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Genetic variability of local maize (*Zea mays* L.) varieties screened under soil Phosphorus deficiency conditions in Benin

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Abstract

Maize (*Zea Mays* L.) is one of the world's most important food crops and a model system for studying genetics, evolution, and domestication. The grain yield depends on the management of nutrients at the production site. The development of plant molecular genetics allowed the identification and selection of Mendelian components combining simple and complex agronomic traits. Seventeen quantitative agro-morphological traits and 12 Simple Sequence Repeat markers flanking quantitative trait loci controlling six agronomic characteristics were used to screen 134 genotypes derived from maize produced in Benin agro-ecological zones. Descriptive analysis and trait variabilities were estimated, including broad-sense heritability, genotypic and phenotypic coefficients of variation,

and genetic gains. Among the studied traits, only harvest ear weight and grain yield per plant fulfilled the conditions to be used for selection of high-performance genotypes under soil phosphorus deficiency conditions. According to the SSR screening findings, genotypes VA1, VA7, and VA16 were pointed out as having three, two, and one tolerance gene, respectively. A combination of the field and molecular data reveals that the genotype VA1 can be extended on farms or used as a candidate to improve maize for its tolerance to phosphorus deficiency.

Keywords: Quantitative trait loci, microsatellites, screening, soil fertility, grain yield.

Introduction

Maize is one of the most important food crops in the world and a key ingredient in animal feed. It is extensively used in industrial products and also in the production of biofuels (Shiferaw et al., 2011). Soil spatial variability in small farms has largely been trivialized when designing technological interventions, but it is widely demonstrated that the variability of the fertility of soils on farms poses major challenges to the efficient use of resources to increase crop productivity (Kurwakumire et al., 2014). Maize average demand in developing countries was estimated at 504 million tons by 2020,while it was expected to exceed wheat and rice demand (Makumbi et al., 2011). According to Kurwakumine et al., (2014), maize productivity is significantly influenced by the management of nutrients at each production site. The fertility of soil can be as important as its ability to maintain plant growth. Besides nitrogen, phosphorus (P) is the most important essential nutrient for cereal production and animals, and its deficiency leads to huge yield losses, up to 67% in maize (Mapiemfu-Lamaré et al., 2011). Phosphate is immobile in soil because it reacts with many chemical and biological soil constituents (Lynch, 2011). Generally considered to have a strong requirement for fertility, maize showed variations in the efficiency of phosphorus acquisition (Zhu et al., 2006). For example, phosphorus deficiency may reduce the synthesis of proteins and nucleic acids, lead to the accumulation of soluble nitrogen compounds in the tissues, and ultimately delay cell growth. The time also required for the expression of phosphorus deficiency during the growth of the plants depends on the phosphorus reserves in the plants (Grant et al., 2001). Visual symptoms of plant phosphorus deficiency are generally neither accurate nor pronounced enough in the field to be properly diagnosed. These symptoms can be confused with other constraints symptoms unless a comparison is made between the application of phosphorus fertilizer and non–application in the same field (Shiferaw et al., 2011). The major challenge for the future is to achieve significant increases in food production without compromising public health, environmental quality, or the sustainability of farming systems (Tilman et al., 2002; Shiferaw et al., 2011).

Recent developments in molecular plant genetics have offered breeders tools to identify and select components that combine simple and complex agronomic traits (Ribaut and Ragot, 2006). As phosphorus deficiency tolerance is known as a quantitative trait, the appropriate method to study the succession of polygenes is the analysis of quantitative trait loci (QTL). A QTL is defined as a region in the genome that is responsible for the variation in the quantitative target trait (Ramaekers et al., 2010).The advent of new molecular markers, the availability of high resolution mapping, and the application of new statistical methods have led to the identification of large QTLs numbers for grain yield and its components in different populations of several species, including maize (Ma et al. 2007). Assuero et al. (2004), recorded a significant increase in plant height with a nitrogen (N) and phosphorus (P) fertilizer application. Although genetic information on the regulation of plant height (PH), ear height (EH), and the PH/EH ratiois scarce (Agrama 2006), more than 280 QTLs controlling plant height in maize were detected (Zhang et al., 2007). Other QTLs related to the characteristics of the roots(Zhu et al., 2005, 2006) and biological properties of the plant (Chen

et al., 2009) were detected in maize. In 2010, Li et al. conducted mapping of QTLs for grain yield and its components during two different field assessments. They identified some markers flanking different detected QTLs that have a major effect on the increase in maize yield and are unevenly distributed on the 10 maize chromosomes.

