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RESEARCH ARTICLE

Tagging, mapping and identification of the QTLs associated with phosphorous utilization for phosphorous- limiting soils in tropical maize

Chibesa E. Mutale, Kalaluka Munyinda, Langa Tembo

Department of Plant Science, University of Zambia, P.O. Box 32379, Lusaka, Zambia

Corresponding authors email Id: langa.tembo@unza.zm

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Abstract

Phosphorus (P) deficiency is one of the major abiotic constraints leading to low maize productivity. Twenty- four SSR markers sequence information was obtained from maize databases (MaizeGDB). These SSRs were selected from targeted regions of the maize genome linked to Quantitative Trait Loci (QTLs) for Phosphorus utilization efficiency. The mapping population consisting 67 F_2 maize plants, were obtained from advancing a bi-parental cross between an efficient line (L354) and an in-efficient maize line (L585). Out of 24 SSRs five identified polymorphic SSR markers were subsequently used for genotyping the 67 tagged F_2 plants. Sixty seven $F_{2:3}$ maize families were used for QTL localization. Inclusive composite interval mapping (ICIM) analysis identified seven QTLs associated with phosphorus utilization on chromosome 5. All the mapped QTLs were more than 5 cM from the nearest molecular marker utilized in the study. The closest mapped QTL, PeSB-5, 7 associated with shoot biomass was at 11.7 cM, from the nearest marker umc2136 on chromosome 5. Therefore, there is need to saturate the map which may prove useful to identify and test several markers near the mapped QTLs. The identified markers could serve as potential SSR markers for QTLs associated with phosphorous utilization for phosphorous- limiting soils in tropical maize.

Keywords: P- limited soil, maize, QTLs, Chromosome, linkage group

Introduction

Maize is grown widely throughout the world in a range of agro-ecological environments. It is the most important cereal crop and a staple food for a large part of the population in sub-Saharan Africa (Tembo *et al.,* 2016; Edmonds *et al.,* 2009). In Zambia, maize is a staple food, mostly used for human consumption and is ranked as the number one cereal (Smale and Jayne, 2003). Apart from human consumption, maize is a major ingredient in stock-feed and it is an important source of carbohydrates, protein, iron, vitamin B, and minerals. Maize is consumed as a starchy base in a wide variety of porridges, grits, and beer. Green maize (fresh on the cob) is eaten parched, baked, roasted or boiled; playing an important role in filling the hunger gap after the dry season. Every part of the maize plant has economic value: the grain, leaves, stalk, tassel, and cob can all be used to produce a variety of food and non-food products.

However, the production of maize is hampered by both biotic and abiotic factors. Among the abiotic stresses, yield losses due to phosphorus (P) deficient are a major factor. Fertilizer application is one major method of replenishing P in depleted soils. However, P fertilizers are costly to small scale farmers and potentially ineffective because of immobilization by the soil. Thus the applied mineral P fertilizer may also possibly be transformed to organic form, a process known as microbial immobilization (Richardson and Simpson, 2011; Holford, 1997). Furthermore, prolonged and excessive use of P fertilizers may lead to environmental pollution. Thus, developing P-efficient maize cultivars that produce reasonably high biomass in low-P soils presents a sustainable approach in this scenario.

Selection in conventional breeding methods for a trait of interest, nutrient efficiency inclusive is a challenge to breeders because of their dependency on environmental effect (Mbwando *et al.,* 2016). Moreover, collection of the data for a trait under investigation can be subjective especially where qualitative scoring is applied (Chilipa *et al.,* 2016). Therefore in selecting for desirable maize genotypes in P-limiting soils, there is need to develop a selection method which is not only reliable, but also independent of environmental effect and subjectivity. Most of the researchers relay on the simple sequence repeats (SSRs) molecular markers due to the fact that their genotypging is easy and are codominant, highly informative, reproducible and are heritable among related species (Vieira *et al.,* 2016). Due to advent in sequence level information in each crop plants there is surges in crop based genomic databases. The community developed database and global web resource for maize genetics and genomics database (MaizeGDB – https://www.maizegdb.org), comprises of genomic information of the maize crop including informative genic and genomic SSRs(Portwood *et al.,* 2018).

