4 to 4.1 mg/g and the inorganic phosphorous varied from 0.2 to 1.1 mg/g (Lorenz et al. 2007). Kasim and Edwards (1998) reported as high as 6.39 g of phytic acid/ 100g dry weight, which is far higher than the same in other food-grains. Maize is one of the major food-grains in India, it is also widely used as feed additive or as a raw feed in poultry. Therefore, reducing phytate content in maize grain stands by higher priority.

2.9 Effect of phytic acid on human and animal nutrition

Maize is an important source of protein, carbohydrate, vitamins and minerals as it is used by 63% of the world as food and poultry feed (Shah et al. 2016). However, phytic acid-anti-nutritional factor in maize reduces the bioavailability of minerals thereby reducing nutritional value. In its physiological pH, phytic acid forms a polyanion (negatively charged) and strong chelator of Fe and Zn making them insoluble and unavailable (Raboy 2000).

On consumption, phytic acid binds to minerals and endogenous minerals in the gut. Due to the lack of phytase enzyme in humans and poultry, unable to digest this phytic acid-mineral complex and hence excreted as salts (Raboy 2000). Therefore, phytic acid is considered as an anti-nutritional factor that affects the micronutrient bioavailability (Zhou and Erdman 1995). This issue is of great concern in the maize dependent poultry sector. In poultry, to enhance the body weight and feed conversion ratio, fungal phytase supplementation is practiced which increase the input cost (Lelis et al. 2012).

2.10 Effect of phytic acid on environment

The multivalent metal ions mainly Zn, Mg, Fe form the complex due the stronger ability of phytic acid to chelate them. The binding can result in highly insoluble salts that are poorly absorbed from the gastrointestinal tract, which results in poor bioavailability of minerals. The monogastric animals such as humans and poultry lack phytase enzyme in digestive track so that when they consume food or feed with phytic acid, they unable to digest and directly expelled into the environment in their manure (Boling et al. 2000; Maenz and Classen 1998; Schroder et al. 1996; Singh et al. 2011; Wodzinski and Ullah 1996). Monogastric animals are releasing nearly 70% of total P in feed in excreta due to inability of digestion (Milko et al. 2008). Accumulation of phytate and inorganic phosphate through leaching or surface run-off, causes eutrophication of surface water and algal blooms (Milko et al. 2008; Turner and Haygarth 2000), hypoxia, death of fish and aquatic animals and production of nitrous oxide, a potent greenhouse gas (Mallin 2000; Naqvi et al. 2000). The livestock industry growth is expected to accelerate such environmental problems. Animal feed supplementation with phytase improves the P bioavailability and lowers the P excretion

(Yano et al. 1999). It lowersP excretion by 30% to 50% (Greiner and Carlsson 2006; Haefner et al. 2005; Lie and Porres 2003; Selle and Ravindran 2007).

2.11 Biosynthesis pathway of phytic acid

In plants, biosynthesis of phytic acid happens by two pathways: a “lipid-dependent” pathway, ubiquitous in eukaryotes, and operates in all plant tissues (Figure 2.1). A highly conserved reaction in which the enzyme, D-myo-inositol 3-phosphate synthase (MIPS) converts D-glucose-6-phosphate into myo-inositol 3-phosphate (Ins(3)P1) requires de novo production of myo-inositol (hereafter referred to as "Ins"). Myo-inositol 3-phosphate is then converted to myoinositol derivatives and finally to phytic acid by series of intermediate steps. Second pathway “lipid-independent” pathway is entirely independent from inositol lipid synthesis and consists of sequential phosphorylation of the Ins ring to InsP6 which finally is converted to phytic acid through the action of inositol phosphate kinases. In vacuoles, phytic acid is stored as globoids and then transported by a specific InsP6 transporter, a multidrug-resistance-associated protein (Sparvoli et al. 2015). Phytic acid biosynthesis pathway is interlinked to several other pathways such as signal transduction, vesicle trafficking and polar auxin transport, biotic and abiotic stress response, nuclear functions and regulation of phosphorus homeostasis and hormonal signalling. Hence, the knowledge on the pathway needs to be carefully considered for developing stable mutants in different crops.

2.12 Biotechnological tools for reduction of phytic acid

Genetic engineering technology can be used to lower phytic acid content through creation of the genetic engineered plants possessing modified phytase. Studies in this are resulted into development of genetic engineered lines in several crops (Brinch-Pedersen et al. 2006; Chen et al. 2008; Kuwano et al. 2009; Nunes et al. 2006).

In this context, genetic engineered rice was developed to over-express phytase encoding genes from *Aspergillus fumigatus* and *Phaseolus vulgaris* for ferritin, and a protein specific to cysteine rich methionine to increase the bioavailability of Fe to humans in rice (Lie and Porres 2003; Lucca et al. 2001). Wheat transgenic plants expressing *A. japonica phytase* gene *(phyA)* in wheat endosperm were developed. The transgenic lines showed an 18% to 99% increase in phytase activity and a 12% to 76% decrease in seed phytic acid content.

Genetic engineered maize (*phyA2* gene from *Aspergillus niger*) revealed in 50-times enhancement in phytase action compared to non-transgenic maize seeds with same per se performance (Chen et al. 2008). Drakakaki et al. (2005) also described expression of *A. niger-phytase* gene in maize resulted in 95% decrease in phytate. Hence, Genetic

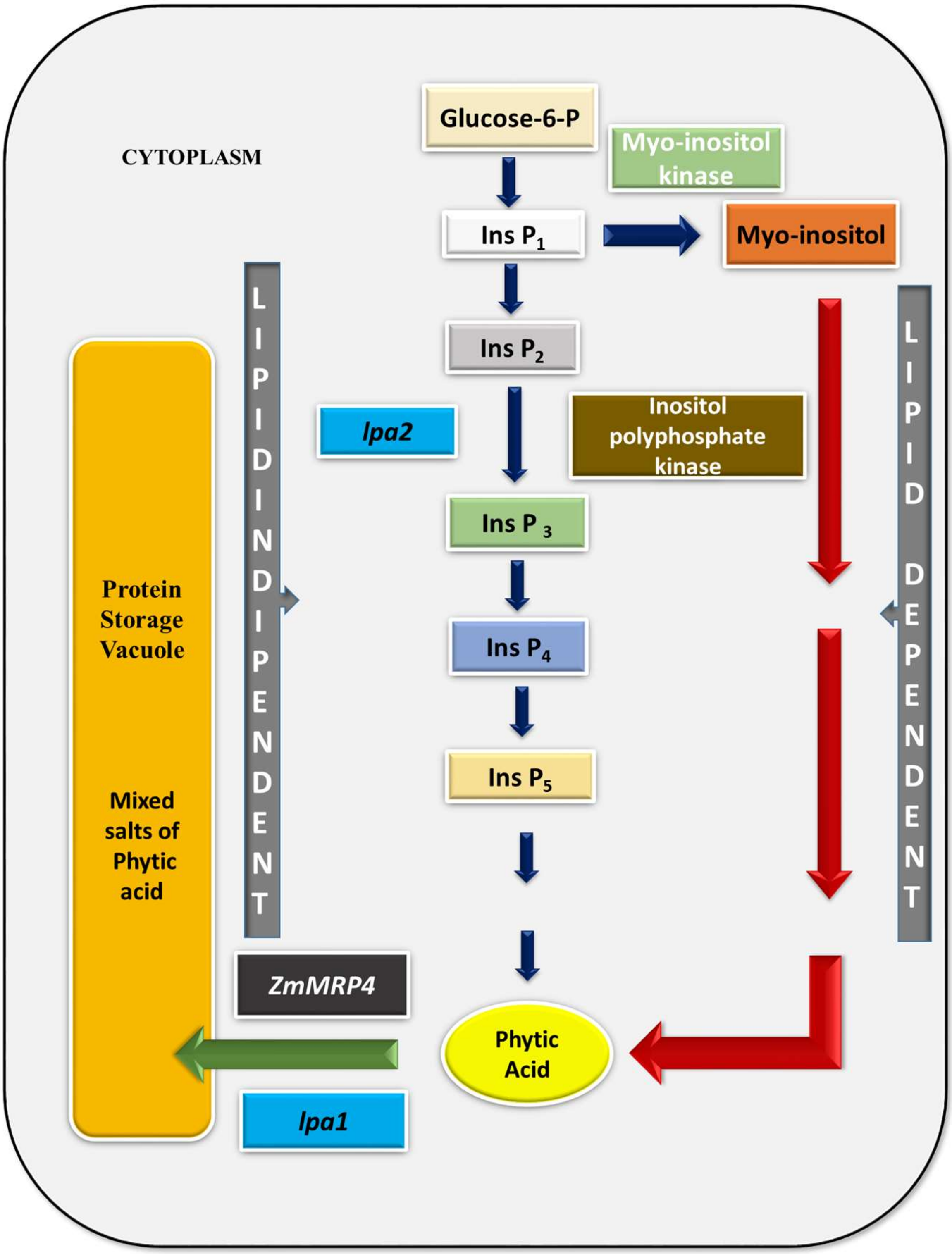

Figure 2.1 Phytic acid biosynthesis pathway in maize

engineering is also promising tool for development of maize lowered phytate. *Srpf6* and *appA* genes from *Selenomonas ruminatium* and *A. japonicas* have also been cloned into rice and 40-60 folds increase in the phytase activity was observed (Hong et al. 2004). In wheat, introduction of these genes reduced 12% to 76% of phytate in the grains (Abid 2016). Another method to reduce phytate is down regulation of genes involved in the phytic acid biosynthesis pathway. In one such attempt silencing of *IPK1* gene using RNAi mechanism in rice was found to be effective in reducing phytate as well as increasing the bioavailability of Fe, Zn and Ca significantly (Ali et al. 2013).

2.13 Breeding tools for the reduction of phytic acid

2.13.1 Use of low phytic acid (lpa) mutants

The development of low phytic acid (*lpa*) grains, resulting in increased micronutrient cation availability is considered to be a major goal in the improvement of the nutritional quality of seed crops.

Cereal *lpa* mutant isolation opens the new avenue for experimenting seed phytic acid biology, and also addressing related environmental and nutritional issues. Seeds produced by *lpa* lines contain normal total P levels, but phytic acid P levels are greatly reduced (Raboyet al. 2000). Raboy et al. (2000) were the first to isolate the *lpa* mutants in maize followed by in barley (Larson et al. 1998; Rasmussen and Hatzack 1998) and in rice (Larson et al. 2000). In maize, three *lpa* mutants have been isolated namely *lpa-1, lpa-2* and *lpa-3*.

2.13.2 lpa mutants in maize

lpa1mutant

The *lpa1* mutation was induced by using a chemical mutagen ethyl methane suphonate (EMS) and the loci mapped into the distal portion of chromosome 1S (Raboy et al. 2000). This mutation is generated by distortion in a gene, which encodes membrane transport protein which transfer phytic acid to storage vacuole (Shi et al. 2007). In mutants, transporters are defective as a result phytic acid cannot be stored inside the protein storage vacuoles, however phytic acid is synthesized and accumulated in the cytoplasm which leads to a negative feedback mechanism that suppresses the expression of genes involved in phytic acid biosynthesis. The *lpa1* mutants have 1.1 mg/g total inositol phosphate and 3.1 mg/g inorganic phosphate compared to the normal maize with 3.4 mg/g total inositol phosphate and 0.3 mg/g inorganic phosphate which is accounted for 66% reduction in the phytic acid. However, the yield of these lines has experienced 5% to 15% reduction compared to high yielding commercial checks (Raboy et al. 2000). Until now four mutant alleles of *lpa1* have been identified in maize namely *lpa1-1, lpa1- 2, lpa1-7* and *lpa1-241.*

lpa2mutant

The *lpa2* is a recessive, EMS induced low-phytic acid mutant, it was mapped on chromosome 1S. It also shows reduced phytic acid content up to 50% (Raboy et al. 2000). The *lpa2* mutant is caused by mutation in an inositol phosphate kinase gene. This mutant was induced by knocking down *ZmIpk* gene by insertion of Mu transposon in target site. In the *ZmIpk* Mu insertion mutants, seed phytic acid content is reduced approximately by 30%, and inorganic phosphate is increased by about 3-folsd (Shi et al. 2003). Cloning and sequencing of the *ZmIpk* gene from *lpa2* in the southern blot revealed that the *lpa2-1* allele is induced by the genomic sequence rearrangement in the *ZmIpk* locus, and the *lpa2-2* allele has a point mutation that produced a stop codon in the N-terminal region of the open reading frame. These results show that *ZmIpk* is one of the kinases responsible for biosynthesis of phytic acids in maize seed development (Shi et al. 2003).

lpa3 mutant

The*lpa3* mutant is a single, recessive *lpa* mutation in maize induced by chemical mutagenesis of pollen, which is mapped on chromosome 1S. Maize *lpa3* gene was found to encode myo-inositol kinase (MIK), is an enzyme which catalyzes the synthesis of Ins(3)P1. The*lpa3* gene is situated closer to *adh1*gene on chromosome 1S. The *lpa3* mutation is induced by insertion of Mu-insertion in the target site and this mutant has a phenotype of reduced phytic acid, lacks significant amounts of myo-inositol phosphate intermediates and increased myo-inositol in seeds. This insertion has disrupted the normal biosynthesis pathway and hence reduced the phytic acid content up to 50%. Throughout the development of embryos, the maize *lpa3* gene is expressed, where phytic acid is rapidly synthesized and accumulates to a significant volume (Shi et al. 2005).

lpa241mutant

The *lpa241* mutant was due to the mutation in the first enzyme of phytic acid biosynthesis pathway, myo-inositol-3-phosphate synthase (MIPS). This mutation has led to the 90% reduction of phytic acid and about a 10-fold increase in seed-free phosphate content though there has been 30% lower germination of mutants as against wild types. The mutation in MIPS causes some negative pleiotropic effects on embryo, seedling and ear growth (Pilu et al. 2003).

2.13.3 Development of low phytic acid maize

Development of low phytic acid maize is very challenging task due to the primary involvement of phytic acid in signal transduction, response to biotic and abiotic stresses (Graham et al. 2001; Welch and Graham 2004).Therefore, reduction of phytic acid is always associated with negative pleiotropic effect on plant and seed performance like reduced germination and emergence rate, susceptibility to various biotic and abiotic stresses, lower seed filling and stunted growth (Doria et al. 2009; Pilu et al. 2005;Raboy et al. 2000). To develop a commercial cultivar, low phytic acid must be accompanied with better yield performance. Thus, the exploitation of mutants like *lpa1* and *lpa2* become more viable option compared to other mutants (Raboy et al. 2000). The first effort to breed low phytic acid maize inbreds and/or hybrids was made by Ertl et al. (1998) through backcrossing methods. 14 near-isogenic maize hybrid pairs, each consisting of non-mutant and *lpa1-1* mutant were developed and evaluated. They observed little or no effect on germination potential, stand establishment, lodging, plant height, ear height, growth rate and grain moisture. However, eight *lpa1-1* hybrids exhibited an average yield loss of 5.5% as compared to the non-mutant hybrids. Recently, Beavers et al. (2015) developed low phytic acid maize population without negatively affecting seed quality parameters through selections (three rounds of selection) in synthetic populations. The *lpa* population thus developed had only 18% more phytic acid than *lpa* mutants. Recurrent selection was an alternative breeding approach to alter phytate levels in maize (Beavers et al. 2015). Phytate levels can change in populations under recurrent selection for oil concentration (Raboy et al. 1989); and phytic acid has genetic variation required to respond to selection (Lorenz et al. 2008).

The *lpa* mutants of cereal crops and legumes were developed because of the nutritional and environmental problems caused by phytate. In maize, these mutants have 30% to 90% reduction in phytate concentration (Raboy 2009). Such mutants had greater Fe-bioavailability leading to better Fe absorption by 49% (Mendoza et al. 1998) and increased utilisation of P in pigs (Hill et al. 2009). However, these kinds of mutants are usually low in seed germination, yield or dry weight, rendering them undesirable for farmers (Raboy 2009). Hence, development of low phytate lines with good agronomic performance is a challenging task for the breeders

2.14 Molecular breeding for low phytate maize

2.14.1 Molecular markers for low phytic acid

Marker-aided selection (MAS) is the best choice for the identification of the target genomic regions and quick improvement of targeted trait (Singh and Singh 2015). Quantifying the content of phytic acid is a disruptive in nature, and requires significant costs, resources and money. It is also difficult to test large numbers of samples in a breeding programme through intensive biochemical study. On the other hand, the utilization of markers to select trait in precise manner in the breeding system not only save the time, but also huge resources. Gene-based molecular markers avoid recombination between marker and gene. Functional markers are more helpful than the linked markers since the polymorphism arises from those sites within the gene which causes trait variation. SNP primers for *lpa1-1* have been designed by Naidoo et al. (2013). Sureshkumar et al. (2014) discovered the microsatellite *umc2230* markerfor the *lpa2-2*gene. But, in MAS considering the possibility of recombination, gene-based marker is better than gene-linked markers.

Recently, Abhijith et al. (2020) has developed and validated two dominant markers each specific to wild type and mutant allele SNP transition in *lpa1-1* gene sequence. Similarly, on comparison between the full-length sequence of *lpa2-1* for wild type and mutant allele revealed one transition mutation which is the basis for co-dominant CAPS marker construction, which further differentiated segregants across F_2generations. These markers for *lpa1-1* and *lpa2-1*were also validated through analysis of maize kernels for phytate and inorganic phosphorous.

2.14.2 Breeding for low phytate maize

Marker assisted backcross breeding (MABB) is an efficient method for introgression of target genes in elite background to develop nutritionally rich maize genotypes. Traditional breeding approach to develop nutritional rich genotypes is a tedious and time consuming. The introgression of recessive gene through traditional backcross method is tedious due to (i) difficulty in tracing recessive allele; (ii) 6 to 7 back cross required and (iii) cumbersome biochemical tests. Whereas MABB improves the precise of selection with less time (Collard and Mackill 2008; Gupta et al. 2013). In MABB programme, marker-assisted foreground selection traces gene of interest and marker-assisted background selection recovers the original parent genome which needs to be improved (Babu et al. 2005; Frisch et al. 1999; Gupta et al. 2013; Hospital et al. 1992; Hossain et al. 2018; Visscher et al. 1996). Use of markers for development of maize hybrids rich in nutritional quality traits viz. QPM (Babu et al. 2005; Gupta et al. 2009; Gupta et al. 2013; Hossain et al. 2018; Sarika et al. 2018) and provitamin A (Chandran et al. 2020; Goswami et al. 2019; Muthusamy et al. 2014; Zunjare et al. 2018;) have been well demonstrated in India. For low phytic acid as well, the *lpa2-2* was successfully introgressed into regionally well-adapted and productive elite inbred lines viz. UMI 395 and UMI 285 through MAS at Tamil Nadu Agricultural University (TNAU), Coimbatore (Sureshkumar et al. 2014; Tamilkumar et al. 2014).

2.15 Molecular characterization of inbreds

In any plant breeding programme, genetic diversity information based on molecular marker data is helpful in efficient management of the germplasm collections. Morphological traits are not ideal for diversity studies due to their limitation in number, effect of environment, expression at specific stage, less polymorphic and low heritability nature (smith and smith 1992), while DNA markers are free from any of the limitation which are mentioned above (Saker et al. 2005; Souza et al. 2008).

Molecular markers based genetic diversity of germplasm could be exploited for various purposes viz., (1) maintenance of existing germplasm and widening the genetic base of genetic resource; (2) classification of genotypes and heterotic grouping; (3) identification of suitable genotypes for hybrid development; and (4) development of segregating population to derive improved recombinant lines (Mohammadi and Prasanna 2003). Simple sequence repeats (SSRs) also known as microsatellite markers are made up of short stretches of tandem repeats with motifs of di-, tri- or tetra-nucleotide. SSRs are ideal for diversity analysis of genotypes due to large scale availability in genome (Matsuoka et al.2002), more variability (Schug et al. 1998;Tautz 1989), and even distribution in the genome (Senior et al. 1996). SSRs are co-dominant markers which can distinguish between homozygote and heterozygote individuals, less labour intensive, inexpensive, easy to use and can be differentiated using simple gel electrophoresis information (Chin et al. 1996 ; Pinto et al. 2003 Senior et al. 1998).About 2000 uniformly distributed SSR markers covering all the 10 chromosomes in maize have been reported and are regularly being utilized by researchers as a useful genomic resource (MaizeGDB). Genetic diversity analysis using SSR markers in maize have been rigorously used by various researchers (Bantte and Prasanna 2003; Choudhary et al. 2014; Das and Singh 2012; Das et al. 2019; Jaiswal et al. 2020; Laborda et al.2005; Mehta et al. 2016;Muthusamy et al.2015; Pandey et al.2015; Pandey et al. 2016; Rakshit et al.2011; Rief et al. 2004; Senior et al.1998; Smith et al.1997; Warburton et al. 2002; Warburton et al. 2008; Zunjare et al. 2014). SSR based genetic distance can also be used for classification of maize inbreds into different heterotic pools (Melchinger and Gumber 1998). In earlier studies, SSR markers have already been reported to identify the heterotic pattern in maize germplasm (Barata and Carena 2006; Enoki et al. 2002; Reif et al. 2004). Besides, molecular characterization of the inbreds using SSRs also helps in DNA fingerprinting essentially required for registration as a unique germplasm in the gene bank (Choudhary et al. 2015; Devi et al. 2017).

3

MATERIALS AND METHODS

3.1 Morphological evaluation of *lpa1*- and *lpa2*-based inbreds

3.1.1 Plant materials

A diverse set of 24 *lpa1*-based inbreds developed at the Maize Genetics Unit, ICAR-Indian Agricultural Research Institute, New Delhi were selected for the study (Table 3.1). Of them, seven inbreds viz. PMI-LP-101 to PMI-LP-107 were developed through marker-assisted backcross breeding, where *lpa1* allele was introgressed in different genetic backgrounds (Bhatt et al. 2018); 16 inbreds viz. PMI-LP-108 to PMI-LP-123 were developed through marker-assisted pedigree selection involving crosses between diverse inbreds and *lpa1* mutants (Abhijith et al. 2020). One inbred PMI-LP-124 has been developed from the cross between two diverse*lpa1* mutants of different genetic backgrounds.