The purpose of this study is to assess the genetic variability of 134 maize genotypes grown in Benin using agromorphological data and known SSR markers linked to phosphorus deficiency tolerance genes in order to identify the most tolerant and improve the plant species.

Material and Methods

Description of material used

The plant material consisted of 134 maize genotypes collected in different production zones in Benin, as mentioned by Bonou-gbo et al. (2017), and two checks, Mo17 (tolerant check), and B73 (susceptible check) obtained from CIMMYT (International Maize and Wheat Improvement Center) in Mexico.

According to a previous study conducted by Bonou-gbo et al. (2017), the samples investigated were 71 local varieties improved by research centers and 63 traditional cultivars. To rename the assessed genotypes, the two initial letters VA were followed by their entry number in the collection. After the seed multiplication in the field, when we notice that an accession has two different seed colors, those seeds with the same color are bulked accordingly. So, for each group of seeds derived from the same accession but having the same color, their names were written using VA followed by the entry numbers, to which the letter b was added to show that they differed by seed color. In order to purify the accession to have the same color of seeds per genotype, three rounds of self-pollination had been completed prior to the field characterization trials.

Field trial

Experimental design and soil screening

The study was conducted at the IITA station located in Abomey-Calavi district in southern Benin. The climate in this area is of the Soudano-Guinean type, characterized by two rainy seasons with an average rainfall between 800 and 1200 mm/year. The experiment consisted 136 genotypes and was established on a split-plot design with 3 replicates, including 3 phosphorus levels such as 0 kg/ha, 50 kg/ha, and 100 kg/ha as P0, P1, and P2, respectively. Each plot consisted of a total of 45 plants from the same genotype. Phosphorus levels and genotypes were, respectively, the main plot and subplot factors, while the replication was the time of the phosphorus applications. Each treatment in the replication was separated by 1.75 m, whereas 3 m was kept between replications. Each genotype was sown on a line of 3 m, with 0.20 m between two consecutive holes and 0.5 m between plots. Three seeds were sown per hole and thinned at 1 plant per hole two weeks after sowing. Nutrients such as nitrogen (N), phosphorus (P), and potassium (K) were then applied using urea, triple superphosphate (TSP), and potassium chloride (KCl) fertilizers, respectively, according to the treatments (+/-). According to Li et al. (2010), 180 and 63 kg/ha of N and K were uniformly applied per replication, respectively.

The top soil samples (0-20 cm) were randomly collected from six different points per replication and dried at room temperature. Samples from the same replication were mixed, homogenized, and put in a bag. At the end, 15 g of the soil per replication was aliquoted in a plastic bag and sent to the laboratory for physiochemical analysis. So, available phosphorus and total nitrogen had been determined according to Bray1 and the micro-Kjeldahl method(Anderson and Ingram, 1993). The exchangeable bases (K^+, Mg^{2+}, and Ca^{2+}) have been extracted by the use of ammonium acetate. The pH (H_2O) of soil has been determined by the potentiometric method in a soil/water ratio of 1:2.5, while the pH of KCl has

been identified using the same method after the addition of KCl to the soil/water solution.

Data collection

Data were randomly recorded on five selected plants per accession per plot among the 45 plants sown per genotype. Seventeen quantitative traits were chosen among these maize descriptors (IBPGR, 1991) and scored. Reproductive stage traits were days to 50% male flowering (DMF), days to 50% female flowering (DFF), length (LL) and width (WL) of ear leaf, plant height (PH), ear height (EH), panicle length (PL), length of panicle peduncle (LPP), number of panicle branches (PBN), and distance of panicle ramification (DPR). Post-harvest stage traits included harvest ear weight (HEW), ear length (EL), ear diameter (ED), row number (RN), kernel number per row (KNR), grain yield (GY), and 100 kernel weight (100-KW).

Marker diversity for phosphorus deficiency tolerance

Maize young leaves collection and genomic DNA extraction

Two weeks after sowing in the field, young leaves from five random plants were aliquoted per genotype in well-labeled 15-ml tubes. Collected samples were kept on ice from the field to the laboratory to be immediately used or stored at -20°C.

About 300 mg of maize young leaf were ground in SDS buffer (200 mM Tris-HCl, 25 mM EDTA, 250 mM NaCl, 0.5% SDS (w/v)). The extraction was performed according to Chen et al., (2010) with a slight modification at the precipitation step as described by Nalini et al., (2004). This modification was performed to acquire a good quality of DNA that can be kept for a long time. The extracted DNA was then suspended in Tris-EDTA (TE) buffer 1X, and its concentration was estimated using a spectrophotometer (Nanodrop 2000). The final concentration of all samples was then adjusted to 25ng/µl for gene amplification during the polymerization chain reaction step.