Identification of quantitative trait loci (QTLs) associated with phosphorus utilization is an initial step in identifying linked molecular markers. Linked molecular markers to associated QTL can be employed in a marker assisted selection (MAS) technique which is independent to environmental effects and management practices. Previous studies mapped QTLs for yield and yield components associated with P- use efficient in temperate maize (Li *et al.,* 2010; Chen *et al.,* 2003). Limited information is available on tagging, mapping and identification of the QTLs associated with phosphorous utilization for phosphorous-limiting soils in tropical maize. Being that tropical maize has a relatively larger genetic base than temperate maize; it may imply that much more genetic information may be observed in mapping tropical maize (Sibov *et al.,* 2013). Hence, by considering all above points the present study was panned with the objectives to tag and map the SSRs markers associated with efficient utilization of phosphorous and also to map the QTLs associated with efficient utilization of phosphorous in P- limited soils.

Materials and methods

Source of SSR makers and synthesis

SSR marker names and primer sequence information were retrieved from SSR maize databases ((MaizeGDB) available at http://www.maizegdb.org/ssr.php. MaizeGDB is rich source of genomic information available for the maize researchers. These SSRs were selected from targeted regions of the maize genome linked to QTLs for Phosphorus utilization efficiency. The twenty- four SSR primer pairs utilised in this study were synthesised from University of Cape Town, Department of Molecular and Cellular Biology (Cape Town, South Africa).

Genotyping of the mapping population and development of linkage map

The mapping population consisting 67 F_2 maize plants, were obtained from advancing a bi-parental cross between an efficient line (L354) and an in-efficient maize line (L585), obtained from Golden Research Trust (latitude 140 40' S; longitude 250 01'E) in Zambia. Efficient of the line entailed ability to utilise phosphorous in P-limited soil. The 67 F_2 genotypes were tagged and young leaf samples; one to two weeks old were cut from each plant for DNA extraction during the 2015/ 16 cropping season. DNA was extracted from the ground leaf material using the cetyltrimethylammonium bromide (CTAB) method (Hoisington *et al.*, 1994). The Lab work was carried out at the University of Zambia molecular Lab.

Primer pair was synthesized as described above. The primers were used as part of the PCR reaction mixture. The final concentrations of reaction components were as follows: 0.2 μM each of SSR forward and reverse primers, 1× PCR buffer, 2.0 mmol MgCl2/L; 0.2 mmol/L each of dATP, dCTP, dGTP, and dTTP; 0.16 U Taq polymerase (BioLabs); and 30 ng genomic DNA and distilled sterile water to a total volume of 20 μL. Initial testing involved screening for polymorphism between the parental DNA (L585 and L354). Five identified polymorphic SSR markers were subsequently used for genotyping the 67 tagged F_2 plants. In the present study in order to to place the markers on chromosomes and to decide the linkage group i.e. for the construction of the linkage map GenStat 14, tool was used (Payne *et al.*, 2011).

Phenotypic evaluation of the mapping population

The phenotypic evaluation was carried out at the University of Zambia (latitude 15°23´S, longitude 28°20´E) field station in an area with P-limited soil (2.93 mg/ kg P) during the 2016/17 cropping season. The optimum P requirement being 10 mg/ kg P. The site has been deliberately built up to be a P- deficient site to be utilized in evaluating genotypic responses. The evaluation was done on 67 F2:3 maize families obtained from advancing the F2 population. Each F2:3 family was appropriately tagged to match with its F_2 extracted DNA. The 67 F2:3 families were planted following a randomized complete-block design with two replications. Plants were spaced at 75 cm × 30 cm in 3 m long rows with 10 plants per row. Two seeds were initially planted per hill, but seedlings were subsequently thinned to one plant per hill, 3 weeks after germination. Phosphorus fertiliser was not applied to the trial at any time. However, the recommended quantities of potassium (30 kg K /ha) and nitrogen (20 kg N/ha) were applied in form of potassium sulphate and urea (46% N) fertilisers at the rates of 64 kg/ha and 25 kg/ha respectively as basal dressing. Urea fertiliser was repeated at 28 days after planting at the rate of 200 kg/ha (92 kg N/ha) as top dressing. Other agronomic practices such as weed management, pest and disease control were carried out according to the recommended practices.