For *lpa2*, a diverse panel of 24 inbreds developed at the Maize Genetic Unit, were also selected (Table 3.2). Of them, seven inbreds viz. PMI-LP-201 to PMI-LP-207 are developed through marker-assisted backcross breeding, where *lpa2* allele was introgressed in different genetic backgrounds (Bhatt et al. 2018) and 17 inbreds viz. PMI-LP-208 to PMI-LP-224 were developed through marker-assisted pedigree selection involving crosses between diverse wild types and *lpa2* mutants (Abhijith et al. 2020).

A set of seven wild type (high phytic acid) inbreds viz. PMI-PV5, PMI-PV6, PMI-PV7, PMI-PV8, PMI-Q1, PMI-Q2 and PMI-Q3 were also been included in the study as checks (Table 3.3).

3.1.2 Field evaluation

The *lpa1*-and *lpa2*-inbreds along with the wild type genotypes were evaluated separately at two diverse locations viz. IARI, experimental farm, New Delhi during *kharif* 2019and IIMR-Winter Nursery Centre, Hyderabad during *rabi* 2019-20. The trials were conducted in a Randomized Complete Bock Design (RCBD); where each inbred was grown in a single row of 3 metre length under two replications at spacing of 75 cm × 15 cm. The entire recommended package of practices was followed to raise the inbreds. The inbreds were evaluated for characters viz., plant height (PH), ear height (EH), days to 50% anthesis (MF), days to 50% silking (FF) and grain yield (GY). 2-3 cobs per inbred were selfed in both the trials at two locations viz. Delhi and Hyderabad for estimation of quality parameters.

3.1.3 Data analyses

3.1.3.1 Analysis of variance

Table 3.1 Details of the *lpa1*-based inbreds evaluated in the study

S. No.	Genotype	Pedigree
1	PMI-LP1-101	(PMI-PV5///A619 lpa1-1)-⊗-⊗-⊗-⊗
2	PMI-LP1-102	(PMI-PV6///A632 lpa1-1)-⊗-⊗-⊗-⊗
3	PMI-LP1-103	(PMI-PV7///A632 lpa1-1)-⊗-⊗-⊗-⊗
4	PMI-LP1-104	(PMI-PV8///A619 lpa1-1)-⊗-⊗-⊗-⊗
5	PMI-LP1-105	(PMI-Q1///A632 lpa1-1)-⊗-⊗-⊗
6	PMI-LP1-106	(PMI-Q2///A619 lpa1-1)-⊗-⊗-⊗
7	PMI-LP1-107	(PMI-Q3///A619 lpa1-1)-⊗-⊗-⊗
8	PMI-LP1-108	(PMI-PV5/A619 lpa1-1)-12-5-⊗-⊗-⊗-⊗
9	PMI-LP1-109	(PMI-PV5/A619 lpa1-1)-33-5-⊗-⊗-⊗-⊗
10	PMI-LP1-110	(PMI-PV5/A619 lpa1-1)-100-2-⊗-⊗-⊗-⊗
11	PMI-LP1-111	(PMI-PV5/A632 lpa1-1)-7-1-⊗-⊗-⊗-⊗
12	PMI-LP1-112	(PMI-PV6/A632 lpa1-1)-3-3-⊗-⊗-⊗-⊗
13	PMI-LP1-113	(PMI-PV6/A632 lpa1-1)-38-4-⊗-⊗-⊗-⊗
14	PMI-LP1-114	(PMI-PV6/A632 lpa1-1)-96-4-⊗-⊗-⊗-⊗
15	PMI-LP1-115	(PMI-PV7/A619 lpa1-1)-60-1-⊗-⊗-⊗-⊗
16	PMI-LP1-116	PMI-PV7/A632 lpa1-1)-8-2-⊗-⊗-⊗-⊗
17	PMI-LP1-117	PMI-PV7/A632 lpa1-1)-67-2-⊗-⊗-⊗-⊗
18	PMI-LP1-118	(PMI-PV8/A619 lpa1-1)-31-2-⊗-⊗-⊗-⊗
19	PMI-LP1-119	(PMI-PV8/A619 lpa1-1)-31-5-⊗-⊗-⊗-⊗
20	PMI-LP1-120	(PMI-PV8/A619 lpa1-1)-100-2-⊗-⊗-⊗-⊗
21	PMI-LP1-121	(PMI-Q3/A619 lpa1-1)-6-1-⊗-⊗-⊗
22	PMI-LP1-122	(PMI-Q3/A619 lpa1-1)-63-4-⊗-⊗-⊗
23	PMI-LP1-123	(PMI-Q3/A619 lpa1-1)-7-2-⊗-⊗-⊗
24	PMI-LP1-124	(A619 lpa1-1/A632 lpa1-1)- ⊗-⊗-⊗-⊗

Table 3.2 Details of the *lpa2*-based inbreds evaluated in the study

S. No.	Genotype	Pedigree
1	PMI-LP2-201	(PMI-PV5///A619 lpa2-1)-⊗-⊗-⊗-⊗
2	PMI-LP2-202	(PMI-PV6///A619 lpa2-1)-⊗-⊗-⊗-⊗
3	PMI-LP2-203	(PMI-PV7///A619 lpa2-1)-⊗-⊗-⊗-⊗
4	PMI-LP2-204	(PMI-PV8///A619 lpa2-1)-⊗-⊗-⊗-⊗
5	PMI-LP2-205	(PMI-Q1///A619 lpa2-1)-⊗-⊗-⊗-⊗
6	PMI-LP2-206	(PMI-Q2///A619 lpa2-1)-⊗-⊗-⊗-⊗
7	PMI-LP2-207	(PMI-Q3///A619 lpa2-1)-⊗-⊗-⊗-⊗
8	PMI-LP2-208	(PMI-PV5/A619 lpa2-1)-13-2-⊗-⊗-⊗-⊗
9	PMI-LP2-209	(PMI-PV5/A619 lpa2-1)-36-3-⊗-⊗-⊗-⊗
10	PMI-LP2-210	(PMI-PV5/A619 lpa2-1)-88-1-⊗-⊗-⊗-⊗
11	PMI-LP2-211	(PMI-PV5/A619 lpa2-1)-88-2-⊗-⊗-⊗-⊗
12	PMI-LP2-212	(PMI-PV7/A619 lpa2-1)-16-4-⊗-⊗-⊗-⊗
13	PMI-LP2-213	(PMI-PV7/A619 lpa2-1)-51-1-⊗-⊗-⊗-⊗
14	PMI-LP2-214	(PMI-PV7/A619 lpa2-1)-75-4-⊗-⊗-⊗-⊗
15	PMI-LP2-215	(PMI-PV7/A619 lpa2-1)-83-2-⊗-⊗-⊗-⊗
16	PMI-LP2-216	(PMI-PV7/A619 lpa2-1)-84-3-⊗-⊗-⊗-⊗
17	PMI-LP2-217	(PMI-PV8/A619 lpa2-1)-20-2-⊗-⊗-⊗-⊗
18	PMI-LP2-218	(PMI-PV8/A619 lpa2-1)-55-2-⊗-⊗-⊗-⊗
19	PMI-LP2-219	(PMI-PV8/A619 lpa2-1)-63-4-⊗-⊗-⊗-⊗
20	PMI-LP2-220	(PMI-Q2/A619 lpa2-1)-33-1-⊗-⊗-⊗-⊗
21	PMI-LP2-221	(PMI-Q2/A619 lpa2-1)-62-1-⊗-⊗-⊗-⊗
22	PMI-LP2-222	(PMI-Q3/A619 lpa2-1)-10-5-⊗-⊗-⊗-⊗
23	PMI-LP2-223	(PMI-Q3/A619 lpa2-1)-12-5-⊗-⊗-⊗-⊗
24	PMI-LP2-224	(PMI-Q3/A619 lpa2-1)-66-5-⊗-⊗-⊗-⊗

Table 3.3 Details of the wild type inbreds evaluated in the study

S. No.	Genotype	Pedigree
1	PMI-PV1	(HKI161/// HP704-23)-⊗-⊗-⊗-⊗-⊗-⊗-⊗-⊗
2	PMI-PV2	(HKI163///HP704-22)-⊗-⊗-⊗-⊗-⊗-⊗-⊗-⊗
3	PMI-PV3	(HKI193-1///HP704-23)-⊗-⊗-⊗-⊗-⊗-⊗-⊗-⊗
4	PMI-PV4	(HKI193-2///HP704-22)-⊗-⊗-⊗-⊗-⊗-⊗-⊗-⊗
5	PMI-Q1	(HKI1105///CML161)-⊗-⊗-⊗-⊗-⊗-⊗-⊗-⊗
6	PMI-Q2	(HKI323///HKI161)-⊗-⊗-⊗-⊗-⊗-⊗-⊗-⊗
7	PMI-Q3	(HKI1128///HKI193-1)-⊗-⊗-⊗-⊗-⊗-⊗-⊗-⊗

Data for all traits recorded across the locations were analysed through WindoStat 8.0 software. The details of analysis of variance (ANOVA) for RCBD for individual location were given as below:

Sources of variation	df	MSS	Expectations for MSS
Genotypes	g-1	M_g	$\sigma^2_e + r\,\sigma^2_g$
Replications	r-1	M_r	$\sigma^2_e + g\,\sigma^2_r$
Error	(g-1) (r-1)	M_e	σ^2_e
Total	(gr-1)	-	-

Where, df = degrees of freedom; g = number of genotypes; M_g = MSS due to genotypes; r = number of replications; MSS = Mean sum of squares; M_r = MSS due to replications; M_e = MSS due to errors.

Appropriate 'F' values were obtained to test the statistical significance of replication MSS and genotypes MSS against error MSS. When F value was found to be significant, pairs of treatment means were tested for significance by calculating the least significant difference (LSD) as follows:

SE difference of Means (SEM) = $\sqrt{(2M_e/r)}$

LSD = SEM ×'t' (at 0.05 level of significance and error df)

3.1.3.2 Pooled analysis of variance

Pooled ANOVA across both the locations was computed using Windostat 8.0 software. Inbreds were considered as fixed effects; whereas, replication and location were considered as random effects. The structure of analysis of variance for the experiment is given below:

Sources of variation	df	MSS	Expectations for MSS
Genotypes (G)	G-1	M_G	$\sigma^2_e + R\,\sigma^2_{Ge} + RE\,\sigma^2_G$
Replications (R)	R-1	M_R	-
Locations (L)	E-1	M_C	
Genotype × Environment (G × E)	(G-1) (E-1)	$M_{G\times E}$	$\sigma^2_e + R\,\sigma^2_{Ge}$
Error	(R-1) (GE-1)	M_e	σ^2_e
Total	(GRE-1)	-	-

Where, df = degrees of freedom; G = number of genotypes; E = number of environments; R = number of replications; MSS = Mean sum of squares; M_G = MSS due to genotypes; M_R = MSS due to replications; M_E = MSS due to environment; $M_{G\times E}$= MSS due to genotype × environment; M_e = MSS due to pooled errors.

Statistical significance of MSS for genotype, replication and environment was tested against MSS for G × E interaction and compared against tabulated value of 'F', whereas the significance of MSS for G × E interaction was tested against the pooled error. Significance of MSS at $P = 0.05$ is indicated by *or significance of MSS at $P = 0.01$ is indicated by **.

3.1.4 Quantification of phytic acid (PA) and inorganic phosphorous (iP)

Selfed seeds of the inbreds of both the *lpa1*- and *lpa2*-based experiments from both the locations were used to quantify the PA and iP as explained by Lorenz (2007) with minor changes. 100 mg powder of maize grains from each genotype was weighed and placed in 2 ml eppendorf tube, and 2 ml of 0.65 M HCl was added. The tubes were then kept on shaker for overnight with 120 rpm at room temperature and afterwards centrifuged at 12,000 revolutions per minute (rpm) for 5 minutes.

A total of 500 µl of the extract of each genotype was transferred to a fresh 15 ml tube for valuation of iPand to a 2 ml eppendorf tube for valuation of PA. Equal volumes of the PA and iP quantitative standards were used. Phytic acid dodecasodium salt from corn (Sigma) and KH_2PO_4 (HiMedia) were used as phytate and Pi standards, respectively. The Pi reagent was made immediately before use and it consisted of two parts of distilled H_2O, one part each of 0.02 M ammonium molybdate, 0.57 M ascorbic acid and 3Msulphuric acid. For the valuation of iP, 1 ml of Pi reagent and 1 ml of distilled H_2O were added to each tube. Once, the blue colour develops after 15 to 20 minutes of incubation at room temperature and then the optical density (OD 820) was measured at 820 nm.

For measurement of phytate, 1.25 ml of Wade reagent was added to each tube and allowed to react for 15 min at room temperature and after the development of pink colour the optical density at 490 nm (OD 490) was measured. Wade reagent consisted of 0.3 g 5-sulfosalycyclic acid, 0.03 g $FeCl_3.6H_2O$ and 80 ml distilled H_2O and could be stored in a refrigerator for 1 month. The above solution was refrigerated overnight and adjusted to a p^H of 3.05 with NaOH the following day. After p^H adjustment, dist. H_2O was added for a final volume of 100 ml. Phytate was converted to phytate P by dividing phytate by 3.55 (Raboy and Dickinson, 1984; Abhijith et al. 2020).

3.2 Molecular characterisation of *lpa1*- and *lpa2*-based inbreds selected in the study

3.2.1 Plant materials

A diverse set of 24 *lpa1*-based inbreds and 24 *lpa2*-based inbreds as mentioned in the section 3.1 were used for characterization by SSR markers.

3.2.2 Isolation and quantification of DNA

The isolation of DNA was carried out using conventional SDS protocol from 2-3 seeds of 24 *lpa1*- and 24 *lpa2*-based inbreds (Dellaporta et al. 1983). The seeds of the inbreds were ground into fine powder and transferred to 2 ml tube. 1.5 ml of SDS buffer extraction was added to each of the tube. These tubes containing samples were placed with intermittent mixing at 65 °C in the water bath for 1 hour. The samples were then added with 300µl of 5 M potassium acetate for incubation for 20 minutes. After centrifugation for 10 min at 12000 rpm, supernatant was transferred to fresh 2 ml tube. 120µl of 3M sodium acetate and 1ml of chilled isopropanol was added, and placed for 10 minutes at -20 °C. The samples were then centrifuged for 10 min at 1000 rpm. After removal of supernatant, pellet was treated with buffer-1 (RB-1) re-suspension, and centrifuged for 10 min at 12000 rpm. Supernatant was then transferred to fresh tube of 2 ml and added with 75µl of 3M sodium acetate and 500µl of crushed isopropanol. The sample was kept at -20°C for 1 hour to permit precipitation. After centrifugation, pellet was gathered for 15 min at 12000 rpm and added with 70% ethanol. It was then again centrifuged for 5 min at 6000 rpm and pellet was gathered and dried with 70µl of RB-2 and RNase for final therapy.

3.2.3 PCR amplification

For the assessment of molecular diversity, 60 SSR markers were used for genotyping *lpa1*-based inbreds (Table 3.4). For *lpa2*-based inbreds, 61 SSR markers were used for molecular characterization (Table 3.5). The details of the SSR primers were obtained from MaizeGDB. The PCR mediated amplifications were carried out in a GenePro-Thermal Cycler with a final quantity of 20 µl of genomic DNA of 100 ng. PCR was conducted in a 20 µl response blend comprising 100 ng of genomic DNA as a template, 1x OnePCRTM Mix (GeneDireX Inc., China) and 0.5 µM of forward and 0.5 µMof reverse primer.

3.2.4 Resolution of amplicon and allele profiling

Using 1.0X TBE buffer the PCR amplified products of each SSR markers were resolved at 90 V for 7 to 9 hour using horizontal electrophoresis. The stained 4% agarose gels of ethidium bromide (10mg/ml) were used to visualize the DNA bands and ladders. To understand the right size of amplified products, DNA ladder 100 bp (G-Biosciences, USA) was loaded and picture was taken in a gel documentation system (SYNGENE, United Kingdom), followed by marker profile scoring. The amplicons have been scored as loci

Table 3.4 List of SSR markers used for genotyping *lpa1*-based inbreds

S. No.	Marker	Bin	SSR repeats	S. No.	Marker	Bin	SSR repeats
1	*bnlg1203*	1.03	(AG)17	31	*umc2309*	6	(CAGG)4
2	*umc1076*	1.05	(CA)N	32	*umc1614*	6.04	(CTG)6
3	*umc1774*	1.1	(GT)7	33	*umc1105*	6.04	(GCC)4
4	*umc2186*	2	(CGG)6	34	*umc1795*	6.05	(GCGCG)4
5	*umc2245*	2.01	(CAA)7	35	*umc1912*	6.06	(GCG)6
6	*mmc0111*	2.02	(GA)34	36	*umc2123*	6.06	(GCT)4
7	*umc1541*	2.04	(GCT)4	37	*umc2170*	6.06	(TAC)4
8	*umc1769*	2.04	(GA)8	38	*umc1666*	7.02	(AG)10
9	*umc2253*	2.06	(TCGC)4	39	*umc1393*	7.02	(GTC)4
10	*umc1560*	2.07	(GC)6	40	*umc2333*	7.05	(CCGT)4
11	*umc2101*	3	(AG)7	41	*umc1858*	8.04	(TA)8
12	*umc2119*	3.04	(AC)7	42	*umc1882*	8.05	(AG)12
13	*umc1495*	3.04	(AGGAC)4	43	*umc2210*	8.05	(AAAAT)4
14	*umc1087*	3.04	(GA)17	44	*umc1846*	8.05	(TC)8
15	*umc1025*	3.04	(CT)11	45	*umc1828*	8.06	(GA)8
16	*umc2262*	3.04	(CATCT)5	46	*umc2031*	8.06	(GCG)5
17	*bnlg1456*	3.05	AG(15)	47	*umc1055*	8.07	(AG)9
18	*bnlg1957*	3.05	AG(10)	48	*umc2134*	9.05	(TTC)6
19	*umc2152*	3.09	(TG)8	49	*umc1078*	9.05	(GT)13
20	*umc1758*	4.01	(CTT)5	50	*umc2089*	9.07	(CGC)4
21	*umc1194*	4.07	GGCC	51	*umc1277*	9.07	(AATA)5
22	*umc2188*	4.08	(TC)8	52	*umc1505*	9.08	(AAAAC)4
23	*umc2365*	4.08	(TGC)4	53	*umc2053*	10.01	(CGA)4
24	*umc2384*	4.08	(GCC)5	54	*umc2114*	10.02	(TCT)4
25	*umc1740*	4.09	(AGT)4	55	*umc1113*	10.02	(CACAG)5
26	*umc1101*	4.09	(CT)6	56	*umc2016*	10.03	(ACAT)4
27	*umc1761*	5.02	(GCA)5	57	*umc1678*	10.04	(TCG)6
28	*umc2216*	5.06	(TCTC)5	58	*umc1246*	10.04	(AAAT)5
29	*umc2308*	5.09	(CGGCG)4	59	*umc1507*	10.05	(CACAA)4
30	*umc1143*	6	AAAAT	60	*umc2203*	10.07	(CGC)6

N: Long stretch of repeats

Table 3.5 List of SSR markers used for genotyping *lpa2*-based inbreds

S. No.	Marker	Bin	SSR repeats	S. No.	Marker	Bin	SSR repeats
1	*umc1689*	1.05	(GCG)5	31	*umc2309*	6	(CAGG)4
2	*umc1914*	1.08	(CAG)4	32	*umc1614*	6.04	(CTG)6
3	*umc1082*	1.09	(GA)16	33	*umc1105*	6.04	(GCC)4
4	*umc1819*	1.12	CAAC)4	34	*umc1795*	6.05	(GCGCG)4
5	*umc1776*	2.03	(TA)7	35	*umc1912*	6.06	(GCG)6
6	*umc1448*	2.04	(GCT)5	36	*umc2123*	6.06	(GCT)4
7	*umc1541*	2.04	(GCT)4	37	*umc1831*	7.02	(AG)8
8	*umc2253*	2.05	(TCGC)4	38	*umc1666*	7.02	(AG)10
9	*umc2101*	3	(AG)7	39	*umc1393*	7.02	(GTC)4
10	*umc1425*	3.04	(TCA)4	40	*umc1359*	7.04	(TC)12
11	*umc1025*	3.04	(CT)11	41	*umc2190*	7.06	(CCT)4
12	*umc1087*	3.04	(GA)17	42	*umc1799*	7.06	(TG)12
13	*umc1012*	3.04	(GA)6	43	*umc1765*	8.03	(GCT)5
14	*bnlg1456*	3.05	(AG)15	44	*umc2210*	8.05	(AAAAT)4
15	*umc2152*	3.09	(TG)8	45	*umc1846*	8.05	(TC)8
16	*umc1869*	4.06	(GGT)6	46	*umc1950*	8.05	not given
17	*umc1329*	4.06	GCC)7	47	*umc2031*	8.06	(GCG)5
18	*umc1194*	4.07	GGCC	48	*umc1055*	8.07	(AG)9
19	*umc1740*	4.09	(AGT)4	49	*umc1647*	9	(GA)10
20	*umc1101*	4.09	(CT)6	50	*bnlg1724*	9.01	(AG)31
21	*umc1445*	5	(AC)7	51	*umc1809*	9.01	(GGA)5
22	*umc1240*	5	(TTG)5	52	*umc2337*	9.03	(AGC)4
23	*umc1761*	5.02	(GCA)5	53	*umc1878*	9.04	(AT)6
24	*umc1687*	5.05	(TGC)6	54	*umc1771*	9.04	(CGTC)4
25	*umc2216*	5.06	(TCTC)5	55	*umc1310*	9.06	(GCG)5
26	*umc1537*	5.07	(TCG)4	56	*umc2053*	10.01	(CGA)4
27	*umc2143*	5.08	(TTC)4	57	*umc2114*	10.02	(TCT)4
28	*umc2308*	5.08	(CGGCG)4	58	*umc2016*	10.03	(ACAT)4
29	*umc2209*	5.09	(CT)6	59	*umc1246*	10.04	(AAAT)5
30	*umc1143*	6	AAAAT	60	*umc1507*	10.05	(CACAA)4
				61	*umc2203*	10.07	(CGC)6

alleles. Allele scoring was performed manually. Base pair sizes of alleles were determined by comparing the 100bp ladder running parallel to the inbred lines. To estimate their frequencies, scored alleles were numbered as' l', '2' etc., with the lowest products as 1 and the next biggest as 2. The presence of a band in a genotype was stated for each allele by (1) and absence of band as (0).