Microsatellite marker selection

The main goal of producers is to have a huge yield, even under constraints. For this purpose, 12 SSR primers (Table 1) flanking yield QTLs and their components associated with tolerance to phosphorus deficiency previously detected by Li et al. (2010) in the maize genome were used for this study. The specific reason for the choice of these markers is their association with high grain yield (GY) expression, and its components such as 100 kernel weight (KW), ear diameter (ED), ear length (EL), kernel numbers (KN), and row number (RN) in phosphorus deficiency conditions (Table 1). These markers were synthesized based on the sequence provided on the maize GDB website (http://www.maizegdb.org).

Table 1: Expected microsatellite marker profile compared to the checks

		Sequences (5'-3')								
		F	R							
Primers	**Chr.**			**Dist. (cM)**	**GY**	**RN**	**KN**	**ED**	**100-KW**	**EL**
umc1166	1	CGATCAGATCATACACAACCTTGC	GAGGATCGATTCTTGGCGAGT	131.8	1	0	0	0	1	0
bnlg1429	1	CTCCTCGCAAGGATCTTCAC	AGCACCGTTTCTCGTGAGAT	143.5	0	1	0	1	0	1
umc1298	1	AGCTGAACAAAATAAACGGAACGA	AGGACAAGAAAAAGAAGAAGCACG	845.51	1	0	0	0	0	0
bnlg1331	1	TGGTGATAACTGTCAAGCGC	TTGGGGCATTGGCCTATATA	847	1	0	0	0	0	0
umc1587	5	ATGCGTCTTTCACAAAGCATTACA	AGGTGCAGTTCATAGACTTCCTGG	128.7	0	0	0	0	1	0
umc1264	5	AGATAGCTGCACATGGAAACACTT	GACACTAGCCTGGAATCAGTTTCA	366	0	1	0	0	0	0
umc1155	5	TCTTTTATTGTGCCCGTTGAGATT	CCTGAGGGTGATTTGTCTGTCTCT	370.9	0	1	0	0	0	0
umc1792	5	CATGGGACAGCAAGAGACACAG	ACCTTCATCACCTGCAACTACGAC	625.8	1	0	1	0	1	0
phi059	10	AAGCTAATTAAGGCCGGTCATCCC	TCCGTGTACTCGGCGGACTC	104.4	0	0	1	0	0	0
umc2349	10	TACAACAAGAAACGAAAACGGCTT	CCTATTGCTGCGCATACCTAACTAA	227.9	0	0	1	0	0	0
bnlg1839	10	AGCAGACGGAGGAAACAAGA	TCTCCCTCTCCCTCTTGACA	466.4	0	1	0	0	0	0
bnlg1360	10	TCTGCTCATCCACAACTTGC	AGAACGTGAAGCTGAGCGTT	469.7	0	0	1	1	0	0
Total				**4727.61**	**4**	**4**	**4**	**2**	**3**	**1**

Chr.: chromosome; Dist.: genetic distance; 0: Absence of tolerance allele for the character; 1: Presence of tolerance allele for the character

Polymerase chain reaction

The mix PCR volume was 25µl, which consisted of 2µl of DNA (25ng/µl), 2.5µl of PCR buffer 10X, 1µl of 10µM primer (mixture of forward and reverse); and 0.1µl of Taq DNA polymerase, and was adjusted to the reaction volume with 18.4µl of purified distilled water (dH_2O). Two sets of primers were registered. The first one has an annealing temperature of 55°C, and the second one has a temperature of 62°C. the PCR program performed started with an initial denaturation at 94°C for 2 min; followed by 35 cycles consisting of a denaturation at 94°C for 1 min, primer annealing at 55°C or 62°C for 45 sec, and polymerization at 72°C for 1 min. All those cycles were followed by a final incubation at 72°C for 7 minute and storage at 4°C for an indefinite period.

Agarose gel electrophoresis and allele detection

A 3% agarose gel was prepared for this purpose and mixed with 3% GelRed to allow visualization of the DNA amplicons under UV. Next, 10 µl of loading dye was added to 25µl of PCR product, and 10 µl of this mixture was loaded at 150V for 2 hours. A molecular weight marker of 100 bp was also loaded in first well of each line, followed by both checks (tolerant Mo17 and susceptible B73) in order to better characterize the collection and estimate the size of the amplicons. The fragments were separated and visualized through UV using the gel documentation device AlphaImager, and the images were saved on a computer for further analysis.

Data analysis

The recorded data were first submitted to a descriptive analysis. So, the mean, minimum, maximum, standard deviations, and coefficient of variation for the set of traits were determined.