Phenotypic assessment

Several parameters were evaluated. They are as under-listed below:

1. Grain yield (GY) was determined as the average total weight of shelled grain harvested from 3 m long rows in grams;
2. 100 grain weight (100GW) was measured as the average weight of 100 grains from the ear (cob) from the plot in grams;
3. Plant height (PH) was measured as an average of plants in a row using a tape, measuring from ground level to flag leaf in centimetres

4. Root biomass (RB) was determined by weighing roots taken from the plot and finding the average in grams;

5. Shoot biomass (SB) was determined by weighing the above ground total dry matter including stalk and ears harvested from the plot and later finding the average in grams and

6. Plant biomass (PB) was determined by adding the measured root biomass and shoot biomass.Statistical analysis

The combined data for genotypic and phenotypic was utilised in construction of a linkage map and QTL analysis. Parameters for forward regression analysis were set at a window size of 10 cM, a walk speed of 2 cM and probability threshold of 0.05 each for the partial F test for both marker inclusion and exclusion. Significance threshold for QTL detection was calculated by 1,000 random permutations of the phenotypic data at 5 % level and logarithm of odds (LOD) thresholds were set at 2.5 for all traits.QTL positions were assigned at the point of maximum LOD score in the region under consideration. Naming of identified QTL was done as by Rabiei 2007. All analysis was performed using inclusive composite interval mapping (ICIM) QTL Ici Mapping version 4.1 software (Wang *et al.*, 2012).

Results and discussion

Response of genotypes in P- limited soil

There were significant differences ($P < 0.001$) among genotypic responses in P- limited soils with regards to grain yield, 100 seed weight, plant height, shoot biomass, root biomass and plant biomass (Table 1). Implying that genotypic family responses within a population sufficiently differed in all measured traits to undergo further molecular mapping analysis.

Single marker analysis and QTL detection

Single marker analysis is one of a most popularly used method used by plant breeders to identify the quantitative trait locus (QTL) that can detect associations between molecular markers and traits of interest in a question, such as disease resistance, increased yield, or may be quality related traits. Single marker analysis associated with phosphorus efficient traits revealed that of the 5 identified polymorphic markers, umc1194 exhibited the highest phenotypic variation explained (R^2) of 18.9 % ($P < 0.01$) (Table 2) associated with root biomass. Five identified polymorphic SSR markers were subsequently used for genotyping the 67 tagged F_2 plants. This gave a total of 335 molecular data points. A map with one linkage group was constructed with 3 SSR markers from chromosome 5 (Fig. 1). Polymorphic markers umc1194 and umc1112 located on chromosomes 4 and 7 respectively were not accounted for on the linkage map as they did not associate with any of the other markers on same chromosome. QTL detection using inclusive composite interval mapping (ICIM) revealed seven QTLs located on a linkage group (chromosome 5) (Fig. 1, Table 3). The finding of few (seven) QTLs detected could be attributed to few (only five) SSR markers used during genotyping. The number of identified QTLs could have been more if more SSR primer pairs, greater than the utilised 24 (may be >50) were employed in the initial phase (screening for polymorphism). In turn, the number of polymorphic SSR markers identified and utilised during genotyping could have been increased which may have led to more detection of QTLs associated with efficient utilization of phosphorous in P- limited soils.

From the seven QTLs identified, three QTLs associated for responses to root biomass, two to plant height; one to grain yield and one to shoot biomass in P- limited soil were detected on chromosome 5. Chen *et al.,* (2008) identified thirty-one clusters of QTLs for phosphorus utilization traits on chromosome 5 in maize. It therefore appears that linkage group 5 holds many loci that have coevolved to adapt maize plants for association to phosphorus deficiency. The phenotypic variation explained (PVE) by the QTLs ranged from 11.14 to 19 % with LOD score ranging from 3.22 to 5.15. The QTL associated with shoot biomass (PeSB-5, 7) was computed with highest PVE (19%).

The finding on negative additive effects implies that the substitution effect of a favourable allele (efficient at utilizing phosphorous) with a non-favourable allele enhances plant inefficiency at utilizing phosphorous in P- limiting medium.

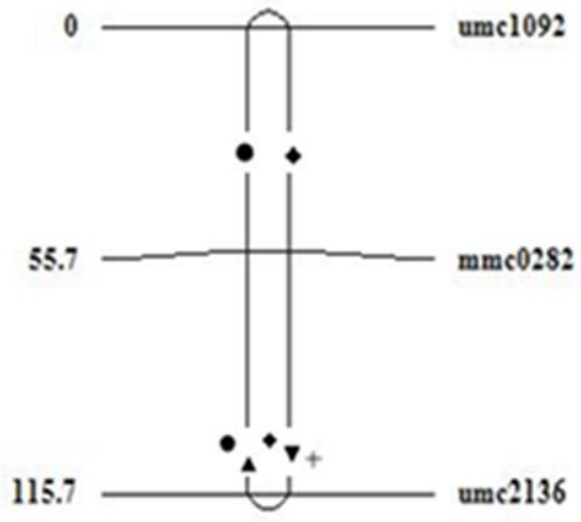
PVE is the proportion of phenotypic variance (also designated as R^2) which is explained by a predictor of a quantitative trait and is formed using estimated effects of all markers. This depends on the number of independently measured genomic variants associated with the trait, the proportion of the total variance they explain and the sample size in the discovery sample (Wray *et al.,* 2013).The additive effects ranged from -2.35 to 4.68 (Table 3).