Because SSR markers are co-dominant in nature, it is possible to distinguish heterozygous and homozygous lines for the specific loci. Genotype was scored as heterozygous with two bands of equal intensity. In case of superimposed conditions and variable intensities of bands, it was considered as heterogeneous. Higher intensity was used for scoring where heterogeneous situation occurred and reduced intensity band was dismissed. Any group of artefacts or diffused bands were regarded to be 'missing information', designated as '9' (in comparison with '1' for presence and '0' for absence). The missing information was not regarded in the information matrix while analyzing genetic similarities. Specific markers that generate 'null' allele in a genotype even after re-runs with particular controls were regarded to be the lack of a band (referred to as' 0') that emerged owing to the lack of the genotype SSR primer binding site. In order to estimate fragment size in base pairs, the migration distance of amplified fragments was inferred. In the Windows-Office Excel spreadsheet, generated molecular data were entered, in which rows were assigned to microsatellite alleles and column genotypes.

3.2.5Molecular marker data analysis

The polymorphism information content (PIC) was determined for each of the SSR marker as given by Senior et al. (1998). The PIC values range from '0' (monomorphic) to '1' (highly polymorphic: many alleles of equal frequencies) provides an assessment of a marker's discriminatory strength. For example, if a marker locus reveals four alleles, but one of the alleles are found at a higher frequency (say, frequency= 0.9), the overall discriminative capacity is lower than that of the maker locus with four more or less equal frequencies alleles. Any allele that appears only in single genotype termed as unique allele and allele with a frequency of less than 0.5 is rare. Genetic dissimilarity was calculated using the coefficient of Jaccard to compare genotypes in pairs. The neighour-joining method used in DARwin-6.0 was used to build up the dendogram (Perrier et al. 2003). To calculate the mean PIC value, the allelic frequency obtained through different loci SSRs was used. Then, to estimate parameters viz. total number of identified alleles, significant frequency of alleles, gene diversity, heterozygosity and content of polymorphic data (PIC) PowerMarker V3.0 was used (Liu and Muse 2005).

4

RESULTS

In the present investigation, experiments were conducted at two locations viz. Delhi and Hyderabad representing the two different zones of India. Each set of 24 *lpa1-1* and *lpa*-2 based maize inbreds along with seven wild type inbreds were evaluated for various morphological and biochemical characteristics. The obtained data on various parameters in each category were used for analysis and the results are presented below under different headings.

4.1 Evaluation of *lpa*-based inbreds for yield and yield attributes

4.1.1Variation for yield and other morphological characters among the *lpa1-1*based inbreds

The results of the analysis of variance (ANOVA) revealed that significant genetic variation existed for yield and other morphological characters like plant height, ear height, days to 50% male flowering and days to 50% female flowering among the *lpa1-1* based and wild type inbreds at both the locations (Table 4.1). The obtained results signified that the wide range of variability existed among the set of *lpa1-1* based inbreds which provided scope for selection of the desired genotype.

The data pertaining to mean performance of the yield and yield attributing characters at both the locations viz. Delhi and Hyderabad are presented in table 4.2. The plant height and ear height are considered to be very much critical characters in terms of describing the potential to use an inbred as male or female parent in the hybrid development programme. The mean plant height of *lpa1-1* mutants is 165.5 cm, which ranged between 129.8 to 188.0 cm at Delhi; and for Hyderabad, the mean plant height was 172.2 cm with the range of 143.5 to 192.5 cm. However, the wild type inbreds had the mean plant height of 157.0 cm and 163.0 cm at Delhi and Hyderabad locations, respectively (Table 4.2). The average ear height for *lpa1-1* mutants is 74.2 cm and 78.3 cm at Delhi and Hyderabad locations, respectively. The wild type inbreds had the mean ear height of 71.5 cm and 75.7 cm at Delhi and Hyderabad, respectively. Ear height for *lpa1-1* mutants varied from60.2 to 85.2 cm at Delhi; and 63.8 to 88.5 cm at Hyderabad, whereas the ear height of the wild type inbreds ranged from 65.2 to 81.0 cm at Delhi and 69.8 to 83.8 cm at Hyderabad (Table 4.2). The obtained data signified that there were no statistical differences for ear height among the *lpa1-1* mutants and wild type inbreds across the locations.

The days to male and female flowering are the most critical in deciding the synchrony of flowering during hybrid seed production. The average days to 50% male flowering for *lpa1-1* mutants was found to be 57.2 days with the range of 48.5 to 67 days at Delhi; and for Hyderabad, it varied between 61.5 to 77 days with the mean of 71.7 days

Table 4.1 Analysis of variance for yield and other morphological characters among the *lpa1-1* based inbreds evaluated in the study

Source of variation	*df*	Mean sum of squares									
		PH		EH		MF		FF		YLD	
		Delhi	Hyderabad	Delhi	Hyderabad	Delhi	Hyderabad	Delhi	Hyderabad	Delhi	Hyderabad
Replication	1	2.57	0.45	0.00	1.12	0.58	5.23	1.61	2.73	1307.33	2250.04
Genotype	30	472.93**	401.78**	81.00**	75.21**	36.17**	30.68**	36.09**	28.08**	200653.15**	206264.93**
Error	30	3.87	3.76	3.17	2.321	2.01	1.66	2.48	2.33	17118.91	22221.91
Total	61	234.53	199.45	41.40	38.15	18.79	15.99	19.00	15.00	107122.48	112407.55

PH: plant height; EH: ear height; MF: days to 50% male flowering; FF: days to 50% female flowering; YLD: yield; *: Significant at 5% and **: 1% level of significance

Table 4.2 Mean performance for yield and other morphological characters among the *lpa1-1* based inbreds evaluated in the study

Genotype	PH (cm)		EH (cm)		MF (days)		FF (days)		YLD (kg/ha)	
	Delhi	Hyderabad	Delhi	Hyderabad	Delhi	Hyderabad	Delhi	Hyderabad	Delhi	Hyderabad
***lpa1-1* mutants**										
PMI-LP1-101	185.2	190.0	84.5	88.3	55.0	71.0	58.5	74.0	3146.7	3297.8
PMI-LP1-102	172.0	179.5	73.7	76.2	63.5	74.0	66.0	77.5	3271.1	3288.9
PMI-LP1-103	161.2	168.3	69.2	74.3	59.5	76.0	63.0	78.5	2488.9	2666.7
PMI-LP1-104	170.5	179.0	73.2	79.0	57.5	72.5	61.0	75.5	2471.1	2613.3
PMI-LP1-105	139.5	148.2	70.5	77.2	48.5	63.0	52.0	66.5	2808.9	2951.1
PMI-LP1-106	129.8	143.5	69.7	72.8	51.5	66.0	54.0	69.5	2826.7	2755.6
PMI-LP1-107	159.2	166.0	79.0	82.5	57.5	75.5	61.0	79.0	2826.7	2915.6
PMI-LP1-108	174.7	181.5	77.2	78.5	52.0	70.0	55.0	72.5	2933.3	2720.0
PMI-LP1-109	188.0	192.5	85.2	87.5	58.0	73.5	62.0	75.5	3208.9	2862.2
PMI-LP1-110	170.0	173.7	78.0	82.0	52.5	68.5	56.0	71.0	2880.0	2951.1
PMI-LP1-111	177.2	181.0	77.7	80.3	53.5	71.5	55.0	74.5	2844.4	3093.3
PMI-LP1-112	177.2	180.2	77.7	78.7	60.5	71.0	65.0	74.5	2755.6	3057.8
PMI-LP1-113	182.2	184.5	83.2	84.8	67.0	75.5	69.5	79.0	3146.7	2951.1
PMI-LP1-114	167.0	170.5	68.7	73.5	63.5	74.0	65.5	77.0	2862.2	3093.3
PMI-LP1-115	157.5	160.5	63.5	66.5	57.5	72.5	62.0	75.5	2168.9	2293.3
PMI-LP1-116	171.2	177.3	72.0	74.0	56.0	77.0	59.5	79.5	2542.2	2346.7

PMI-LP1-117	161.8	171.5	68.2	72.7	60.5	74.0	63.5	77.5	2364.4	2506.7
PMI-LP1-118	167.3	172.5	70.3	74.5	54.0	67.5	58.5	70.5	2133.3	2008.9
PMI-LP1-119	180.2	182.5	79.5	82.0	58.5	74.5	61.5	78.0	2560.0	2720.0
PMI-LP1-120	168.2	177.0	77.3	79.3	59.5	72.5	63.5	75.0	2435.6	2346.7
PMI-LP1-121	156.7	171.0	76.5	88.5	60.0	70.0	63.0	72.5	2560.0	2808.9
PMI-LP1-122	149.2	157.0	68.3	77.2	55.5	72.5	58.0	76.5	2524.4	2346.7
PMI-LP1-123	157.7	170.5	78.8	85.3	58.0	76.0	60.5	78.5	3093.3	3093.3
PMI-LP1-124	148.2	154.5	60.2	63.8	53.0	61.5	56.5	65.5	2275.6	2506.7
Mean	**165.5**	**172.2**	**74.2**	**78.3**	**57.2**	**71.7**	**60.4**	**74.7**	**2713.7**	**2758.1**
Wild type inbreds										
PMI-PV1	180.5	185.0	81.0	83.8	53.5	69.0	56.0	72.5	3066.7	3200.0
PMI-PV2	176.5	182.0	78.2	80.5	62.5	72.5	65.5	76.0	3324.4	3253.3
PMI-PV3	133.0	139.2	66.5	72.3	50.0	65.0	53.0	68.5	2897.8	2791.1
PMI-PV4	135.5	140.0	65.2	69.8	53.0	68.0	56.5	71.0	2880.0	2915.6
PMI-Q1	154.0	162.0	73.0	77.8	58.5	75.0	62.0	76.5	2951.1	2773.3
PMI-Q2	155.0	159.5	65.5	70.2	59.0	74.0	62.0	77.5	2755.6	2631.1
PMI-Q3	164.5	173.0	71.0	75.5	58.0	74.5	62.0	77.0	2702.2	2560.0
Mean	**157.0**	**163.0**	**71.5**	**75.7**	**56.4**	**71.1**	**59.6**	**74.1**	**2939.7**	**2874.9**
CD	**4.02**	**3.96**	**3.64**	**3.11**	**2.90**	**2.63**	**3.22**	**3.11**	**267.21**	**304.44**

PH: plant height; EH: ear height; MF: days to 50% male flowering; FF: days to 50% female flowering; YLD: yield; CD: Critical difference at 5% level of significance

(Table 4.2). The wild type inbreds had the average days to 50% male flowering of 56.4 days with the range of 50 to 62.5 days at Delhi, however for Hyderabad, the mean of 71.1 days with the range of 65 to 75 days was observed for days to 50% male flowering. The mean days to 50% female flowering was 60.4 days at Delhi and 74.7 days at Hyderabad and it ranged between 52 to 69.5 days and 65.5 to 79.5 days, respectively. On the other hand, the wild type inbreds had the mean of 59.6 days and 74.1 days at Delhi and Hyderabad, respectively; with the values ranging between 53 to 65.5 days and 68.5 to 77.5 days in the respective locations (Table 4.2). From the evaluation, it was clear that the mean days to 50% male and female flowering for *lpa1-1* mutants were in the similar duration for both the wild type and the mutant inbreds in both the locations.

The grain yield is a complex character being influenced by different factors. In the current study, the grain yield of *lpa1-1* mutants ranged between 2133.3 to 3271.1 kg/ha with the mean of 2713.7 kg/ha at Delhi, however it ranged between 2008.9 to 3297.8 kg/ha with the mean of 2758.1 kg/ha at Hyderabad (Table 4.2). Whereas, the wild type genotypes recorded mean grain yield of 2939.7 kg/ha with the range of 2702.2 to 3324.4 kg/ha at Delhi, however at Hyderabad, grain yield varied from 2560.0 to 3253.3 kg/ha with the mean of 2874.9 kg/ha (Table 4.2).

Among the set of 24 *lpa1-1* based inbreds, PMI-LP1-102, PMI-LP1-109, PMI-LP1-101, PMI-LP1-113 and PMI-LP1-123 were found to be the top performing inbreds for grain yield compared to the remaining *lpa1-1* based inbreds at Delhi location. Whereas the inbreds, PMI-LP1-101, PMI-LP1-102, PMI-LP1-111, PMI-LP1-114 and PMI-LP1-123 were found to be the top ranking *lpa1-1* based inbreds for grain yield at Hyderabad. Considering both the locations, *lpa1-1* inbreds viz. PMI-LP1-102, PMI-LP1-101 and PMI-LP1-123 recorded the highest grain yield among the *lpa1-1* based inbreds evaluated in the study.

4.1.2 Combined ANOVA for yield and other morphological characters among the *lpa1-1* based inbreds

The data obtained from the evaluation of the same set of 24 *lpa1-1* mutants at both the locations were used for the combined ANOVA for yield and other morphological characters and the results are presented in table 4.3.

The results of the pooled ANOVA indicated that highly significant differences existed among the *lpa1-1* mutants and wild type inbreds across both the locations (Delhi and Hyderabad) for all the characters studied. All the characters under study significantly differed among the genotypes across the locations except for the grain yield (Table 4.3). In addition to this, the interaction effect of genotype with location also showed significant differences among the genotypes for all the characters studied except for the grain yield. The performance of the genotypes for the grain yield was stable, indicating that the effect of

Table 4.3 Combined Analysis of variance for yield and other morphological characters across locations among the *lpa1-1* based inbreds evaluated in the study

Source of variation	*df*	Mean sum of squares				
		PH	EH	MF	FF	YLD
Replication	1	0.43	0.60	4.65	4.27	3493.78
Location	1	1329.78^{**}	523.20^{**}	6561.32^{**}	6402.27^{**}	12125.50
Genotype	30	864.99^{**}	151.01^{**}	57.37^{**}	55.03^{**}	377437.68^{**}
Genotype × Location	30	9.73^{**}	5.20^{*}	9.47^{**}	9.15^{**}	29480.40
Error genotype	61	3.79	2.71	1.83	2.36	19348.99
Total	123	226.04	43.70	70.59	68.91	108971.19

PH: plant height; EH: ear height; MF: days to 50% male flowering; FF: days to 50% female flowering; YLD: yield; *: Significant at 5% and **: 1% level of significance

environment was minor and irrespective of environments, the genotypes showed stable performance for the grain yield. It was also observed that replications had no influence on the variance among the experimental materials evaluated for yield and other morphological characters studied.

4.1.3 Variation for yield and other morphological characters among the *lpa2-1* based inbreds

The results from the ANOVA revealed that highly significant differences existed among the genotypes evaluated for all the characters (plant height, ear height, days to 50% male flowering, days to 50% female flowering and yield) studied at both the locations (Delhi and Hyderabad) (Table 4.4). The obtained results indicated that the wide range of variability existed among the genotypes across the locations and it provided the scope for selection of desired genotypes to be used in the breeding programme.

The mean plant height of *lpa2-1* based maize inbreds was 159.6 cm and it varied from 127.5 to 188 cm at Delhi, whereas at Hyderabad the mean plant height was 166.9 cm with the range of 136.5 to 191.7 cm (Table 4.5). The wild type inbreds had the mean plant height of 158.4 cm and 164.4 cm at Delhi and Hyderabad locations, respectively. The mean data of plant height indicated non-significant difference between *lpa2-1* and wild type inbreds in their respective locations.

The ear height of *lpa2-1* mutants were 71.8 cm and 76.9 cm at Delhi and Hyderabad respectively. At the same time, the wild type inbreds had the mean ear height of 74.1 cm and 78.3 cm at Delhi and Hyderabad, respectively. The range of ear height for *lpa2-1* inbreds was 57.7 to 85.2 cm at Delhi; and 62 to 89.5 cm at Hyderabad. However, among the wild type inbreds it varied from 67.2 to 85.5 cm at Delhi and 71.8 to 90.5 cm at Hyderabad. There was no significant difference for the mean ear height between the wild type inbreds and *lpa2-1* inbreds at both the locations, though values seemed to be higher in the wild type inbreds.

The mean days to 50% male flowering for *lpa2-1* mutants was 57.4 days with the range of 49 to 65 days at Delhi, whereas at Hyderabad it varied from 60.5 to 77.5 days with the mean of 70.2 days (Table 4.5). The wild type inbreds had the average days to 50% male flowering of 56.6 days, which ranged between 50.0 to 63.0 days at Delhi but for Hyderabad, it was found to be ranging from 65.0 to 76.0 days with a mean of 71.8 days (Table 4.5). The days required for 50% male flowering was statistically similar between *lpa2*-1 based inbreds and the wild type inbreds in their respective locations.

Average of 60.8 days was required for 50% female flowering among *lpa2-1* mutants, which varied between 51.5 to 68.5 days at Delhi, however for Hyderabad, the mutants had the mean days of 73.5 with the range of 65.0 to 80.5 days (Table 4.5). On the

Table 4.4 Analysis of variance for yield and other morphological characters among the *lpa2-1* based inbreds evaluated in the study

Source of variation	*df*	Mean sum of squares									
		PH		EH		MF		FF		YLD	
		Delhi	Hyderabad	Delhi	Hyderabad	Delhi	Hyderabad	Delhi	Hyderabad	Delhi	Hyderabad
Replication	1	7.77	2.69	2.19	4.66	0.79	6.45*	1.03	1.95	23587.22	17734.88
Genotype	30	522.51**	499.48**	96.54**	90.22**	37.22**	39.74**	40.46**	36.55**	149535.52**	253019.67**
Error	30	2.10	3.36	2.74	2.83	1.86	1.12	2.27	1.95	7569.45	8727.51
Total	61	258.13	247.34	48.86	45.84	19.23	20.20	21.03	18.97	77651.38	129018.92

PH: plant height; EH: ear height; MF: days to 50% male flowering; FF: days to 50% female flowering; YLD: yield; *: Significant at 5% and **: 1% level of significance

Table 4.5 Mean performance for yield and other morphological characters among the *lpa2-1* based inbreds evaluated in the study

Genotype	PH (cm)		EH (cm)		MF (days)		FF (days)		YLD (kg/ha)	
	Delhi	Hyderabad	Delhi	Hyderabad	Delhi	Hyderabad	Delhi	Hyderabad	Delhi	Hyderabad
***lpa2-1* mutants**										
PMI-LP2-201	182.8	190.5	80.5	85.5	56	72	59	75.5	3235.6	3448.9
PMI-LP2-202	175.5	182.0	79.0	75.2	61.5	74	65	76.5	3280.0	3102.2
PMI-LP2-203	158.5	166.0	69.7	74.0	61	77.5	65	80.5	2648.9	2853.3
PMI-LP2-204	167.5	181.0	72.0	79.3	58	73.5	61.5	76.5	2648.9	2826.7
PMI-LP2-205	137.5	146.0	68.5	75.5	49	64.5	51.5	67.5	2773.3	2951.1
PMI-LP2-206	136.2	144.2	75.2	80.0	52	66.5	55.5	68.5	2986.7	3200.0
PMI-LP2-207	156.0	162.0	77.0	82.0	58.5	77	62	79.5	2960.0	3102.2
PMI-LP2-208	167.5	175.5	68.7	73.0	53	71	56.5	74.5	3040.0	3182.2
PMI-LP2-209	184.5	190.5	85.2	89.5	52.5	63.5	55.5	66.5	2666.7	2444.4
PMI-LP2-210	170.0	175.7	73.3	78.8	62.5	71.5	66	74.5	2995.6	2728.9
PMI-LP2-211	188.0	191.7	81.7	86.0	58	73.5	61.5	77	3164.4	3288.9
PMI-LP2-212	161.5	168.8	74.5	78.7	65	71.5	68.5	74.5	2497.8	2346.7
PMI-LP2-213	151.0	160.5	62.2	73.8	54	66.5	57.5	72.5	2524.4	2293.3
PMI-LP2-214	165.3	173.7	73.2	77.3	60	71.6	63.2	75	2533.3	2417.8
PMI-LP2-215	155.5	160.5	64.5	67.0	58	67.5	61.5	71	2320.0	2506.7
PMI-LP2-216	150.0	154.3	62.3	67.0	61.5	76	65	78.5	2746.7	2977.8

PMI-LP2-217	175.5	183.8	83.0	88.2	59	72	62.5	75	2417.8	2204.4
PMI-LP2-218	160.0	166.7	70.0	75.5	55	67.5	59.5	70.5	2222.2	2417.8
PMI-LP2-219	164.5	169.8	73.2	77.5	62	75	65.5	79	2622.2	2817.8
PMI-LP2-220	141.0	148.0	61.3	65.8	52	60.5	55	65	2542.2	2435.6
PMI-LP2-221	127.5	136.5	57.7	62.0	53.5	68	56.5	71	2844.4	3004.4
PMI-LP2-222	147.0	159.0	66.5	74.3	63.5	72.5	67	75.5	2764.4	2942.2
PMI-LP2-223	158.7	161.7	77.5	82.2	56.5	63.5	60	66.5	2506.7	2382.2
PMI-LP2-224	149.0	158.0	67.8	76.8	56	68.5	58.5	72.5	2817.8	3031.1
Mean	**159.6**	**166.9**	**71.8**	**76.9**	**57.4**	**70.2**	**60.8**	**73.5**	**2740.0**	**2787.8**
Wild type inbreds										
PMI-PV1	186.2	191.2	85.5	90.5	53	69.5	55.5	72	3111.1	3360.0
PMI-PV2	177.0	183.0	75.2	82.0	63	72.5	66.5	76	3164.4	3377.8
PMI-PV3	154.0	160.8	67.2	71.8	59.5	74.5	63	78.5	2666.7	2968.9
PMI-PV4	166.0	171.7	73.5	79.0	59	75.5	62	77.5	2542.2	2737.8
PMI-Q1	134.2	141.3	74.2	71.8	53	69.5	56	72	3004.4	3217.8
PMI-Q2	133.7	138.5	67.5	73.0	50	65	53	68.5	2755.6	2933.3
PMI-Q3	157.7	164.5	75.5	80.0	59	76	62.5	78	2844.4	3057.8
Mean	**158.4**	**164.4**	**74.1**	**78.3**	**56.6**	**71.8**	**59.8**	**74.6**	**2869.8**	**3093.3**
CD	**2.96**	**3.74**	**3.38**	**3.43**	**2.78**	**2.16**	**3.08**	**2.85**	**177.68**	**190.79**

PH: plant height; EH: ear height; MF: days to 50% male flowering; FF: days to 50% female flowering; YLD: yield; CD: Critical difference at 5% level of significance other hand, the wild type inbreds had the mean days of 59.8 and 74.6 days at Delhi and Hyderabad locations, respectively. However, these values ranged between 53.0 to 66.5 days and 68.5 to 78.5 days at the respective locations (Table 4.5). It was clear here as well that the mean days to 50% female flowering of*lpa2-1* mutants were similar to the wild type inbreds in their respective locations.

The mean grain yield of *lpa2-1* mutant inbreds was 2740 kg/ha and 2787.8 kg/ha at Delhi and Hyderabad locations, respectively, however the grain yield varied between 2222.2 to 3280.0 kg/ha and 2204.4 to 3448.9 kg/ha in the respective locations (Table 4.5). The wild type genotypes had the mean grain yield of 2869.8 kg/ha with the range of 2542.2 to 3164.4 kg/ha at Delhi but at Hyderabad, the mean grain yield of 3093.3 kg/ha with the range of 2737.8 to 3377.8 kg/ha was found (Table 4.5). The observed mean data revealed that the wild type inbreds were having slightly higher grain yield compared to *lpa2-1* based maize inbreds at Hyderabad location but the performance was found to be similar at Delhi location.

Among the set of 24 *lpa2-1* based maize inbreds, PMI-LP2-202, PMI-LP2-201, PMI-LP2-211, PMI-LP2-208 and PMI-LP2-210 were found to be the top performing inbreds for grain yield at Delhi location (Table 4.5). Whereas, PMI-LP2-202, PMI-LP2-222, PMI-LP2-206, PMI-LP2-221, PMI-LP2-201 and PMI-LP2-207 were the top performing*lpa2-1* based maize inbreds for grain yield compared to the remaining inbreds at Hyderabad (Table 4.5). Inbreds, PMI-LP-201 and PMI-LP-202 performed superior for grain yield at both the locations (Table 4.5).

4.1.4 Combined ANOVA for yield and other morphological characters among the *lpa2-1* based inbreds

The combined data from the evaluation of the set of 24 *lpa2-1* mutants were used for the analysis of variance across the locations for yield and other morphological characters. The results obtained from the combined analysis of variance are presented in table 4.6.

The results from the study revealed that the performance of the genotypes across both the locations significantly differed for all the characters (Table 4.6). The *lpa2-1* mutants and the wild type inbreds performed significantly different at both the locations for all the studied characters. In addition to this, the interaction effect of genotype and location was also found to be significantly different for all the characters under study. The results indicating that the performance of genotypes in particular location was unique and provided scope for the selection among the genotypes based on the requirements of the breeding programme.

4.2 Evaluation of *lpa*-based inbreds for biochemical characteristics

Table 4.6 Combined Analysis of variance for yield and other morphological characters across locations among the *lpa2-1* based inbreds evaluated in the study

Source of variation	*df*	Mean sum of squares				
		PH	EH	MF	FF	YLD
Replication	1	9.80	6.61	5.88	2.91	41113.84*
Location	1	1520.26**	732.79**	5515.56**	5409.36**	237072.48**
Genotype	30	1017.05**	179.28**	66.29**	66.63**	370686.92**
Genotype × Location	30	4.93*	7.48**	10.67**	10.38**	31868.28**
Error genotype	61	2.70	2.74	1.49	2.08	8018.31
Total	123	263.04	52.92	64.40	63.81	104422.45

PA: phytic acid; iP: inorganic phosphorous; TP: Total phosphorous; PAP/TP: phytic acid phosphorous to total phosphorous; *: Significant at 5% and **: 1% level of significance

The set of low phytic acid-based mutants along with the wild type inbreds were evaluated for various biochemical characters like phytic acid (PA), inorganic phosphate (iP), total phosphate (TP) and the ratio of phytic acid phosphate to total phosphates (PAP/TP). Each set of 24 *lpa1-1* and *lpa2-1* mutant inbreds along with seven wild type inbreds were evaluated at field in RCBD design with two replications at two locations (Delhi and Hyderabad). The selfed grains obtained from the harvest were used for the biochemical analysis with two replications. The estimated data from the biochemical analysis were used for the further analysis.

4.2.1 Variation for phytic acid and inorganic phosphorous among the *lpa1-1* inbreds and the wild type inbreds

The set of 24 *lpa1-1* based inbreds along with seven wild type inbreds were evaluated at both the locations and biochemical parameters were estimated. The obtained data was used for the analysis of variance and presented in table 4.7. ANOVA results indicated that significant differences existed among the *lpa1-1* inbreds and the wild type inbreds for all the biochemical parameters (PA, iP, TP and PAP/TP) estimated across both the locations (Table 4.7). The content of phytic acid and inorganic phosphorus varied among the genotypes under study in both the locations signifying the scope for the selection of desirable low phytate genotype for higher bioavailability of mineral elements.

The data pertaining to mean performance of biochemical parameters is presented in table 4.8. The *lpa1-1* inbreds possessed the mean PA content of 1.65 mg/g, which varied from 1.56 to 1.85 mg/g for Delhi, whereas at Hyderabad, it ranged from 1.54 to 1.89 mg/g with the mean PA content of 1.70 mg/g (Table 4.8). The mean PA of 2.55 mg/g and 2.67 mg/g was observed at Delhi and Hyderabad locations, respectively in the wild type inbreds and the content varied between 2.29 to 2.66 mg/g and 2.45 to 2.80 mg/g at the respective locations. The mean data indicating that PA content was statistically higher in the wild type inbreds than the *lpa1-1* mutant inbreds. The reduction of 35.3% and 36.2 % in the mean PA content of *lpa1-1* mutants was observed over wild type inbreds at Delhi and Hyderabad locations, respectively (Table 4.8).

The iP content in the *lpa1-1* genotypes varied from 0.69 to 1.07 mg/g with the mean of 0.92 mg/g at Delhi, whereas it varied from 0.83 to 1.24 mg/g with the mean of 1.07 mg/g at Hyderabad (Table 4.8). However, the mean iP in the wild type inbreds was 0.23 and 0.25 mg/g at Delhi and Hyderabad locations, respectively. It was clear that the mean iP was significantly higher in the *lpa1-1* mutant inbreds compared to the wild type inbreds at both the locations under study. The increase of iP to the extent of 3.0 and 3.26 fold in the *lpa1-1* inbreds as compared to wild type inbreds was observed at Delhi and Hyderabad, respectively (Table 4.8).

Table 4.7 Analysis of variance for phytic acid and inorganic phosphorous among the *lpa1-1* based inbreds evaluated in the study

Source of variation	*df*	Mean sum of squares							
		PA		IP		TP		PAP/TP	
		Delhi	Hyderabad	Delhi	Hyderabad	Delhi	Hyderabad	Delhi	Hyderabad
Replication	1	0.01*	0.00	0.00	0.00	0.00	0.01	6.57*	0.02
Genotype	30	0.31**	0.36**	0.19**	0.26**	0.04**	0.05**	282.23**	336.06**
Error	30	0.00	0.00	0.00	0.004	0.00	0.01	1.05	2.01
Total	61	0.15	0.18	0.09	0.13	0.02	0.03	139.43	166.27

PA: phytic acid; iP: inorganic phosphorous; TP: Total phosphorous; PAP/TP: phytic acid phosphorous to total phosphorous; *: Significant at 5% and **: 1% level of significance

Table 4.8 Mean performance of phytic acid and inorganic phosphorous among the *lpa1-1* inbreds and wild type inbreds evaluated in the study

Genotype	PA (mg/g)		iP (mg/g)		TP (mg/g)		PAP/TP (%)	
	Delhi	Hyderabad	Delhi	Hyderabad	Delhi	Hyderabad	Delhi	Hyderabad
***lpa1-1* mutants**								
PMI-LP1-101	1.77	1.80	0.95	1.08	2.72	2.88	65.15	62.57
PMI-LP1-102	1.70	1.82	0.99	1.10	2.68	2.92	63.16	62.34
PMI-LP1-103	1.65	1.54	0.85	1.13	2.50	2.66	65.95	57.64
PMI-LP1-104	1.58	1.71	0.69	1.20	2.27	2.91	69.44	58.73
PMI-LP1-105	1.72	1.59	0.77	0.83	2.49	2.42	68.98	65.84
PMI-LP1-106	1.62	1.69	0.91	1.06	2.54	2.75	64.03	61.57
PMI-LP1-107	1.63	1.80	0.89	1.17	2.52	2.97	64.83	60.58
PMI-LP1-108	1.65	1.82	0.94	1.23	2.60	3.04	63.70	59.73
PMI-LP1-109	1.63	1.89	0.99	1.08	2.62	2.97	62.11	63.69
PMI-LP1-110	1.64	1.70	0.95	1.03	2.59	2.73	63.20	62.32
PMI-LP1-111	1.60	1.54	0.96	1.15	2.56	2.69	62.48	57.23
PMI-LP1-112	1.69	1.79	0.94	1.05	2.63	2.84	64.21	63.18
PMI-LP1-113	1.61	1.71	0.97	1.02	2.58	2.73	62.33	62.61
PMI-LP1-114	1.58	1.69	0.91	1.11	2.49	2.80	63.57	60.32
PMI-LP1-115	1.73	1.57	0.97	1.04	2.70	2.60	64.21	60.16
PMI-LP1-116	1.66	1.63	1.02	1.11	2.68	2.74	61.80	59.49

PMI-LP1-117	1.56	1.79	0.88	1.13	2.45	2.91	63.98	61.45
PMI-LP1-118	1.66	1.67	0.93	1.24	2.60	2.91	64.08	57.41
PMI-LP1-119	1.63	1.56	0.86	1.02	2.48	2.58	65.56	60.45
PMI-LP1-120	1.67	1.71	0.89	0.91	2.56	2.62	65.21	65.44
PMI-LP1-121	1.65	1.66	0.89	1.02	2.54	2.69	64.96	61.96
PMI-LP1-122	1.60	1.64	0.91	0.87	2.50	2.51	63.89	65.29
PMI-LP1-123	1.85	1.76	1.00	1.00	2.86	2.75	64.84	63.85
PMI-LP1-124	1.58	1.74	1.07	1.16	2.65	2.91	59.69	60.07
Mean	**1.65**	**1.70**	**0.92**	**1.07**	**2.58**	**2.77**	**64.22**	**61.41**
Wild type inbreds								
PMI-PV1	2.64	2.62	0.28	0.30	2.92	2.92	90.37	89.86
PMI-PV2	2.62	2.78	0.18	0.21	2.81	2.98	93.49	93.05
PMI-PV3	2.66	2.73	0.22	0.27	2.88	3.00	92.36	91.13
PMI-PV4	2.47	2.45	0.27	0.31	2.74	2.76	90.04	88.83
PMI-Q1	2.64	2.80	0.18	0.25	2.82	3.05	93.71	91.82
PMI-Q2	2.56	2.77	0.27	0.24	2.83	3.02	90.59	91.90
PMI-Q3	2.29	2.51	0.20	0.17	2.49	2.68	92.03	93.51
Mean	**2.55**	**2.67**	**0.23**	**0.25**	**2.78**	**2.92**	**91.80**	**91.44**
CD	**0.11**	**0.08**	**0.07**	**0.13**	**0.13**	**0.16**	**2.10**	**2.90**

PA: phytic acid; iP: inorganic phosphorous; TP: Total phosphorous; PAP/TP: phytic acid phosphorous to total phosphorous; CD: Critical difference at 5% level of significance

The mean TP content was 2.58 mg/g and 2.77 mg/g at Delhi and Hyderabad locations, whereas the wild type inbreds recorded the mean TP of 2.78 mg/g and 2.92 mg/g in the respective locations (Table 4.8). Even though the content of PA and iP varied between the *lpa1-1* and wild type inbreds but the TP content was found to be similar, indicating that the decrease in the content of PA led to the increase in the content of iP or vice-versa, and the TP content remained unaffected.

The PAP/TP ratio for *lpa1-1* varied from 59.69 to 69.44 % with the mean of 64.22 % for Delhi, whereas for Hyderabad, it varied from 57.23 to 65.84% with the mean of 61.41% (Table 4.8). However, the ratio for the wild type inbreds varied from 90.04 to 93.71% with the mean ratio of 91.80 % for Delhi and for Hyderabad, it varied from 88.23 to 93.51 % with the mean of 91.44%. The data clearly indicated that the PAP/TP ratio was statistically higher in the wild type inbreds compared to *lpa1-1* inbreds, signifying the direct contribution of PA content to the PAP/TP ratio.

Among the set of 24 *lpa1-1* inbreds, PMI-LP1-124, PMI-LP1-116, PMI-LP1-109, PMI-LP1-113 and PMI-LP1-111 at Delhi, and PMI-LP1-111, PMI-LP1-118, PMI-LP1-103, PMI-LP1-104 and PMI-LP1-116 at Hyderabad were found to have the lowest ratio of PAP/TP. These top *lpa1-1* inbreds supposed to have higher bioavailability of mineral elements compared to remaining inbreds.

4.2.2 Combined ANOVA for phytic acid and inorganic phosphorous among the *lpa1-1* based inbreds

The combined data from both the locations were used for the analysis to find the performance of genotypes across the locations. The results for the combined analysis of variance for biochemical parameters are presented in the table 4.9. The results of the combined analysis of variance revealed that the performance of the *lpa1-1* mutants and the wild type inbreds varied across the locations for all the characters studied. The interaction effect of genotype and location was also highly significant for all the parameters. The environment was also influenced the performance of genotypes for all the biochemical parameters evaluated in the *lpa1-1* based inbreds and the wild type inbreds.

4.2.3 Variation for phytic acid and inorganic phosphorous from the *lpa2-1* inbreds and wild type inbreds

The panel of 24 *lpa2-1* based inbreds along with seven wild type inbreds were evaluated at Delhi and Hyderabad, and biochemical parameters were estimated. The obtained data from the estimation was used for the analysis of variance and presented in table 4.10. The results from the analysis of variance indicated that highly significant differences among the *lpa2-1* inbreds and the wild type inbreds for all the biochemical parameters (PA, iP, TP and

Table 4.9 Combined Analysis of variance for phytic acid and inorganic phosphorous among the *lpa1-1* based inbreds evaluated in the study

Source of variation	*df*	Mean sum of squares			
		PA	IP	TP	PAP/TP
Replication	1	0.01^{*}	0.00	0.01	2.95
Location	1	0.12^{**}	0.44^{**}	1.03^{**}	157.59^{**}
Genotype	30	0.65^{**}	0.43^{**}	0.07^{**}	610.05^{**}
Genotype × Location	30	0.01^{**}	0.01^{**}	0.03^{**}	8.24^{**}
Error genotype	61	0.00	0.00	0.00	1.57
Total	123	0.16	0.11	0.03	152.89

PA: phytic acid; iP: inorganic phosphorous; TP: Total phosphorous; PAP/TP: phytic acid phosphorous to total phosphorous; *: Significant at 5% and **: 1% level of significance

Table 4.10Analysis of variance for phytic acid and inorganic phosphorous among the *lpa2-1* based inbreds evaluated in the study

Source of variation	***df***	**Mean sum of squares**							
		PA		**IP**		**TP**		**PAP/TP**	
		Delhi	**Hyderabad**	**Delhi**	**Hyderabad**	**Delhi**	**Hyderabad**	**Delhi**	**Hyderabad**
Replication	1	0.00	0.00	0.00	0.00	0.00	0.00	1.56	0.01
Genotype	30	0.17^{**}	0.17^{**}	0.13^{**}	0.12^{**}	0.04^{**}	0.04^{**}	162.93^{**}	170.94^{**}
Error	30	0.00	0.00	0.00	0.004	0.00	0.01	1.84	2.98
Total	61	0.09	0.09	0.06	0.06	0.02	0.02	81.06	85.53

PA: phytic acid; iP: inorganic phosphorous; TP: Total phosphorous; PAP/TP: phytic acid phosphorous to total phosphorous; *: Significant at 5% and **: 1% level of significance

PAP/TP) estimated across the locations. The obtained results signified the scope for the selection of desired low phytate genotype for higher bioavailability of mineral elements.

4.2.4 Mean performance of phytic acid and inorganic phosphorous among the *lpa2-1* inbreds and the wild type inbreds

The diverse set of 24 *lpa2-1* inbreds along seven wild type inbreds were evaluated for the mean performance of the various biochemical parameters at two locations (Delhi and Hyderabad). The data obtained from the estimation of various biochemical parameters are presented in table 4.11. The *lpa2-1* inbreds possessed the mean PA content of 1.97 mg/g, which ranged from 1.77 to 2.13 mg/g for Delhi, whereas at Hyderabad, it ranged from 1.59 to 2.01 mg/g with the mean content of 1.83 mg/g (Table 4.11). However, the wild type inbreds possessed the mean PA content of 2.63 mg/g, which ranged between 2.54 to 2.71 mg/g at Delhi and the mean of 2.49 mg/g with the range of 2.32 to 2.62 mg/g at Hyderabad (Table 4.11). The data on PA indicated that the mean PA content was significantly lesser in *lpa2-1* inbreds compared to the wild type inbreds. It signified the effect of *lpa2-1* allele on the phytic acid content. There was reduction of 25% and 26.6% in the PA content in the *lpa2-1* inbreds compared to the wild type inbreds at Delhi and Hyderabad locations, respectively (Table 4.11).

The iP content in the *lpa2-1* genotypes varied from 0.63 to 1.08 mg/g with the mean of 0.78 mg/g at Delhi, whereas it varied from 0.62 to 1.02 mg/g with the mean of 0.83 mg/g at Hyderabad (Table 4.11). However, the mean content in the wild type inbreds was 0.24 and 0.29 mg/g at Delhi and Hyderabad locations respectively; the content ranged from 0.12 to 0.32 mg/g and 0.23 to 0.38 mg/g in the respective locations. The mean iP was significantly higher in the *lpa2-1* mutant inbreds compared the wild type inbreds at both the locations. The increase of iP to the extent of 2.25 and 1.86 fold in the *lpa2-1* inbreds as compared to wild type inbreds was observed at Delhi and Hyderabad, respectively (Table 4.11).

The mean TP content was 2.76 mg/g and 2.66 mg/g at Delhi and Hyderabad locations respectively, whereas the wild type inbreds possessed the mean TP of 2.87 mg/g and 2.78 mg/g in the respective locations (Table 4.11). The content of phytic acid and inorganic phosphate varied between the *lpa2-1* and wild type inbreds, however the total phosphorus content was similar in the *lpa2-1* and the wild type inbreds.

The PAP/TP ratio for *lpa2-1* ranged from 64.17 to 76.14 % with the mean of 71.62 % for Delhi, whereas for Hyderabad, it varied from 63.52 to 74.51% with the mean of 68.85% (Table 4.11). However, the ratio of the wild type inbreds varied from 88.97 to 95.51% with the mean ratio of 91.80 % for Delhi. At Hyderabad, it varied from 86.52 to 91.50 % with the mean of 89.58%. The data clearly indicated that the PAP/TP ratio was higher in the wild type inbreds compared to *lpa2-1* inbreds; this signified the direct

Table 4.11 Mean performance of phytic acid and inorganic phosphorous among the *lpa2-1*inbreds and the wild type inbreds evaluated in the study

Genotype	PA (mg/g)		iP (mg/g)		TP (mg/g)		PAP/TP (%)	
	Delhi	Hyderabad	Delhi	Hyderabad	Delhi	Hyderabad	Delhi	Hyderabad
***lpa1-1* mutants**								
PMI-LP2-201	1.88	1.77	0.95	1.02	2.83	2.79	66.57	63.52
PMI-LP2-202	1.98	1.93	0.72	0.79	2.70	2.71	73.46	70.92
PMI-LP2-203	1.91	1.84	0.70	0.69	2.61	2.53	73.03	72.66
PMI-LP2-204	2.13	1.99	0.71	0.68	2.84	2.67	75.06	74.51
PMI-LP2-205	1.90	1.82	0.87	0.88	2.76	2.70	68.61	67.45
PMI-LP2-206	1.96	1.76	0.68	0.65	2.64	2.41	74.15	73.09
PMI-LP2-207	1.98	1.78	0.80	0.88	2.78	2.66	71.36	66.99
PMI-LP2-208	1.85	1.79	0.77	0.88	2.62	2.67	70.62	67.00
PMI-LP2-209	1.99	1.90	0.73	0.83	2.72	2.73	73.29	69.53
PMI-LP2-210	1.92	1.88	0.63	0.73	2.56	2.61	75.23	72.14
PMI-LP2-211	1.93	1.84	0.73	0.84	2.67	2.68	72.47	68.58
PMI-LP2-212	1.86	1.77	0.75	0.79	2.61	2.56	71.11	69.31
PMI-LP2-213	1.94	1.83	1.08	1.01	3.02	2.83	64.17	64.45
PMI-LP2-214	1.92	1.78	0.75	0.92	2.67	2.70	71.91	65.99
PMI-LP2-215	1.96	1.72	0.65	0.62	2.61	2.34	75.10	73.61
PMI-LP2-216	2.12	1.79	0.66	0.76	2.78	2.54	76.14	70.27

PMI-LP2-217	2.13	1.86	1.00	0.94	3.13	2.80	67.95	66.40
PMI-LP2-218	1.98	1.97	0.74	0.89	2.72	2.86	72.78	69.00
PMI-LP2-219	2.13	2.01	0.81	0.76	2.94	2.77	72.38	72.66
PMI-LP2-220	2.05	1.85	0.87	0.89	2.92	2.74	70.15	67.54
PMI-LP2-221	2.12	1.90	0.71	0.89	2.83	2.78	74.95	68.17
PMI-LP2-222	1.77	1.72	0.82	0.82	2.60	2.54	68.27	67.51
PMI-LP2-223	1.99	1.59	0.83	0.88	2.82	2.47	70.65	64.35
PMI-LP2-224	1.94	1.78	0.85	0.89	2.79	2.67	69.52	66.75
Mean	**1.97**	**1.83**	**0.78**	**0.83**	**2.76**	**2.66**	**71.62**	**68.85**
Wild type inbreds								
PMI-PV1	2.59	2.44	0.32	0.38	2.91	2.82	88.97	86.52
PMI-PV2	2.70	2.62	0.25	0.34	2.96	2.96	91.48	88.42
PMI-PV3	2.66	2.49	0.24	0.23	2.90	2.72	91.70	91.50
PMI-PV4	2.59	2.52	0.17	0.28	2.76	2.80	93.76	90.13
PMI-Q1	2.64	2.51	0.12	0.24	2.77	2.75	95.51	91.12
PMI-Q2	2.71	2.50	0.29	0.28	3.00	2.78	90.34	89.98
PMI-Q3	2.54	2.32	0.26	0.28	2.79	2.60	90.82	89.36
Mean	**2.63**	**2.49**	**0.24**	**0.29**	**2.87**	**2.78**	**91.80**	**89.58**
CD	**0.10**	**0.09**	**0.08**	**0.13**	**0.11**	**0.15**	**2.77**	**3.52**

PA: phytic acid; iP: inorganic phosphorous; TP: Total phosphorous; PAP/TP: phytic acid phosphorous to total phosphorous; CD: Critical difference at 5% level of significance contribution of PA content to the PAP/TP ratio. Hence, the bioavailability of the mineral elements was high in the low phytic acid based inbreds compared to the wild type inbreds.