Table 2: Phenotypic variation explained (R^2) by each marker for association to phosphorous utilization in P- limited soil

Marker	Chromosome	Plant biomass	Shoot biomass	Root biomass	Plant height	100 seed weight	Grain yield
umc 1194	4	15.1***	13.3**	18.9***	12.6**	10.3**	12.6**
Umc 0282	5	7.8*	9.4*	1.4	6.9*	7.7*	7.9*
umc 1092	5	12.2**	13.1**	6.8*	6.8*	8.6*	14.6***
umc 2136	5	10.2**	10.9**	5.9*	3.4	7.0*	10.7**
umc 1112	7	12.2**	11.4**	14.5***	15.2***	10.1**	10.6**

*, **, *** R^2 Significant at P= 0.05, P=0.01 and P= 0.001 respectively

Figure 1: Linkage map on chromosome 5 exhibiting detected QTLs



● ◆▼+▲: Shapes from left to right on the linkage map represents plant height, root biomass, plant biomass, grain yield and shoot biomass respectively

Table 3: QTL analysis for traits associated with phosphorus utilization in P-limited soil

QTL[a]	Linkage[b] Group	Position[c] (cM)	Left marker	Right marker	Bin	LOD[d] score	PVE[e] (%)	Additive[f] effect
PePH-5, 1	5	27	umc1092	mmc0282	5.04-5.05	3.22	14.69	-2.35
PeRB-5, 2	5	28	umc1092	mmc0282	5.04-5.05	3.76	11.49	-0.51
PeRB-5, 3	5	37	mmc0282	umc2136	5.05-5.08	5.15	11.12	0.84
PePH-5, 4	5	38	mmc0282	umc2136	5.05-5.08	4.22	14.61	1.27
PePH-5, 5	5	43	mmc0282	umc2136	5.05-5.08	4.32	18.28	2.97
PePH-5, 6	5	45	mmc0282	umc2136	5.05-5.08	4.56	16.34	1.38
PePH-5, 7	5	48	mmc0282	umc2136	5.05-5.08	4.06	19.00	4.68

Note: QTL identified between two markers, that is, between left marker and right marker

a- have been named from the trait abbreviation followed by the measured parameter abbreviation and then by the chromosome number where detected. The second number is added to show the order and the closest to zero gets position 1. Example PeRB-5, 2 means the QTL associated to phosphorus use efficiency was mapped for root biomass on chromosome 5 and it is in the second position (in terms of distance from 0 on linkage map) to another detected QTL on the same chromosome.

b -Chromosome number

c- The position of the QTL measured from the distance of the left marker listed on the linkage map

d- Logarithm of odds likelihood equivalent to $-Log_{10}$ likelihood

e-The amount of phenotypic variance explained by the detected QTL

f- Additive gene effect of detected QTL

Molecular-assisted selection is more efficient when mapped QTLs are tightly linked to the markers and the tighter the linkage, the higher the probability for a marker to be inherited together with the detected QTL. For the marker to be efficient, a distance of less than 5 cM between the marker and the QTL is recommended (Collard *et al.,* 2008). Meaning a more than 95 % probability that the marker and QTL are inherited together during meiosis. In this study, the closest mapped was QTL PeSB-5, 7 associated with shoot biomass and was at 11.7 cM, from the nearest marker umc 2136. However, the significant phenotypic differences (Table 1) and variations in R^2 values associated with single markers analysis implies that these single markers with inclusion of others can reliably be utilized in cluster analysis (Tembo and Munyinda, 2014).

Conclusion

Seven QTLs were identified on chromosome 5 with phenotypic variations explained (PVE) ranging from 11 % to 19 %. All the markers used were more than 5 cM (recommended distance for utilization in MAS) from the mapped QTLs. The closest marker, umc2136 was 11.7 cM away from the mapped QTL PeSB-5, 7. Therefore, there is need to utilize the maize genomic map to identify and test several markers near the mapped QTL, in order to locate more reliable molecular markers for utilization in MAS.

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