Among the set of 24 *lpa2-1* inbreds, the inbreds with the lowest ratio of PAP/TP were PMI-LP2-213, PMI-LP2-201. PMI-LP2-217, PMI-LP2-222 and PMI-LP2-205 at Delhi, whereas PMI-LP2-201, PMI-LP2-223, PMI-LP2-213, PMI-LP2-214 and PMI-LP2-217 were found to had the lowest ratio of PAP/TP at Hyderabad location. These top *lpa2-1* inbreds also believed to have higher bioavailability of mineral elements compared to remaining inbreds.

4.2.5 Combined ANOVA for phytic acid and inorganic phosphorous among the *lpa2-1* based inbreds

The combined data from both the locations were considered for the analysis to find the performance of genotypes across the locations for the biochemical parameters. The results obtained from the combined analysis of variance for biochemical parameters are presented in the table 4.12. The results of the combined analysis of variance revealed that the performance of the *lpa2-1* mutants and the wild type inbreds varied across the locations for all the characters studied. The interaction effect of genotype and location was significant for all the parameters studied. The role of environment on the performance of genotypes was also significant. It was also observed that replications had no influence on the variance among the genotypes evaluated for biochemical parameters.

4.3 Molecular characterization of inbreds

4.3.1 Molecular characterization of *lpa1-1* based low phytic acid maize inbreds using microsatellite markers

Genetic relationship assessment among inbreds is important for the success of the hybrid breeding programme. Genetic diversity analyses based on molecular markers enables to study the genetic relatedness between the genotypes more efficiently. A diverse panel of 24 *lpa1*-based inbreds varying for kernel phytic acid were analyzed using 60 genome-wide SSRs with three to ten markers per linkage group. The details of the microsatellite markers used for the diversity studies among the *lpa1-1*inbred lines are represented in table 3.4.

In the present study, total of 172 alleles were generated with mean of 2.87 and a range of 2 to 5 alleles per SSR locus. Out of the 60 SSR marker loci, 29 loci produced 2 alleles, 15 has given 3 alleles, 12 gave 4 alleles and finally 4 loci gave 5 alleles. The size of the allele ranged from 60bp (*umc2053*) to 550bp (*bnlg1957*), which indicated the presence of high level of genetic diversity (Table 4.13). Representative gels depicting SSR polymorphism are shown in figure 4.1. Allele present in the maximum number of

Table 4.12 Combined Analysis of variance for phytic acid and inorganic phosphate among the *lpa2-1* based inbreds evaluated in the study

Source of variation	***df***	**Mean sum of squares**			
		PA	**IP**	**TP**	**PAP/TP**
Replication	1	0.00	0.00	0.00	0.65
Location	1	0.65^{**}	0.07^{**}	0.30^{**}	217.25^{**}
Genotype	30	0.34^{**}	0.25^{**}	0.06^{**}	330.03^{**}
Genotype × Location	30	0.01^{**}	0.00^{*}	0.01^{**}	3.83
Error genotype	61	0.00	0.00	0.00	2.38
Total	123	0.09	0.06	0.02	84.38

PA: phytic acid; iP: inorganic phosphorous; TP: Total phosphorous; PAP/TP: phytic acid phosphorous to total phosphorous; *: Significant at 5% and **: 1% level of significance

Table 4.13 Details of SSR genotyping among the 24 *lpa1-1* inbreds used in the study

S. No.	Marker	Bin	SSR repeat	No. of alleles	Major allele frequency	Gene diversity	Heterozygosity	PIC
1	*bnlg1203*	1.03	(AG)17	2	0.74	0.41	0.11	0.36
2	*umc1076*	1.05	(CA)N	3	0.5	0.59	0.13	0.51
3	*umc1774*	1.1	(GT)7	2	0.91	0.17	0	0.15
4	*umc2186*	2	(CGG)6	2	0.98	0.04	0.04	0.04
5	*umc2245*	2.01	(CAA)7	3	0.54	0.6	0	0.53
6	*mmc0111*	2.02	(GA)34	3	0.52	0.6	0.17	0.53
7	*umc1541*	2.04	(GCT)4	2	0.88	0.23	0	0.21
8	*umc1769*	2.04	(GA)8	2	0.58	0.49	0	0.37
9	*umc2253*	2.06	(TCGC)4	2	0.52	0.5	0.04	0.37
10	*umc1560*	2.07	(GC)6	2	0.92	0.15	0	0.14
11	*umc2101*	3	(AG)7	4	0.39	0.7	0.05	0.64
12	*umc2119*	3.04	(AC)7	2	0.64	0.46	0	0.36
13	*umc1495*	3.04	(AGGAC)4	4	0.5	0.65	0.04	0.6
14	*umc1087*	3.04	(GA)17	2	0.76	0.36	0.13	0.3
15	*umc1025*	3.04	(CT)11	2	0.88	0.22	0.17	0.19
16	*umc2262*	3.04	(CATCT)5	3	0.44	0.63	0.25	0.56
17	*bnlg1456*	3.05	AG(15)	3	0.5	0.62	0	0.55
18	*bnlg1957*	3.05	AG(10)	4	0.8	0.35	0.18	0.33
19	*umc2152*	3.09	(TG)8	3	0.81	0.32	0.17	0.3

20	*umc1758*	4.01	(CTT)5	2	0.57	0.49	0	0.37
21	*umc1194*	4.07	GGCC	3	0.63	0.5	0.08	0.41
22	*umc2188*	4.08	(TC)8	3	0.67	0.47	0.08	0.39
23	*umc2365*	4.08	(TGC)4	3	0.6	0.52	0.13	0.44
24	*umc2384*	4.08	(GCC)5	2	0.75	0.38	0.17	0.3
25	*umc1740*	4.09	(AGT)4	2	0.88	0.22	0	0.19
26	*umc1101*	4.09	(CT)6	4	0.6	0.57	0.25	0.53
27	*umc1761*	5.02	(GCA)5	4	0.54	0.63	0	0.58
28	*umc2216*	5.06	(TCTC)5	4	0.56	0.61	0.08	0.56
29	*umc2308*	5.09	(CGGCG)4	3	0.42	0.64	0	0.57
30	*umc1143*	6	AAAAT	2	0.67	0.44	0	0.35
31	*umc2309*	6	(CAGG)4	3	0.46	0.63	0	0.56
32	*umc1614*	6.04	(CTG)6	2	0.77	0.35	0.04	0.29
33	*umc1105*	6.04	(GCC)4	4	0.42	0.66	0.17	0.6
34	*umc1795*	6.05	(GCGCG)4	2	0.5	0.5	0	0.38
35	*umc1912*	6.06	(GCG)6	2	0.63	0.47	0	0.36
36	*umc2123*	6.06	(GCT)4	4	0.46	0.68	0.13	0.63
37	*umc2170*	6.06	(TAC)4	2	0.54	0.5	0.17	0.37
38	*umc1666*	7.02	(AG)10	3	0.63	0.53	0.13	0.46
39	*umc1393*	7.02	(GTC)4	3	0.48	0.56	0.21	0.46
40	*umc2333*	7.05	(CCGT)4	2	0.61	0.48	0	0.36

41	*umc1858*	8.04	(TA)8	2	0.52	0.5	0	0.37
42	*umc1882*	8.05	(AG)12	2	0.75	0.38	0	0.3
43	*umc2210*	8.05	(AAAAT)4	2	0.67	0.44	0	0.35
44	*umc1846*	8.05	(TC)8	4	0.33	0.73	0.13	0.68
45	*umc1828*	8.06	(GA)8	2	0.58	0.49	0	0.37
46	*umc2031*	8.06	(GCG)5	4	0.71	0.47	0.29	0.44
47	*umc1055*	8.07	(AG)9	4	0.43	0.66	0.2	0.59
48	*umc2134*	9.05	(TTC)6	2	0.63	0.47	0	0.36
49	*umc1078*	9.05	(GT)13	5	0.5	0.65	0.18	0.6
50	*umc2089*	9.07	(CGC)4	2	0.83	0.28	0	0.24
51	*umc1277*	9.07	(AATA)5	2	0.62	0.47	0	0.36
52	*umc1505*	9.08	(AAAAC)4	3	0.92	0.16	0.08	0.15
53	*umc2053*	10.01	(CGA)4	5	0.52	0.62	0.29	0.56
54	*umc2114*	10.02	(TCT)4	2	0.86	0.24	0	0.21
55	*umc1113*	10.02	(CACAG)5	5	0.52	0.66	0.35	0.62
56	*umc2016*	10.03	(ACAT)4	2	0.83	0.28	0	0.24
57	*umc1678*	10.04	(TCG)6	3	0.5	0.54	0.08	0.43
58	*umc1246*	10.04	(AAAT)5	3	0.71	0.45	0.21	0.4
59	*umc1507*	10.05	(CACAA)4	4	0.6	0.57	0.1	0.52
60	*umc2203*	10.07	(CGC)6	5	0.52	0.67	0.36	0.63
	Mean			**2.87**	**0.63**	**0.48**	**0.09**	**0.41**

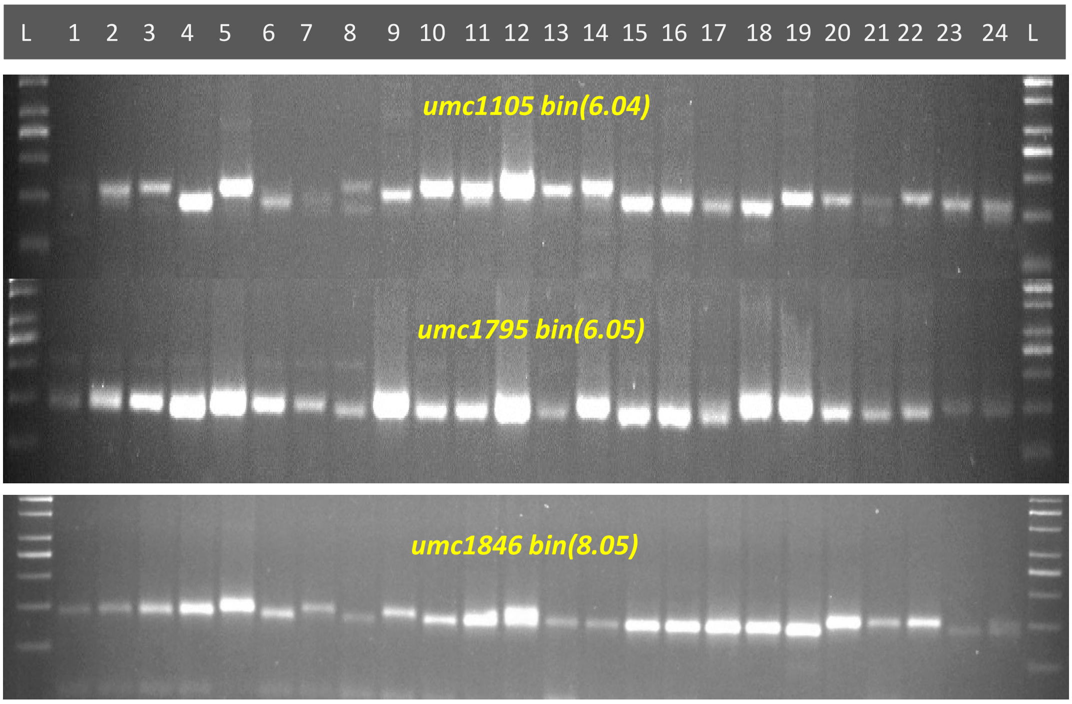

Figure 4.1 Genotyping of *lpa1-1* inbreds using SSR markers

individuals in the population is regarded as the major allele. The average frequency of major alleles was 0.63 with the range of 0.33 (*umc1846*) to 0.98 (*umc2186*). The locus with low major allelic frequency indicates highly diverse locus. Gene diversity defined as the probability that two randomly selected alleles are different from the population. The highest gene diversity was for the SSR marker *umc1846*(0.73) and the lowest gene diversity observed was for the SSR marker *umc2186*(0.04) with a mean gene diversity of 0.48 (Table 4.13).

The polymorphism information content (PIC) ranged between 0.04(*umc2186*) to 0.68 (*umc1846*) among the diverse panel of *lpa1-1*based maize inbreds with a mean PIC of 0.41 (Table 4.13). Of the 60 SSR loci, 21 loci had a PIC value of more than 0.5, indicating the greater capacity of these SSR markers to distinguish the genotypes. The analysis also identified 4 unique and 17 rare alleles which offered the prospect of unequivocal identification of inbreds.

In addition to understanding the purity of the seed lot, SSR-based assessment of heterozygosity effectively measures the period of inbred development. The co-dominant SSR markers can differentiate heterozygotes from homozygotes genotypes. The mean heterozygosity observed by all SSR markers was 0.09 which ranged between 0.00 to 0.36, indicating that most loci were homozygous and alleles were fixed. Some loci however, such as *umc2262*(0.25), *umc2031*(0.29), *umc2053*(0.29), *umc1113* (0.35*), umc2203*(0.36) and *umc1393*(0.21) showed significant heterozygosity (Table 4.13).

Cluster analysis for molecular data was done using neighbor-joining method, which was based on the genetic dissimilarities. The cluster analysis was carried-out for the set of 24 *lpa1*-based maize inbreds. The pair-wise genetic dissimilarity ranged from0.23 to 0.81, with a mean of 0.64 which indicated the studied inbreds were diverse in nature at genetic level. Cluster analysis grouped the selected set of 24 genotypes into three distinct major clusters namely A, B and C (Figure 4.2). Cluster A had the highest number of genotypes of 10, which werePMI-LP1-104,PMI-LP1-106, PMI-LP1-107,PMI-LP1-118, PMI-LP1-119, PMI-LP1-120, PMI-LP1-121,PMI-LP1-122,PMI-LP1-123and PMI-LP1-124.Cluster B had only six genotypes(PMI-LP1-101,PMI-LP1-105,PMI-LP1-108,PMI-LP1-109, PMI-LP1-110andPMI-LP1-111).Finally, the cluster C consisted of remaining eight genotypes (PMI-LP1-102, PMI-LP1-103, PMI-LP1-112, PMI-LP1-113, PMI-LP1-114, PMI-LP1-115, PMI-LP1-116 and PMI-LP1-117) (Figure 4.2).

These obtained results of cluster analysis have been further validated through principle co-ordinate analysis (PCoA). In the PCoA, the inbreds belongs to same quadrants showed the similar pedigree, while inbreds belong to different cluster possessed different pedigree. In the present study, a set of 24 *lpa1*-based inbreds lines were derived from the seven parental inbreds lines (PMI-PV1, PMI-PV2, PMI-PV3,

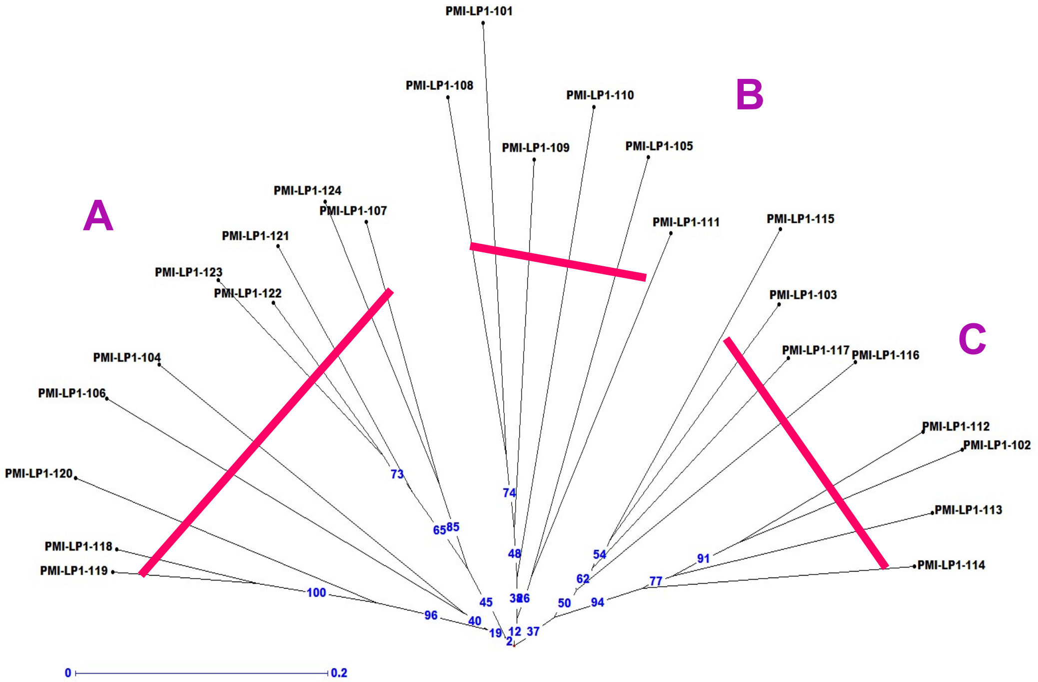

Figure 4.2 Cluster analysis depicting genetic relationship among 24 *lpa1*-based inbreds

PMI-PV4, PMI-Q1, PMI-Q2 and PMI-Q3) and these were the parents of already released commercial hybrids; and they were well distributed both in the cluster analysis and PCoA (Figure 4.3). The lines(PMI-LP1-112,PMI-LP1-113,PMI-LP1-114 and PMI-LP1-102) belonged to cluster C,were present in the below right quadrangle and derived from the one parental line PMI-PV6;whereas the lines(PMI-LP1-107,PMI-LP1-121,PMI-LP1-122 and PMI-LP1-123) belonged to cluster A, were present in above left quadrangle and derived from the other parental line PMI-Q3, of already released hybrid. Hence, the lines derived from these two respective parental inbreds also belonged to two different quadrangles.

4.3.2 Molecular characterization of *lpa2-1* based low phytic acid maize inbreds using microsatellite markers

Here a set of selected 24 *lpa2*-based inbreds differed for kernel phytic acid was analyzed using 61 SSR markers, which are distributed across the genome with four to nine markers per linkage group. From the 61 SSR markers, a total of 181 alleles were generated with mean of 2.97 alleles; and the number of alleles ranged from 2 to 6. Presence of high level of locus diversity was indicated by size of the allele, which ranged from 50bp (*umc1012*) to 450bp (*umc1765*). Out of 61 SSR loci, 24markers have given 2 alleles, 21 markers have given 3 alleles,12 markers have given 4 alleles, two marker loci have given 5 alleles and other two marker loci have produced 6 alleles. The list of details of SSRs which are used in the study are presented in table 4.14and the representative of gel image depicting polymorphism is shown in figure 4.4.

The major allele frequency ranged from 0.28 (*umc1012*) to 0.98 (*umc1537*) with a mean of 0.65. The allele, which is having low value of major allele frequency indicates that the locus is highly diverse. The mean gene diversity was 0.46 with the gene diversity ranged from 0.04 (*umc1537*) to 0.76 (*umc1012*). The PIC value ranged among *lpa2*-based inbreds from0.04 (*umc1537*) to 0.72 (*umc1012*) with the mean of 0.40. Among the 61 SSR marker loci, 19 SSR loci showed the PIC value of 0.5 and above which indicated the higher ability of SSRs to distinguish inbred lines (Table 4.14).

The analysis from the present study also identified 13 unique alleles and 24 rare alleles, which offered the prospect of explicit separation of one inbred line from others. Co-dominant nature of SSR marker can differentiate the heterozygotes from homozygotes. In the selected *lpa2*-based inbreds, the heterozygosity was detected with mean of 0.07 and the range varied from 0.00 to 0.39, which indicates that most of loci have attained the homozygosity. SSR-based assessment of heterozygosity effectively measures the period of inbred development. However, some loci namely *umc1914* (0.21),

Table 4.14 Details of SSR genotyping among the 24 *lpa2-1* inbreds used in the study

S. No.	Marker	Bin	SSR repeats	No. of alleles	Major allele frequency	Gene diversity	Heterozygosity	PIC
1	*umc1689*	1.05	(GCG)5	4	0.63	0.54	0.14	0.48
2	*umc1914*	1.08	(CAG)4	4	0.48	0.67	0.21	0.62
3	*umc1082*	1.09	(GA)16	3	0.54	0.58	0	0.5
4	*umc1819*	1.12	CAAC)4	2	0.92	0.15	0.17	0.14
5	*umc1776*	2.03	(TA)7	4	0.88	0.23	0.17	0.22
6	*umc1448*	2.04	(GCT)5	3	0.44	0.65	0	0.58
7	*umc1541*	2.04	(GCT)4	2	0.77	0.35	0.04	0.29
8	*umc2253*	2.05	(TCGC)4	2	0.67	0.44	0	0.35
9	*umc2101*	3	(AG)7	2	0.91	0.17	0	0.15
10	*umc1425*	3.04	(TCA)4	2	0.83	0.28	0	0.24
11	*umc1025*	3.04	(CT)11	2	0.79	0.33	0.33	0.28
12	*umc1087*	3.04	(GA)17	3	0.75	0.38	0.12	0.32
13	*umc1012*	3.04	(GA)6	5	0.28	0.76	0.27	0.72
14	*bnlg1456*	3.05	(AG)15	4	0.59	0.58	0.04	0.52
15	*umc2152*	3.09	(TG)8	4	0.8	0.35	0.27	0.33
16	*umc1869*	4.06	(GGT)6	2	0.79	0.33	0	0.28
17	*umc1329*	4.06	GCC)7	2	0.54	0.5	0	0.37
18	*umc1194*	4.07	GGCC	3	0.38	0.66	0	0.59
19	*umc1740*	4.09	(AGT)4	2	0.77	0.36	0.04	0.31

20	*umc1101*	4.09	(CT)6	3	0.63	0.54	0.1	0.49
21	*umc1445*	5	(AC)7	2	0.77	0.35	0	0.29
22	*umc1240*	5	(TTG)5	2	0.57	0.49	0	0.37
23	*umc1761*	5.02	(GCA)5	4	0.46	0.66	0	0.59
24	*umc1687*	5.05	(TGC)6	3	0.58	0.57	0	0.51
25	*umc2216*	5.06	(TCTC)5	3	0.5	0.57	0.08	0.48
26	*umc1537*	5.07	(TCG)4	2	0.98	0.04	0.04	0.04
27	*umc2143*	5.08	(TTC)4	2	0.92	0.15	0	0.14
28	*umc2308*	5.08	(CGGCG)4	3	0.46	0.6	0	0.52
29	*umc2209*	5.09	(CT)6	2	0.82	0.29	0	0.25
30	*umc1143*	6	AAAAT	3	0.67	0.49	0	0.42
31	*umc2309*	6	(CAGG)4	3	0.45	0.63	0	0.55
32	*umc1614*	6.04	(CTG)6	3	0.67	0.49	0.08	0.42
33	*umc1105*	6.04	(GCC)4	4	0.43	0.68	0.15	0.63
34	*umc1795*	6.05	(GCGCG)4	3	0.45	0.64	0.05	0.57
35	*umc1912*	6.06	(GCG)6	2	0.68	0.43	0	0.34
36	*umc2123*	6.06	(GCT)4	4	0.79	0.35	0.04	0.33
37	*umc1831*	7.02	(AG)8	2	0.92	0.15	0	0.14
38	*umc1666*	7.02	(AG)10	6	0.46	0.73	0.21	0.7
39	*umc1393*	7.02	(GTC)4	3	0.46	0.58	0.39	0.48
40	*umc1359*	7.04	(TC)12	2	0.57	0.49	0	0.37

41	*umc2190*	7.06	(CCT)4	3	0.45	0.64	0	0.57
42	*umc1799*	7.06	(TG)12	6	0.5	0.68	0.21	0.64
43	*umc1765*	8.03	(GCT)5	5	0.44	0.72	0.16	0.67
44	*umc2210*	8.05	(AAAAT)4	2	0.96	0.08	0	0.08
45	*umc1846*	8.05	(TC)8	3	0.52	0.54	0.1	0.44
46	*umc1950*	8.05	not given	3	0.53	0.54	0.1	0.44
47	*umc2031*	8.06	(GCG)5	3	0.75	0.39	0.05	0.33
48	*umc1055*	8.07	(AG)9	4	0.76	0.4	0.09	0.37
49	*umc1647*	9	(GA)10	2	0.96	0.08	0.08	0.08
50	*bnlg1724*	9.01	(AG)31	4	0.5	0.61	0.33	0.54
51	*umc1809*	9.01	(GGA)5	3	0.6	0.5	0.18	0.4
52	*umc2337*	9.03	(AGC)4	3	0.5	0.62	0	0.55
53	*umc1878*	9.04	(AT)6	2	0.7	0.42	0	0.33
54	*umc1771*	9.04	(CGTC)4	4	0.42	0.7	0	0.65
55	*umc1310*	9.06	(GCG)5	2	0.81	0.31	0	0.26
56	*umc2053*	10.01	(CGA)4	2	0.92	0.15	0	0.14
57	*umc2114*	10.02	(TCT)4	2	0.55	0.5	0	0.37
58	*umc2016*	10.03	(ACAT)4	4	0.69	0.47	0.13	0.42
59	*umc1246*	10.04	(AAAT)5	3	0.52	0.55	0.17	0.45
60	*umc1507*	10.05	(CACAA)4	2	0.89	0.19	0	0.17
61	*umc2203*	10.07	(CGC)6	3	0.62	0.53	0	0.46
	Mean			**2.97**	**0.65**	**0.46**	**0.07**	**0.40**

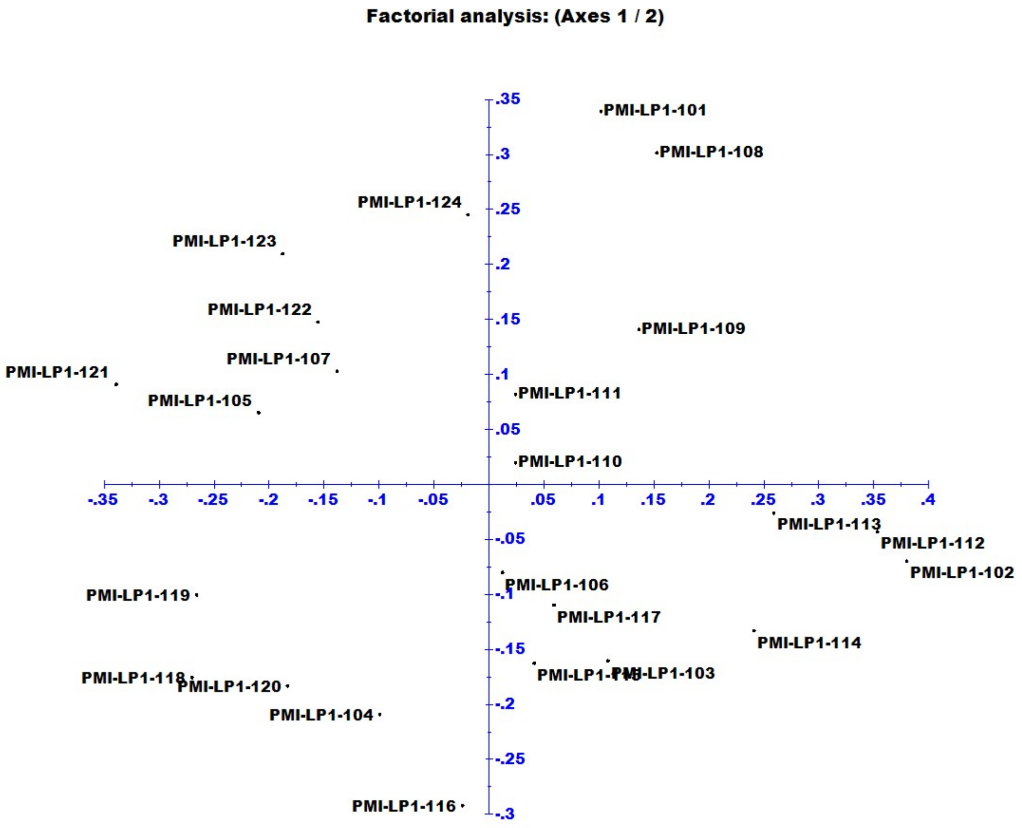

Figure 4.3 PCoA depicting genetic relationship among 24 *lpa1*-based inbreds

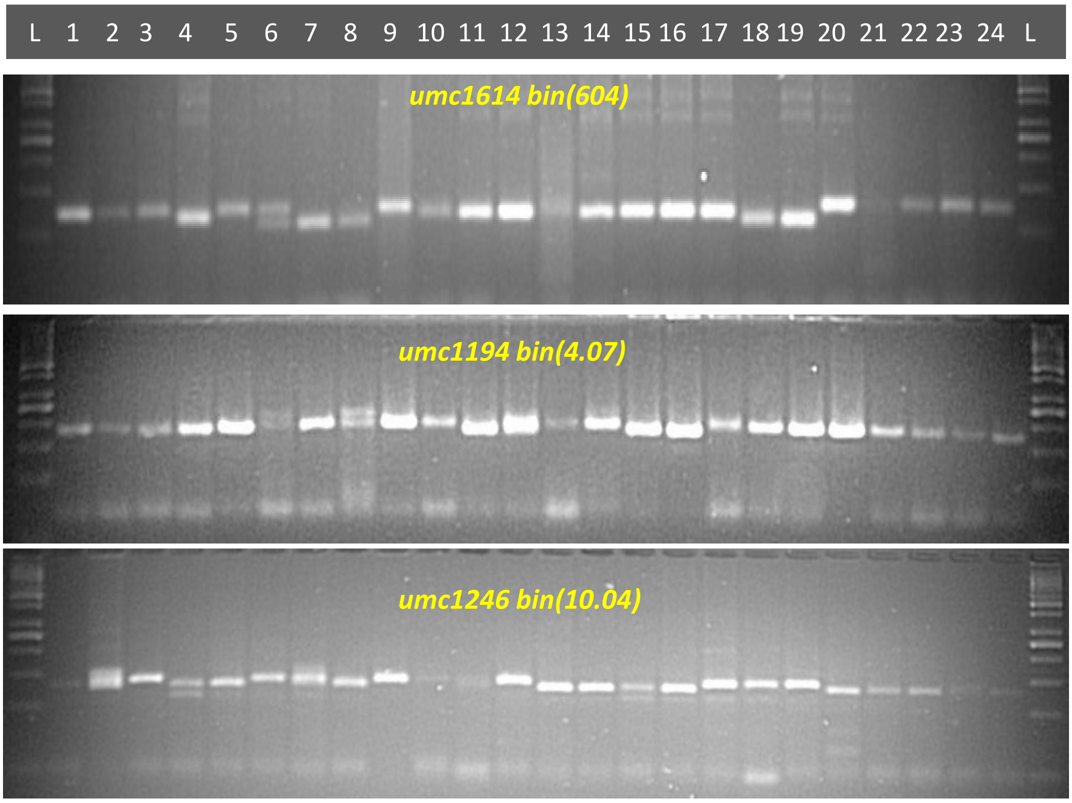

Figure 4.4 Genotyping of *lpa2-1* inbreds using SSR markers

umc1799 (0.21), *umc1666* (0.21), *umc2152* (0.27), *umc1025* (0.33), *bnlg1724* (0.33), *umc1393* (0.39) and *umc1012* (0.27) had shown high heterozygosity (Table 4.14).

The selected set of 24 *lpa2*-based inbreds was subjected to cluster analysis based on genetic dissimilarity by using neighbor-joining method. The pair wise genetic dissimilarity ranged between 0.27 to 0.79 with a mean of 0.61. Cluster analysis grouped selected set of inbreds into three major clusters, namely A, B and C (Figure 4.5). Cluster A consisted of 10 genotypes (PMI-LP2-205, PMI-LP2-206, PMI-LP2-207, PMI-LP2-218, PMI-LP2-219, PMI-LP2-220, PMI-LP2-221, PMI-LP2-222, PMI-LP2-223 and PMI-LP2-224).Cluster B comprised of nine genotypes (PMI-LP2-202, PMI-LP2-203, PMI-LP2-204, PMI-LP2-212, PMI-LP2-213, PMI-LP2-214, PMI-LP2-215, PMI-LP2-216 and PMI-LP2-217); and the cluster C had only five genotypes (PMI-LP2-201,PMI-LP2-208,PMI-LP2-209,PMI-LP2-210 and PMI-LP2-211) (Figure 4.5).

The results of cluster analysis were further supported though the principle co-ordinate analysis (PCoA). In PCoA, the inbreds belongs to same quadrants showed the similar pedigree, while inbreds belong to different cluster possessed different pedigree (Figure 4.6). In the present study, a set of 24 *lpa2*-based inbreds lines were derived from the seven parental inbreds lines (PMI-PV1, PMI-PV2, PMI-PV3, PMI-PV4, PMI-Q1, PMI-Q2 and PMI-Q3) and these are the parents of already released commercial hybrids. The lines (PMI-LP2-211, PMI-LP2-208, PMI-LP2-209 andPMI-LP2-210) belonged to cluster C, were present in the below left quadrangle and the lines viz., PMI-LP2-204, PMI-LP2-203, PMI-LP2-215, PMI-LP2-216 and PMI-LP2-217 belonged to cluster B, were present in above right quadrangle (Figure 4.6).

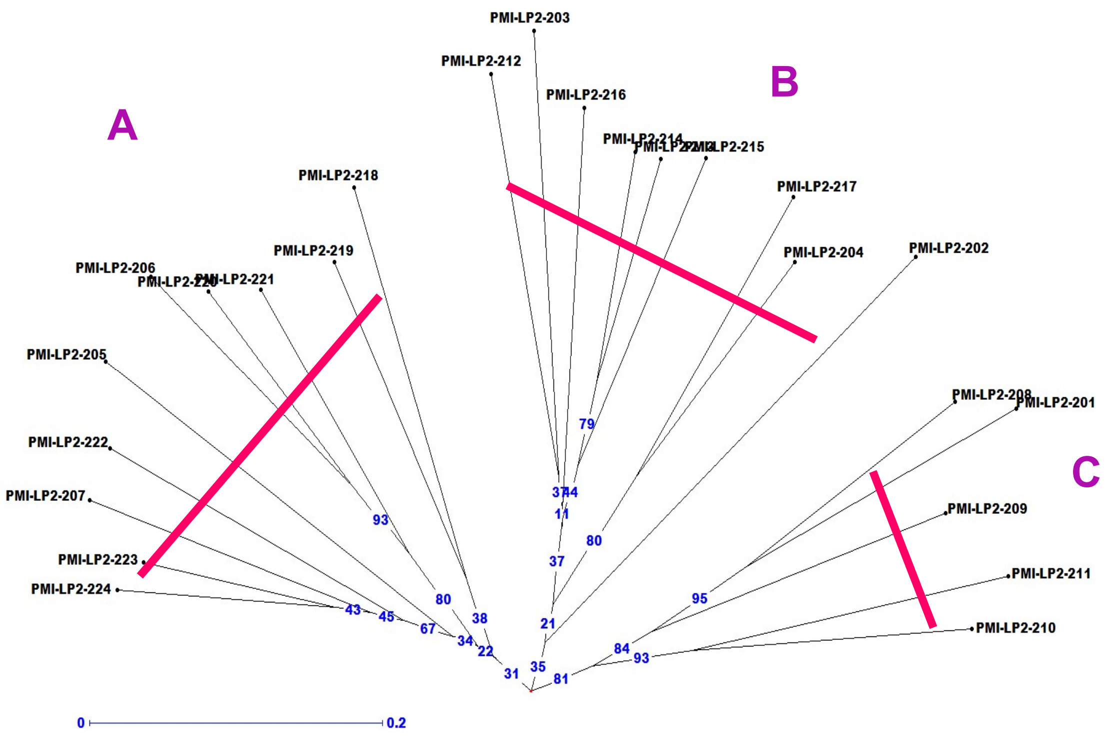

Figure 4.5 Cluster analysis depicting genetic relationship among 24 *lpa2*-based inbreds

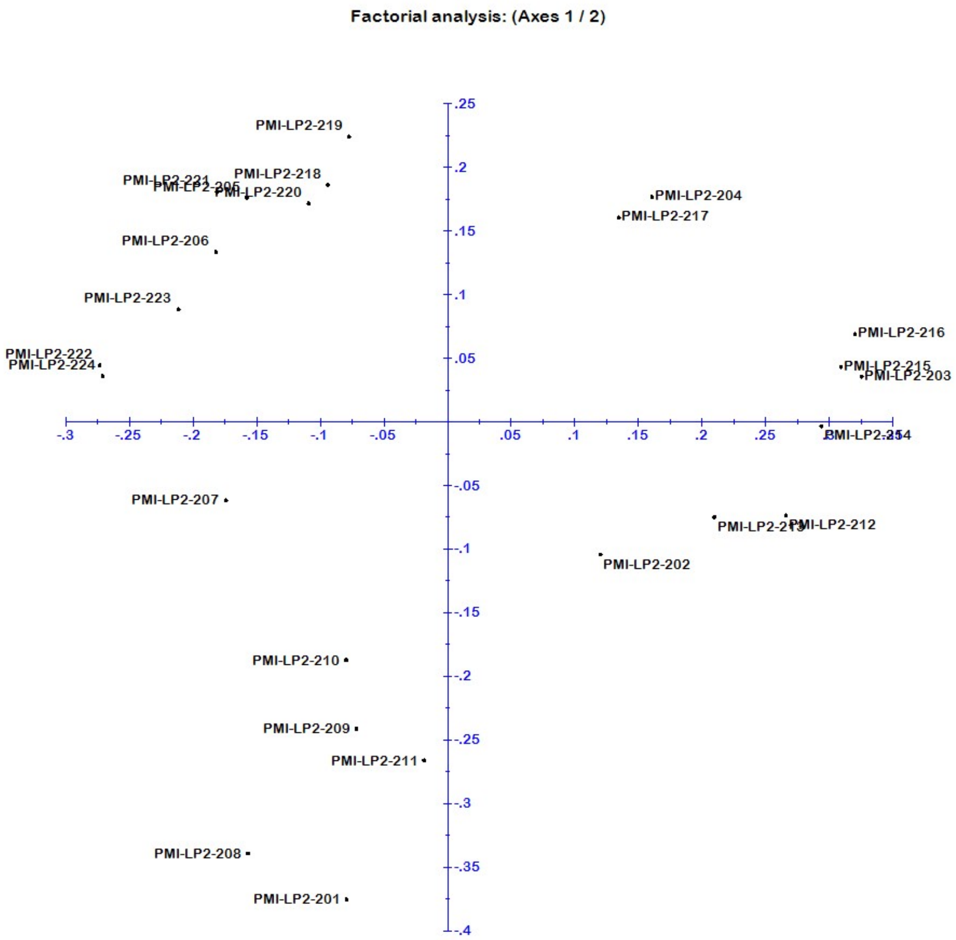

Figure 4.6 PCoA depicting genetic relationship among 24 *lpa2*-based inbreds

5

DISCUSSION

Approximately two billion people suffer from micronutrient malnutrition which accounts one in three people worldwide (Herrington et al. 2019; Prasanna et el. 2020). The majority of the global population depends on plant-based foods, which are often low in key micronutrients and even not able to meet the recommended daily allowances (RDA) (Jha and Warkentin 2020).The deficiencies of key micronutrients may lead to serious illnesses such as poor growth, perinatal complications, intellectual impairments and increased risk of morbidity and mortality (Bailey et al. 2015 ; Guerrant et al. 2000;Kapil and Bhavna 2008). Existence of micronutrient deficiencies for a longer period can impair functioning of immune system and increase chances of susceptibility to infectious disease. Even the present day, COVID-19 has had an instant direct effect on food systems in developing countries. “Many of those people at highest risk during the COVID-19 pandemic are also the populations at highest risk of micronutrient deficiencies and poorer overall nutrition” (McAuliffe et al. 2020).

Alleviation of micronutrient deficiencies has been found to be effective in several infectious diseases and it has been shown to promote favourable clinical outcomes. Biofortification is one such proven solution and nutrition-sensitive agricultural intervention that works in coexistence with other complementary intervened strategies like fortification, supplementation and dietary diversification (Herrington et al. 2019). Nutrition via biofortified crops such as potato, sweet potato, wheat, maize and beans can play a more significant role to provide key micronutrients especially vitamin A, iron (Fe) and zinc (Zn) at large scale (Heck et al. 2020).

Maize is considered as one of the six key staple food crops that are the focus of an international effort to combat micronutrient malnutrition in the developing countries through biofortification (Sureshkumar et al. 2014). Maize being ‘queen of cereal’ also popularly known as ‘poor man’s nutri-cereal’ due to possession of various fractions of nutrients in it. Phosphorous is the most dominant mineral in the maize kernel, where it is found as phytate salts of Mg, Fe, Ca, and Zn. Micronutrients are present in very less amount in maize grain as in the case of other cereals (Gupta et al. 2015a & b).

Considering the global widespread occurrence of micronutrient deficiency especially the mineral elements like Fe and Zn, severe socio-economic loss is encountered (Bouis 2018), and deficiency caused by these mineral elements is found to be quite prominent (Abhijith et al. 2020). The estimated world’s population of 30% and 60% are deficient in Zn and Fe, respectively (White and Broadley 2009). Even though, the genetic improvement of these target micronutrient is the most viable approach but enhancement of Fe and Zn in maize limited due to complexity of these traits like polygenic inheritance, higher influence of genotype × environment interaction and cumbersome phenotyping (Gupta et al. 2015).In addition to these factors, the availability of these elements is also restricted mainly due to the presence of anti-nutritional factor like phytic-acid in maize. The anti-nutritional factor chelates the mineral elements that lead to cause of micronutrient deficiency. Hence, the reduction in the anti-nutritional factors is an essential parameter for higher bioavailability of mineral elements (Gupta et al. 2015a).

The first report of low phytic acid mutations and their role in lowering the phytic acid in maize endosperm is established by Raboy et al. (2000). Majority of these mutants are in the genetic background of temperate genotypes but very few *lpa*-based maize genotypes are available in the locally adapted genetic background (Abhijith et al. 2020); and this posed a serious limitation in the breeding programme for low phytic acid maize genotypes. In this regard, the efforts are being made at ICAR-Indian Agricultural Research Institute (IARI), New Delhi to develop *lpa*-based maize genotypes through marker-assisted selection (Bhatt et al. 2018). The low phytic acid being a grain related trait, destructive nature of phenotyping for the trait posed demand for labour, time and resources. Gene based functional markers have been developed and reported for *lpa1-1* and *lpa2-1* genes (Abhijith et al. 2020). Further, several *lpa*-based inbreds have been developed from the F_2 segregants generated between normal and *lpa*-donor inbreds (Abhijith et al. 2020).

In addition, the evolving facts of COVID-19 provide an opportunity to focus on agricultural innovations, including development and delivery of biofortified crops. The development of new and more specifically "jobs-to-done" crops should be designed through the food systems. This will help to bridge some current disruptions in supply and demand for food system and even it will help for future crises (Heck et al. 2020). Coincidently, our present investigation is also aimed to enhance the bioavailability of the micronutrients in the human diet and it can serve as one of the alternate options to improve immune system of human beings thereby preventing from the infectious pandemic conditions.

The present study was conducted to characterise the developed *lpa*-based inbreds for their agronomic, yield and yield contributing traits and biochemical parameters. The set of inbreds were subjected to analysis considering mean and range for all the studied traits. Mean and range are the important aspects for selection of desired genotype. The mean can serve as a basis for eliminating undesirable genotypes in the population. The variability present among the genotypes help to choose a potential genotype/cross/progeny since variability indicates the extent of recombination for initiating effective selection procedures. Hence, insights into the extent of genetic variability present in a population is of paramount importance to a plant breeder for initialising a judicious breeding programme (Singh and Narayanan 2006). Further, molecular characterization of these inbreds have also been done using SSR markers for their precise usage in maize hybrid breeding. The results obtained from the study are discussed below.

5.1 Morphological characterisation of *lpa*-inbreds

5.1.1 Evaluation of *lpa-1-1* based inbreds for yield and yield attributes and across the locations

The set of 24 *lpa1-1* based inbreds along seven wild type inbreds were evaluated for yield and other morphological characters like plant height, ear height, days to 50% male flowering and days to 50% female flowering at two locations.

ANOVA results revealed that significant differences for all the studied characters among all the *lpa1-1* based inbreds and wild types inbreds evaluated at both the locations (Delhi and Hyderabad). These results signify that the existence of wide range of genetic variability for all studied characters among the genotype evaluated. For any plant breeding activity, existence of genetic variability is a pre-requisite factor and it provides an opportunity for breeders to develop the new and/or improved cultivar with desired traits (Bhandari et al. 2017; Bhargava and Srivastava 2019; Govindaraju et al. 2015). Selection can only act on the pre-existing variability and it will not create new variability. Hence, the existence of wide genetic variability among the genotypes provides an opportunity to select the desired genotype.

Further, the mean data pertaining to various yield and other morphological traits have been collected, analysed and compared among the *lpa1-1* mutants and wild type inbreds to draw the conclusive outcomes. Critical difference is an important statistical tool most commonly used to compare the observed differences among different genotypes. If the difference is greater than critical difference, which is considered as significant and vice-versa. The plant height and ear height are considered to be critical characters in terms of describing the potential to use an inbred as male or female parent in the hybrid development programme (Gyenesh-Hegyi and Kizmus 2002). The mean plant height of *lpa1-1* based inbreds are slightly higher compared to the wild type inbreds at their respective locations. The ear height is non-significant among the *lpa1-1* based inbreds and wild type inbreds (Naidoo et al. 2012; Sureshkumar et al. 2014; Tamilkumar et al. 2014;). The mean agronomic performance for 50% male and female flowering for *lpa1-1* mutants are having similar duration for the wild type and mutant inbreds at both the locations. In any plant breeding programme, yield is considered to be the primary target trait for the genetic improvement of crops. The mean grain yield value of the wild type inbreds seems to higher compared to the *lpa1-1* based mutant genotypes at both the locations but statistically the performance is same (Naidoo et al. 2012; Raboy 2000; Sureshkumar et al. 2014; Tamilkumar et al. 2014).

The results of the Combined Analysis of variance indicated that highly significant differences exist among all the *lpa1-1* mutants and the wild type inbreds across the locations (Delhi and Hyderabad) for all the characters under study. The differential performance across the locations for all the studied characters indicated the tremendous scope to select suitable genotype for particular location with desired traits.

5.1.2 Evaluation of *lpa-2-1* based inbreds for yield and yield attributes and across the locations

As like in the case of *lpa1-1* mutants, the diverse set of 24 *lpa2-1* based inbreds along with seven wild type inbreds were also evaluated for yield and other morphological characters like plant height, ear height, days to 50% male flowering and days to 50% female flowering at two locations (Delhi and Hyderabad).

The results from the analysis of variance revealed that significant differences for all the studied characters among all the *lpa2-1* based inbreds and wild types inbreds evaluated at both the locations. The obtained results indicated that presence of wide range of genetic variability for all studied characters among the genotype evaluated. Since, existence of genetic variability is a pre-requisite factor for any plant breeding activity, and it provides great opportunity for breeders to design the new or improved cultivar with desired traits (Bhandari et al. 2017; Bhargava and Srivastava 2019; Govindaraju et al. 2015).

The data collected from *lpa2-1* mutants and wild type inbreds on various yield and other morphological traits were subjected to critical difference analysis to compare and selecting the best performing genotype in their respective locations. The obtained mean data on yield and other morphological traits indicated non-existence of critical difference among the mean values of *lpa2-1* and wild type inbreds (Naidoo et al. 2012; Raboy 2000; Sureshkumar et al. 2014; Tamilkumar et al. 2014).

In addition, the data from both the locations for all the traits under study were subjected to combined analysis of variance. The results indicated that highly significant differences existed among all the *lpa2-1* based inbreds and the wild type inbreds across both the locations (Delhi and Hyderabad) for all the characters under study. The differential performance across the locations for all the studied characters providing greater chance to select best suited genotype for particular location with desired traits. The performance of the genotypes was found to similar even in both the replications in the respective locations.

5.2 Biochemical characterisation of *lpa*-inbreds

5.2.1 Evaluation of *lpa-1-1* based inbreds for phytic acid and inorganic phosphorous

The set of 24 *lpa1-1* low phytic acid-based mutants along with seven wild type inbreds were evaluated at two locations (Delhi and Hyderabad) and were phenotyped for kernel characters like phytic acid (PA), inorganic phosphate (iP), total phosphate (TP) and the ratio of phytic acid phosphate to total phosphates (PAP/TP). The estimated data from the biochemical analysis were used for the further analysis.

The ANOVA results revealed that highly significant differences among the *lpa1-1*inbreds and the wild type inbreds for all the biochemical parameters (PA, iP, TP and PAP/TP) estimated across both the locations. The phytic acid and inorganic phosphorus contents significantly varied among the genotypes under study in both the locations indicating the scope for the selection of desirable low phytate genotype for higher bioavailability of mineral elements.

The *lpa1-1* mutant is an EMS mutagen created recessive mutation by Raboy (2000) and mapped on to chromosome 1S. The *lpa1-1* encodes a multi-drug resistance associated protein (MRP) transporter (Shi et al. 2007). The effect of *lpa1-1*mutant allele is highly noticeable that the recessive homozygotes showed lower PA and higher iP, as against to wild type. The same trend of lesser PA and higher iP in the *lpa1-1* based inbreds at both the locations were observed. The first report of *lpa1-1* mutant by Raboy (2000) found the recessive nature of mutant. In further study, Raboy (2009) reported the utility of *lpa1-1* mutation for lowering the PA followed by concomitant increase in the iP content in the maize kernels.

From the mean data on PA, it is very much clear that the reduction of 35.3% and 36.2% in the mean PA content of *lpa1-1* mutants compared to the wild type inbreds was observed at Delhi and Hyderabad locations, respectively. The similar results were also reported from Abhijith et al. (2020). Even though there was reduction trend of PA was observed in *lpa1-1* mutants compared to wild type inbreds, the reduction proportion is low compared to the previous research (Naidoo et al. 2012; Raboy et al. 2000; Raboy 2009; Shi et al. 2007).

The mean iP content is significantly higher in the *lpa1-1* mutant inbreds compared to the wild type inbreds at both the locations studied. It is observed that increase of iP to the extent of 3.0 and 3.26 fold in the *lpa1-1*inbreds as compared to wild type inbreds at Delhi and Hyderabad, respectively. The data clearly indicated that the increase in iP content with reduction in the PA content was due to recessive*lpa1-1* allele. The obtained results also follow same trend as that of Abhijith et al. (2020).

Even though there is a decrease in PA content and increase in iP content in *lpa1-1* mutants; higher PA and lower content of iP was observed in the wild type inbreds. However, the TP content was observed to be similar, suggesting that the decrease in the content of PA leads to the increase in the content of iP due to presence of *lpa1-1* mutant allele. The PAP/TP ratio was statistically higher in the wild type inbreds compared to *lpa1-1*inbreds, which signify the direct contribution of PA content to the PAP/TP ratio. The genotypes with higher PAP/TP ratio are said to lower the bioavailability of mineral elements (Fe and Zn) in the diet and vice-versa. The *lpa1-1* based inbreds having lower PAP/TP ratio possess more bioavailability of mineral elements in the diet.

The mean data obtained from both the locations were subjected to analysis for finding the performance of genotypes across the locations. The results from combined analysis of variance revealed that the performance of the *lpa1-1* mutants and the wild type inbreds varied across the locations for all the biochemical characters studied. The interaction effect of genotype and location was also highly significant for all the parameters. The environment also influenced the performance of genotypes for all the biochemical parameters evaluated among the *lpa1-1* based inbreds and the wild type inbreds.

5.2.2 Evaluation of *lpa-2-1*based inbreds for phytic acid and inorganic phosphorous

The *lpa2-1* based inbreds along with wild type inbreds were also phenotyped for kernel characters like phytic acid (PA), inorganic phosphate (iP), total phosphate (TP) and the ratio of phytic acid phosphate to total phosphates (PAP/TP). The estimated data from the biochemical analysis were used for the further analysis.

Mean sum of square values from the analysis revealed that highly significant differences existed among the *lpa2-1*inbreds and the wild type inbreds for all the biochemical parameters (PA, iP, TP and PAP/TP) estimated across both the locations. The phytic acid and inorganic phosphorus contents significantly varied among the genotypes under study in both the locations indicating the wider scope for the selection of low phytate genotype for higher bioavailability of mineral elements.

The *lpa2-1* mutant is also an EMS mutagen created recessive mutation by Raboy (2000) and mapped on to chromosome 1S. It is caused by mutation in an inositol phosphate kinase (*ZmIPK*) gene. The effect of *lpa2-1*mutant allele is also more prominent that the recessive homozygotes showed significantly lesser PA and higher iP as compared to the wild type inbreds. The estimated data on PA and iP contents in *lpa2-1*also revealed the lesser PA and higher iP contents but in the wild type inbreds the content of PA was significantly higher and iP content was significantly lower as that of the results of *lpa1-1*. Raboy (2000) first studied the effects of *lpa2-1* on phytic acid. Further, Raboy (2009) reported the utility of *lpa1-1* mutation for lowering the PA followed by concomitant increase in the iP content in the maize kernels.

The mean values of PA content in the *lpa2-1*signifies the effect of *lpa2-1* allele on the PA content. There was mean reduction of 25% and 26.6% in the PA content in the *lpa2-1*inbredsas compared to the wild type inbreds at Delhi and Hyderabad locations, respectively. The similar trend in the obtained results were also reported by Abhijith et al. (2020). Similarly, Abhijith et al. (2020) and Shi et al. (2003) also reported 26-30% less phytic acid in the *lpa2*-1 mutants of maize. However, low phytic acid in the recessive *lpa2-1* genotypes evaluated in the present study, the extent of reduction is relatively low compared to the previous research (Raboy et al. 2000; Raboy 2009). The mean inorganic phosphorous content is significantly higher in the *lpa2-1* mutant inbreds compared to the wild type inbreds at both the locations studied. The increase of iP to the extent of 2.25 fold and 1.86 fold in the *lpa2-1*inbreds as compared to wild type inbreds was observed at Delhi and Hyderabad, respectively. The data clearly indicates that the increase in iP content while reduction in the PA content due to recessice*lpa2-1* allele. The obtained results are on for with that of Abhijith et al. (2020). The content of phytic acid and inorganic phosphate varied between the *lpa2-1*and wild type inbreds, however the total phosphorus content is similar in the *lpa2-1* and the wild type inbreds. The PAP/TP ratio is higher in the wild type inbreds compared to *lpa2-1*inbreds; this signifies the direct contribution of PA content to the PAP/TP ratio. Hence, the bioavailability of the mineral elements is high in the low phytic acid based inbreds compared to the wild type inbreds.

The combined data from both the locations were analysed to find the performance of genotypes across the locations. The results from combined analysis of variance revealed that the performance of the *lpa2-1* mutants and the wild type inbreds varied across the locations for all the characters studied. The interaction effect of genotype and location is also highly significant for all the parameters studied. The role of environment on the performance of genotypes is significant.

5.3 Impact of low phytate maize on Fe- and Zn- bioavailability

The maize genotypes with variable concentration of Fe and Zn are available. The inbreds possessing maximum up to 40 ppm Fe in kernel is available (Pandey et al. 2015; Prasanna et al. 2011) but inbreds with target level of 60 ppm is quite uncommon (Gupta et al. 2015b). Likewise, inbreds with nearly 30 ppm Zn in kernels is available (Pandey et al. 2015; Prasanna et al. 2011), but inbreds with the target level of 37 ppm to meet RDA is also not generally available (Gupta et al. 2015b). Complex nature Fe and Zn limit the increment in their content through breeding programme (Gupta et al. 2015a). However, the bioavailability of Fe and Zn is very less in the gut due the presence of anti-nutritional factors like phytic acid and not even meet RDA (Bouis et al.2011). On the other hand, the low phytic acid maize developed in the present study offers many opportunities to meet the RDA due to its profound effects on reduction of phytic acids (Raboy et al. 2000; Raboy 2009).

Thus, the low phytate maize inbreds evaluated under this study can be potentially used for the development of low phytic acid maize hybrids that would offer potential scope for biofortification of kernel Fe and Zn in maize. Even higher bioavailability of micronutrients can mitigate the issue of hidden hunger in the coming days. Finally, the increased bioavailability of micronutrients in turn helps to improve immune system of human beings.

5.4 Molecular characterisation of *lpa*-inbreds

5.4.1 Characterisation of *lpa1-1* based inbreds using SSR markers

5.4.1.1 Genetic diversity among lpa1-based inbreds

Genetic diversity analyses based on molecular markers enables to study the genetic relatedness between the genotypes more efficiently. SSR markers were the chosen marker for the study of diversity in maize as shown by previous reports (Bantte and Prasanna 2003; Choudhary et al., 2016; Muthusamy et al. 2015b; Pandey et al., 2016;Smith et al., 1997; Warburton et al., 2002; Zunjare et al. 2015).In the present investigation, 60 SSR markers mostly of di- and tri-nucleotide repeats which are distributed across the genome are used for the genotyping of set of *lpa1*-based inbreds. The study showed wide diversity among the *lpa1*-based inbreds.

In the present study the mean number of alleles identified (2.87) per locus was low as compared to Choudhary et al. (2015), Mehta et al. (2018), Muthusamy et al. (2015b) Sivaranjani et al. (2014). Sivaranjani et al. (2014) detected an average of 4 alleles/locus by using 36 SSR markers. Muthusamy et al. (2015b) detected an average of 3.88 alleles/locus. Choudhary et al. (2015) detected an average of 4.12 allele by using 65 SSR markers and Mehta et al. (2018) detected a mean of 3.8 allele/locus by using 56 SSR markers. However, In the present study, the mean number of alleles identified (2.87) per locus was higher when compared to Das et al. (2018) and Jaiswal et al. (2019). Das et al. (2018) detected an average of 2.71 allele/locus by using 80 SSR markers and Jaiswal et al. (2019) detected 2.32 alleles/ locus. In the present study, the electrophoresis based on agarose gel was used to resolve the alleles and it has low resolving power compared to polyacrylamide gel or capillary electrophoresis (Bracco et al., 2009; Kumar et al., 2008; Mehta et al.2017; Muthusamy et al.2015;Sibov et al., 2003).Although we used agarose to resolve the alleles, the numbers of alleles are quite comparable and also alleles of the selected markers gave clear-cut polymorphism and separated well from each other. Agarose gel electrophoresis is highly desired because it is less cumbersome and less costly and highly useful for routine analysis (Mehta et al. 2017; Rakshit et al. 2011).

Frequency of the major alleles tells the diverse nature of genotypes being characterised. The locus with low major allelic frequency indicates highly diverse nature of genotypes for that locus. Of the 60 SSR marker loci only a less than half of SSR loci showed >0.5 major allelic frequency. The polymorphism information content(PIC) value depends on the nature of the inbred lines used in study; because closely related lines showed less polymorphism compared with genetically diverse lines (Choudhary et al. 2015;Sivaranjani et al. 2014).In the present study out of the 60 SSR loci, 21 loci had a PIC value of more than 0.5, suggesting the higher capacity of these SSR loci to distinguish the inbred lines. The obtained mean PIC in the present study is 0.41 comparable to previous findings (Das et al. 2018; Duo et al.2019; Jaiswal et al.2019). It was however comparatively smaller than recorded by others (Choudhary et al.2015; Mehta et al.2017; Muthusamy et al.2015b; Pandey et al. 2016).

Alleles present only in a particular genotype and absent in other genotypes are called unique alleles. The occurrence of unique and rare alleles depends on the number of studied genotypes and the type of SSR markers used in the analysis (Muthusamy et al. 2015b; Rakshit et al. 2011; Zunjare et al. 2015). Many studies have reported the occurrence of unique and rare alleles in maize (Bantte and Prasanna 2003; Choudhary et al. 2015; Das et al. 2018; Jaiswal et al. 2019; Kumar et al. 2008; Mehta et al. 2017; Muthusamy et al. 2015b; Pandey et al. 2015; Pandey et al. 2016; Rakshit et al. 2011; Sharma et al. 2010; Sivaranjani et al. 2014).Owing to high levels of mutations in SSR repeat motifs as well as inclusion of alleles from some diverse germplasm, unique allele may occur (Choudhary et al.2015; Zunjare et al. 2015).The present analysis identified 4 unique alleles amplified by the SSR markers *umc2186*,*bnlg1957*,*umc1393* and *umc2053*; and 17 rare allele**s** which offered the prospect of unequivocal separation of the respective inbred lines from others (Choudhary et al. 2015; Muthusamy et al. 2015b; Pandey et al. 2015; Sivaranjani et al. 2014).The markers that produced unique alleles could prove useful as they often associate with trait expression and can be used in fingerprinting of the inbreds (Choudhary et al. 2015; Muthusamy et al. 2015b; Sivaranjani et al. 2014).

SSR-based estimation of heterozygosity among the inbred lines is best to assess the period of inbred development. SSR markers which are co-dominant in nature can differentiate heterozygotes from homozygotes for gene (Bantte and Prasanna, 2003; Choudhary et al. 2015; Muthusamy et al. 2015b; Pandey et al. 2015; Sivaranjani et al. 2014, Zunjare et al. 2015). The mean heterozygosity observed by all SSR loci was 0.09 in the present study, indicating that most loci were homozygous and alleles were fixed. Some loci however, such as *umc2262* (0.25), *umc2031*(0.29), *umc2053*(0.29), *umc1113* (0.35*),* *umc2203* (0.36) and *umc1393*(0.21) showed significant heterozygosity. This could be pointed to residual heterozygosity, due to which inbreds continue to segregate for certain loci irrespective of repeated selfing (Kaur et al. 2011). Conventionally bred inbreds as compared to doubled haploid based inbreds, often show some degree of heterozygosity (Sivaranjani et al. 2014). Other potential factors that lead to heterozygosity include mutation at particular SSR locations. Duplication also leads to amplification of related sequences from different genomic regions (Kumar et al. 2008; Semagn et al. 2006).

5.4.1.2 Genetic relationships among lpa1-based inbreds

Assessment of genetic diversity among the inbreds is important for exploitation of heterosis. Molecular marker approaches have been used to estimate genetic distance (GD) in maize and classify germplasm into heterotic groups (Prasanna et al., 2010). In the present investigation, the pair-wise genetic dissimilarity ranged from 0.23 to 0.81, with a mean of 0.64 which indicates the studied inbreds are diverse in nature at genetic level and also inbreds are developed from diverse pool. Similar reports has been recorded in the earlier investigations (Choudhary et al. 2015; Das et al. 2018; Lia et al. 2009; Mehta et al. 2017; Muthusamy et al. 2015b; Nepolean et al. 2013; Pandey et al. 2015; Rakshit et al. 2011; Senior et al. 1998; Sivaranjani et al. 2014; Smith et al. 1997; Vaz-Patto et al. 2004).Cluster analysis from molecular data has been done using neighbour-joining method, which is based on the genetic dissimilarities. The cluster analysis grouped the set of 24 *lpa1*-based maize inbreds into three distinct clusters. These obtained results of cluster analysis have been further validated through principle Co-ordinate Analysis (PCoA). In the PCoA, the inbreds were distributed across four quadrangles and the distribution was highly consistent with the pedigree. The inbreds likePMI-LP1-118, PMI-LP1-119, PMI-LP1-120 belong to cluster A, and are derived from the same cross (PMI-PV8 × A619 *lpa1-1*). Similarly, PMI-LP1-121, PMI-LP1-122 and PMI-LP1-123 belonging to Cluster A are derived from the PMI-Q3 × A619 *lpa1-1*. The genotypes which belong to cluster B (PMI-LP1-108, PMI-LP1-109 andPMI-LP1-110, PMI-LP1-111) are derived from PMI-PV5 × A619 *lpa1-1*. Finally, the genotypes which belongs to Cluster C (PMI-LP1-102, PMI-LP1-112, PMI-LP1-113, PMI-LP1-114) are derived from the cross PMI-PV6 × A632 *lpa1-1* and the genotypes (PMI-LP1-103, PMI-LP1 115, PMI-LP1-116 and PMI-LP1-117) are derived from the cross PMI-PV7 × A632 *lpa1-1*.

5.4.1.3 Identification of potential cross combination among lpa1-based inbreds for low phytic acid

Based on tree diagram of the genotypes, promising heterotic hybrid combinations can be elucidated among *lpa1*-based inbreds and those combinations can be used in the breeding programme to exploit heterosis and also while generating heterotic hybrid combinations it is advisable that both the parents should have lower concentration of kernel phytic acid besides being genetically distant for exploitation of heterosis for grain yield with low kernel phytic acid.

The inbred PMI-LP1-105, present in cluster A is derived from the cross PMI-Q1 × A632 *lpa1-1* and in this cross the female parentPMI-Q1 is developed from the cross betweenHKI1105 × CML161.PMI-LP1-106, present in cluster B, is derived the cross PMI-Q2 × A619 *lpa1-1*,in this the parent PMI-Q2 is developed from the parental lines(HKI323 ×

HKI161). However, these two parental genotypes (HKI1105 and HKI323) are parent for already released commercial hybrid HM4; and they are present in two different clusters. So, the lines derived from these two parents by introgression of *lpa1-1*gene from (A619*lpa1-1* and A632*lpa1-1*) can be used to produce potentially heterotic hybrids which are having low phytic acid. Similarly, PMI-LP1-105 × PMI-PL1-107, PMI-LP1-101 × PMI-PL1-102, PMI-LP1-101 × PMI-PL1-103, PMI-LP1-107 × PMI-PL1-102 and PMI-LP1-102 × PMI-PL1-104are the parental version of already released hybrids (HM9, HQPM-5, HQPM-7, HM11 and HM10). This validates the importance of cluster analysis in selecting potential crosses. Thus, inbreds of cluster-A, -B and -C can be inter-crossed to generate new potential hybrids with low phytate.

5.4.2 Characterisation of *lpa2-1* based inbreds using SSR markers

5.4.2.1 Genetic diversity among lpa2-based inbreds

In this study a total of 61 SSR markers distributed across the genome were used for analyzing genetic variability among the selected set of 24 *lpa2*-based inbreds. Most of SSR markers used were of di- and tri-nucleotide repeats. The study revealed wide diversity among the *lpa2*-based inbreds used in the study. In this study, the average number of allele (2.97) generated was higher when compared to Das et al.(2018) and Jaiswal et al.(2019).Das et al.(2018) detected average of 2.71 allele per locus by using 80 SSR markers while working on molecular diversity and genetic variability of kernel tocopherols among maize inbreds possessing favourable haplotypes of γ-tocopherol methyl transferase (*vte4*). Jaiswal et al. (2019) detected 2.32 allele per locus while characterizing the maize genotypes using 58 microsatellite markers associated with QTLs for kernel iron and zinc. However, in the present study the average number of alleles identified (2.97) per locus was low as compared to Sivaranjani et al. (2014), Muthusamy et al. (2015b), Choudhary et al. (2015) and Mehta et al. (2018). Sivaranjani et al. (2014) detected an average of four allele per locus by using 36 SSR markers while characterising 24 maize inbred lines varying for kernel carotenoids. Muthusamy et al. (2015) detected an average of 3.88 alleles per locus while working on molecular characterization of exotic and indigenous maize inbreds for biofortification with kernel carotenoids. Choudhary et al. (2015) detected an average of 4.12 allele using 65 SSR markers while characterising a set of 24 diverse inbreds with rare allele of *β-carotene hydroxylase* (*crtRB1*) gene causing higher β-carotene in maize kernel. Mehta et al. (2018) detected a mean of 3.8 allele per locus by using 56 SSR markers. In this study, agarose gel-based electrophoresis is used to resolve the alleles and it has low resolving power as compared to polyacrylamide gel or capillary electrophoresis (Bracco et al., 2009; Kumar et al., 2008; Sibov et al., 2003), but the number of alleles are quite comparable with other reports and also the selected markers gave distinct polymorphism.

Lesser the frequency of the major allele, diverse is the genotypes for that locus. Nearly 16 loci, out of 61 SSRs analysed showed major allele frequency of <0.5, indicating comparable diversity in the selected *lpa2*- based inbreds. The polymorphism information content (PIC) value depends on the nature of the inbred lines used in study. In the present study, nearly 18 SSR loci showed PIC value >50 with mean PIC of 0.40, suggesting that these SSR loci are more capable of distinguishing the inbred lines. The obtained mean PIC in the present study is comparable to previous findings. Das et al. (2018) has reported the mean PIC of 0.41, Jaiswal et al. (2019) has got the mean PIC of 0.40 and Duo et al. (2019) has obtained the mean PIC of 0.43. However, it was low compared to reports by earlier studies like Muthusamy et al. (2015) who reported mean PIC of 0.52, Choudhary et al. (2015) reported a mean PIC of 0.58, Pandey et al. (2016) obtained a mean PIC of 0.56 and Mehta et al. (2017) obtained a mean PIC of 0.50.

Many studies have reported the occurrence of unique and rare alleles in maize (Bantte and Prasanna, 2003; Choudhary et al., 2015; Das et al.2018; Jaiswal et al., 2019; Mehta et al.2017; Muthusamy et al., 2015; Pandey et al., 2015;Pandey et al.2016;Sivaranjani et al., 2014).The present analysis identified 13 unique for the markers viz., *umc1689*,*umc1776*,*umc2251*,*umc1087*,*bnlg1456*,*umc1740*,*umc1101*,*umc1537*,*umc2123*,*umc1799*,*umc2031*,*umc1055* and*umc1809*. The unique alleles along with the 24 rare alleles gave the possibility of an unambiguous separation of the respective inbred lines from others (Choudhary et al., 2015; Muthusamy et al., 2015; Pandey et al. 2015;Sivaranjani et al. 2014).SSR markers which were co-dominant in nature can differentiate heterozygotes from homozygotes (Bantte and Prasanna, 2003; Choudhary et al., 2015; Muthusamy et al., 2015; Pandey et al., 2015;Sivaranjani et al., 2014).The mean heterozygosity in the present study was 0.07,indicating that most loci were homozygous and alleles were fixed. However, some loci such as *umc1914*(0.21), *umc1799*(0.21), *umc1666*(0.21), *umc2152*(0.27), *umc1025*(0.33), *bnlg1724*(0.33), *umc1393*(0.39) and *umc1012*(0.27) showed significant heterozygosity. It could be due to the residual heterozygosity present in the inbreds. Maize is a highly cross-pollinated crop and some heterozygosity remains inherent because breeders look for morphological uniformity while developing the inbred lines. Some of the cryptic genetic variation at DNA levels may continue to exist, which segregates even after repeated generations of selfing and maintains the heterozygosity in the inbreds (Kumar et al. 2008).

5.4.2.3 Genetic relationships among lpa2-based inbreds

The wide range of genetic dissimilarity (0.27 to 0.79) was observed in the genotypes, which indicates the studied inbreds are diverse in nature at genetic level and also inbreds are developed from diverse pool. Previous investigations have also revealed similar range of genetic dissimilarity (Choudhary et al. 2015; Das et al. 2018; Mehta et al. 2017; Muthusamy et al. 2015b; Pandey et al. 2015; Senior et al. 1998; Sivaranjani et al. 2014; Smith et al. 1997; Vaz-Patto et al. 2004; Zunjare et al. 2015). The clustering of the genotypes based on the markers was closely related with their pedigree information. The cluster analysis grouped the set of 24 *lpa2*-based maize inbreds into three distinct major clusters namely cluster -A, -B and -C. These obtained results of cluster analysis have been further validated through principle Co-ordinate Analysis (PCoA). In the PCoA, the inbreds were distributed across four quadrangles and the distribution was highly consistent with the pedigree. The inbred genotypes belonged to cluster A(PMI-LP2-206, PMI-LP2-220, PMI-LP2-221) are derived from the cross PMI-Q2 × A619 *lpa2-1*;whilePMI-LP2-222, PMI-LP2-223, PMI-LP2-224 are derived from the cross PMI-Q3 × A619 *lpa2-1*.The genotypes belong to cluster B (PMI-LP2-204, PMI-LP2-212, PMI-LP2-213, PMI-LP2-214, PMI-LP2-215 and PMI-LP2-216)are derived from (PMI-PV8 × A619 *lpa2-1*. Finally, the genotypes PMI-LP2-201, PMI-LP2-208, PMI-LP2-209, PMI-LP2-210 and PMI-LP2-211) present in cluster C are derived from PMI-PV5 × A619 *lpa2-1*.

5.4.2.4 Identification of potential cross combination among lpa2-based inbreds for low phytic acids

Based on tree diagram of the genotypes, promising heterotic hybrid combinations can be elucidated and these combinations can be used to exploit heterosis in the breeding program. A diverse panel of 24*lpa2*-based inbreds developed at the Maize Genetic Unit. Of them, seven inbreds viz. PMI-LP-201 to PMI-LP-207 are developed through marker-assisted backcross breeding of *lpa2* allele in different genetic backgrounds (Bhatt et al. 2018) and 17 inbreds viz. PMI-LP-208 to PMI-LP-224 are developed through marker-assisted pedigree selection involving crosses between diverse wild types and *lpa2* mutants HKI161PV × A619 *lpa2-1*,HKI163PV × A619 *lpa2-1*,HKI193-1PV × A619 *lpa2-1*,HKI193-2PV × A619 *lpa2-1*,HKI323Q × A619 *lpa2-1*,HKI1105Q × A619 *lpa2-1*,HKI1128Q × A619 *lpa2-1* (Abhijith et al. 2020). These parental lines (PMI-PL2-201 × PMI-PL2-202, PMI-PL2-201 × PMI-PL2-203, PMI-PL2-201 × PMI-PL2-204,PMI-PL2-205 × PMI-PL2-201 and PMI-PL2-202 × PMI-PL2-207) which were used to derive *lpa2*-based genotypes belonged to different clusters and were parents for already released commercial single cross hybrids viz., HQPM-5, HQPM-7, HQPM-4, HM8 and HM11. Hence the potential low phytate hybrids can be produced by crossing inbreds between cluster-A, -B and -C.

6

SUMMARY AND CONCLUSION

Micronutrient malnutrition or hidden hunger affects large population in developing and developed countries worldwide. Majority of the global population depends on plant-based foods, which are often low in key micronutrients. Maize serves as food to millions of people and valuable source of various fractions of nutrients especially energy, proteins, vitamins and mineral elements. It also possesses higher concentration of phytic acid (PA)which acts as an anti-nutritional factor. In humans, PA chelates gut mineral elements especially iron (Fe) and zinc (Zn), reduces their absorbability, and causes micronutrient malnutrition. Hence, the reduction in the anti-nutritional factor is an essential target for higher bioavailability of mineral elements. Biofortification of maize through genetic manipulation for reduction of PA is appeared to be promising strategy. In this direction, the present study was carried out to characterise diverse sets of *lpa1-1* and *lpa2-1* based maize inbreds for their agronomic, yield and yield contributing traits and biochemical parameters. Further, molecular characterization of these inbreds have also been done using SSR markers for their effective utilization in hybrid breeding programme. The salient findings from the study are as follows:

- Significant differences were observed among the set of 24 *lpa1-1* and 24 *lpa2-1* based inbreds along with seven wild type inbreds for plant height, ear height, days to 50% male flowering, days to 50% female flowering and grain yield across the locations (Delhi and Hyderabad).
- The mean performance for ear height, days to 50% male flowering, days to 50% female flowering and grain yield were similar between the *lpa1-1*/ *lpa2-1*mutants and wild type inbreds.
- Combined ANOVA indicated that the *lpa1-1*/ *lpa2-1* mutants and the wild type inbreds differed significantly at both the locations for all the studied characters and even the interaction effect of genotype and location was also significantly different for majority of characters.
- Considering both the locations, PMI-LP1-102, PMI-LP1-101 and PMI-LP1-123among the *lpa1-1* based inbreds, and PMI-LP2-201 and PMI-LP2-202 among *lpa2-1*inbredsperformed superior for grain yield at both the locations.
- The content of phytic acid (PA) and inorganic phosphorus (iP) significantly varied among the *lpa1-1*/ *lpa2-1* based inbreds and wild type inbreds under study in both the locations signifying the scope for the selection of desirablelow phytate genotype for higher bioavailability of mineral elements.

- The mean performance of *lpa1-1/ lpa2-1* genotypes indicated that PA content was significantly higher in the wild type inbreds than the *lpa1-1/ lpa2-1* mutant inbreds. It was also observed that reduction of 35.3% and 36.2 % in the mean PA content of *lpa1-1*mutants over wild type inbreds at Delhi and Hyderabad locations, respectively. The reduction for *lpa2-1* was 25.0% and 26.6% in the mean PA at Delhi and Hyderabad, respectively.
- The mean iP content was significantly higher in the *lpa1-1* mutant inbreds compared to the wild type inbreds at both the locations. The increase of iP to the extent of 3.0 and 3.26 fold in the *lpa1-1* inbreds as compared to wild type inbreds was observed at Delhi and Hyderabad, respectively. The same in the *lpa2-1* inbreds was of 2.25 fold and 1.86 fold as compared to wild type inbreds at Delhi and Hyderabad, respectively.
- Even though the content of PA and iP varied between the *lpa1-1/ lpa2-1* and wild type inbreds, the total phosphorous (TP) content remained unaffected.
- The PAP/TP ratio was found to be higher in the wild type inbreds compared to *lpa1-1/ lpa2-1* inbreds, signifying the direct contribution of PA content to the PAP/TP ratio.
- Sixty SSRs distributed across the genome with three to ten markers per linkage group generated 172 alleles with mean of 2.87 alleles per SSR locus among the diverse panel of 24 *lpa1-1* based inbreds. The average frequency of major alleles was 0.63 and mean gene diversity was of 0.48. The average polymorphism information content (PIC) was 0.41 and the pair wise genetic dissimilarity was ranged from 0.23 to 0.81, with a mean of 0.64.
- Sixty-one SSRs distributed throughout the genome generated 181 alleles with a mean of 2.97 alleles per locus among the set of 24 diverse *lpa2-1* based inbreds. The average major allele frequency was 0.65 and the mean gene diversity was 0.46. The PIC value ranged among *lpa2-1* based inbreds from 0.04 to 0.72 with the mean of 0.40 and the pair wise genetic dissimilarity ranged between 0.27 to 0.79 with a mean of 0.61.
- Cluster analyses grouped 24 *lpa1-1/ lpa2-1*based inbreds into three major clusters, and principal coordinate analysis depicted the diverse nature of the genotypes consistent with their pedigree.
- The study identified potential hybrid combinations for low phytate (both *lpa1-1* and *lpa2-1*) with good agronomic performance that can be directly utilized in the biofortification programme.

- Genetically diverse and phenotypically contrasting inbred lines can be used in developing segregating mapping populations for identifying modifier loci/QTL influencing low phytic acid.
- Superior *lpa1-1* and *lpa2-1*based inbreds identified in the study can be used as potential donor for respective genes in future breeding programme.
- Development of *lpa1-1* × *lpa2-1* will serve as a potential germplasm resource for basic genetic studies and future breeding applications.

ABSTRACT

Two billion people are estimated to suffer from micronutrient deficiencies. Majority of the global population depends on plant-based foods, which are often low in key micronutrients. The deficiencies of key micronutrients especially iron (Fe) and zinc (Zn) lead to serious illnesses; possess profound effects on human health. Maize serves as food to millions of people worldwide. However, it possesses higher concentration of phytic acid (PA), which is an anti-nutritional factor that drastically reduces the bioavailability of mineral elements (Fe and Zn); thereby causes micronutrient malnutrition. Thus, reduction of PA in maize genotypes through genetic manipulation assumes great importance in enhancing the bioavailability of mineral elements. The present study was aimed to evaluate a set of *lpa1-1* and *lpa2-1* based maize inbreds for their agronomic performance, yield and yield contributing traits, biochemical parameters and molecular characters. Each set of 24 *lpa1-1* and *lpa2-1* based inbreds along with seven wild type inbreds were evaluated separately at two locations (Delhi and Hyderabad) through replicated trials. Significant variation for plant height, ear height, male and female flowering and grain yield was observed among the *lpa1-1* and *lpa2-1* based inbreds at both the locations. The mean performance of both the sets indicated that there is no significant difference between the *lpa*-genotypes and wild type inbreds for all the characters studied at both locations. The inbreds PMI-LP1-102, PMI-LP1-101 and PMI-LP1-123 among the *lpa1-1*, and PMI-LP2-201 and PMI-LP2-202 among the *lpa2-1* inbreds performed superior for grain yield across both locations. The content of PA and inorganic phosphorus (iP) significantly varied among both the sets of *lpa*- based and wild type inbreds. It was observed that reduction in mean PA content was 35.3% and 36.2 % in *lpa1-1*, and 25.0% and 26.6% in *lpa2-1* mutants over wild type inbreds at Delhi and Hyderabad, respectively. The mean iP content was significantly higher in both *lpa*- set mutants compared to wild type inbreds at both locations. The increase of mean iP was to the extent of 3.00-fold and 3.26-fold in the *lpa1-1*, and 2.25-fold and 1.86-fold in the *lpa2-1* inbreds as compared to wild type inbreds at Delhi and Hyderabad, respectively. Even though PA and iP content varied between the *lpa*- and wild type inbreds but the total phosphorous (TP) content found to be remained unaffected. The mean PAP/TP ratio was found to be higher in the wild type inbreds compared to *lpa1-1* and *lpa2-1*inbreds.The molecular characterisation studies using 60 and 61 SSRs distributed across the genome generated 172 and 181 alleles among the diverse set of *lpa1-1* and *lpa2-1* based inbreds, respectively. Further cluster analysis grouped each set of 24 *lpa1-1* and *lpa2-1* inbreds into three major clusters in both sets and principal coordinate analysis depicted the diverse nature of the genotypes consistent with their pedigree. Superior *lpa1-1* and *lpa2-1* based inbreds identified in the study can be used as potential donor for respective genes in future breeding programme. The study also identified potential hybrid combinations for low phytate (both *lpa1-1* and *lpa2-1*) with good agronomic performance that can be directly utilized in the biofortification programme.

Key words: Maize; phytic acid; bioavailability; SSRs; diversity

सार

दो बिलियन लोगों को सूक्ष्म पोषक तत्वों की कमी से पीड़ित होने का अनुमान है। वैश्विक आबादी का अधिकांश भाग पौधों पर आधारित खाद्य पदार्थों पर निर्भर करता है, जो अक्सर महत्वपूर्ण सूक्ष्म पोषक तत्वों में कम होते हैं। मुख्य सूक्ष्म पोषक तत्वों की कमी विशेष रूप से लोहा (Fe) और जस्ता (Zn) गंभीर बीमारियों को जन्म देती है; जिसका मानव स्वास्थ्य पर गहरा प्रभाव है। मक्का दुनिया भर में लाखों लोगों को भोजन का एक श्रोत है। हालांकि, इसमें फाइटिक एसिड (पीए) की उच्च सांद्रता है, जो एक पोषण-विरोधी कारक है जो खनिज तत्वों (Fe और Zn) की जैव उपलब्धता को काफी कम कर देता है; जिससे सूक्ष्म पोषक कुपोषण होता है। इसीलिए, आनुवंशिक हेरफेर के माध्यम से मक्का जीनोटाइप में पीए की कमी खनिज तत्वों की जैवउपलब्धता को बढ़ाने में काफी महत्व रखती है। वर्तमान अध्ययन का उद्देश्य *lpa1-1* और *lpa2-1* आधारित मक्का इनब्रेड्स के उनके अग्रोनॉमिक प्रदर्शन, उपज और उपज योगदान लक्षण, जैव रासायनिक मापदंडों और आणविक पात्रों के लिए मूल्यांकन करना था। 24 *lpa1-1* और 24 *lpa2-1* के प्रत्येक सेट में सात ओरिजिनल प्रकार के इनब्रेड्स के साथ इनब्रेड्स का मूल्यांकन दो स्थानों (दिल्ली और हैदराबाद) में अलग-अलग परीक्षणों के माध्यम से किया गया था। दोनों स्थानों पर *lpa1-1* और *lpa2-1* आधारित इनब्रेड्स के बीच पौधे की ऊंचाई, भुट्टे की ऊंचाई, नर और मादा फूल और अनाज की उपज के लिए महत्वपूर्ण भिन्नता देखी गई। दोनों सेटों के औसत प्रदर्शन ने संकेत दिया कि दोनों स्थानों पर अध्ययन किए गए सभी पात्रों के लिए lpa- और ओरिजिनल प्रकार के इनब्रेड्स के बीच कोई महत्वपूर्ण अंतर नहीं है। *lpa1-1* के इनब्रेड्स PMI-LP1-102, PMI-LP1-101 और PMI-LP1-123, और *lpa2-1* इनब्रेड्स के PMI-LP2-201 और PMI-LP2-202 ने दोनों स्थानों पर अनाज की उपज के लिए बेहतर प्रदर्शन किया है। पीए और अकार्बनिक फॉस्फोरस (आईपी) की सामग्री दोनों lpa- आधारित और ओरिजिनल प्रकार की इनब्रेड्स के सेट के बीच काफी भिन्न है। यह देखा गया कि पीए सामग्री में कमी क्रमशः 35.3% और 36.2% *lpa1-1* में, और 25.0% और 26.6% *lpa2-1* में, दिल्ली और हैदराबाद, ओरिजिनल प्रकार के इनब्रेड्स की तुलना मे थी। दोनों स्थानों पर ओरिजिनल प्रकार की इनब्रेड्स की तुलना में दोनों प्रकार के सेट में आईपी सामग्री काफी अधिक थी। औसत आईपी की वृद्धि क्रमशः *lpa1-1* इनब्रेड्स में 3.00 और 3.26-गुना, और *lpa2-1* इनब्रेड्स में 2.25- और 1.86-गुना दिल्ली और हैदराबाद में ओरिजिनल प्रकार के इनब्रेड्स की तुलना में की सीमा तक थी। भले ही पीए और आईपी सामग्री lpa- और ओरिजिनल प्रकार की इनब्रेड्स के बीच भिन्न हो लेकिन कुल फॉस्फोरस (टीपी) सामग्री अप्रभावित पाई गई। इसका मतलब है कि पीएपी / टीपी अनुपात *lpa1-1* और *lpa2-1* इनब्रेड्स की तुलना में ओरिजिनल प्रकार के इनब्रेड में अधिक पाया गया। 60 और 61 आणविक पात्रों (SSRs) का उपयोग करते हुए अध्ययनों ने क्रमशः 172 और 181 एलील को *lpa1-1* और *lpa2-1* इनब्रेड्स के विविध सेटों के बीच पाया गया। इसके अलावा क्लस्टर विश्लेषण में 24 *lpa1-1* और 24 *lpa2-1* इनब्रेड्स को तीन प्रमुख समूहों में बांटा गया और प्रिंसिपल समन्वय विश्लेषण ने उनके वंशावली के अनुरूप जीनोटाइप की विविध प्रकृति को दर्शाया। बेहतर *lpa1-1* और *lpa2-1* इनब्रेड्स अध्ययन में पहचाने गए जो भविष्य के प्रजनन कार्यक्रम में संबंधित जीन के लिए संभावित दाता के रूप में इस्तेमाल किया जा सकता है। इस अध्ययन में अच्छे एग्रोनॉमिक प्रदर्शन के साथ कम फाइटेट (दोनों *lpa1-1* और *lpa2-1*) के लिए संभावित हाइब्रिड संयोजनों की पहचान की गई, जो सीधे बायोफोर्टिफिकेशन प्रोग्राम में उपयोग किए जा सकते हैं।

मुख्य शब्द: मक्का; फाइटिक एसिड; जैव उपलब्धता; आणविक पात्रों; विविधता

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