In general, knowledge of the combination of genetic parameters is essential for understanding trait effects and using them in breeding programs.

For this study, we considered each treatment (phosphorus dose)as an environment, while the software META-R version 5.0 (Multi-Environment Trial Analysis R with Heart Windows) (Alvarado et al., 2015)was used to define the different parameters linked to the variability of the characters in different treatments. Phenotypic and genotypic correlations (Pliura et al., 2014) between the different traits and the threshold of the interaction genotypes X environment had been analyzed. Broad-sense heritability (H^2) was estimated for each evaluated trait considering each treatment as an environment and genotype x environment interaction as described by different authors (Bello et al., 2012; Estaghvirou et al., 2013; Gonçalves et al., 2013; Johnson et al., 1955; Piepho and Möhring, 2007). Genotypic (GCV) and phenotypic coefficients of variation (PCV) and genetic gain were estimated with 5% of selection intensity. All these analyses were performed to determine the high-performance genotypes under phosphorus deficiency conditions.

From the marker analysis, gels were scored three times, twice visually and once based on the molecular weight indicator integrated in the AlphaImager gel documentation system. This last replication was assessed to reduce reading errors. The strips were read following a 0 or 1 code, which corresponded to the absence or presence of an allele linked to tolerance to the phosphorus deficiency gene, with a check of reference allele position. A genotype cluster was made according to UPGMA (Unweighted Pair Group Method based on Arithmetic Average) to show the different groups according to their tolerance level. Analyses were performed using Microsoft Excel® and PowerMarker 3.25 software.

Results

Variability between agro-morphological traits

The experimental site was a ferrallitic soil type characterized by a high acidity (pH (H_2O) =4.924, pH (KCl) = 4.188) and a low organic compounds, which was 0.578%. Available phosphorus per plant was 24.796 ppm with exchangeable bases K^{2+}, Mg^{2+}, and Ca^{2+}concentrations of 0.472 Cmol/kg, 2.189 Cmol/kg, and 0.569 Cmol/kg of soil, respectively.

The descriptive statistic of quantitative traits are summarized in Table 1. An important gap was found between minima and maxima for all considered traits from one treatment to another. On the phosphorus dose, the smallest mean was obtained with the phosphorus level P0 trait except for the days to 50% female flowering (DFF) and male flowering (DMF), width of the ear leaf (WL), and length of the panicle peduncle (LPP). These variabilities were confirmed by the high variability noticed in coefficients of variation found between traits per dose of phosphorus. The coefficients of variation (CV) values range from 6.827 (DMF) to27.976 (GY) at P0, 6.002% (DFF) to 39.638% (WL) at P1, and 6.431 (DFF) to 24.574 (DRP) at P2, showing a large variation between traits with regard to phosphorus level (Table 2).

Correlation analysis between traits

The genotypic and phenotypic correlations between characters per phosphorus level(P0, P1, and P2) are summarized in Tables 3, 4, and 5, respectively. For positive values, the genotypic correlations are mostly high compared to the phenotypic correlations of the same pair of traits. These correlations were lower for negative values. At the level P0, no significant genotypic correlation was found between row number and each of the characters such as grain yield per plant (GY), ear diameter (ED),ear length (EL), harvest ear weight (HEW), kernel number per row (KNR), and weight of 100 kernels(100-KW), while a weak and significant positive phenotypic correlation was only noticed between RN*vs.*HEW (rp = 0,20) and RN*vs.*ED (rp = 0,29) (Table 3).

The same results were reported for genotypic correlations except that between ear length and 100 kernel weight, which became weak but significant at level P1. The grain yield per plant showed a strong positive and significant correlation with kernel number per row, while it was weak between 100-KW and GY (Table 4). At P2, genotypic and phenotypic correlations were significant for the set of combinations implying the grain yield and its components except RN (Table 5). Otherwise, except for positive phenotypic correlation at P0, the two types of correlation between these characters are negative in all treatments. An analysis of the combined treatments showed similar correlations to some of those recorded at P0 and P1 (Table 6), but at a very high threshold level.

Table 2: Descriptive statistic of evaluated traits in each of tree environment

Trait	Minimal			Maximal			Mean			SD			CV (%)		
Treatment	**P0**	**P1**	**P2**	**P0**	**P1**	**P2**	**P0**	**P1**	**P2**	**P0**	**P1**	**P2**	**P0**	**P1**	**P2**
DMF	48.675	48.831	48.930	64.089	62.000	60.665	56.836	56.102	56.099	2.765	2.517	2.698	6.827	6.085	6.716
DFF	53.981	53.222	52.655	70.207	65.842	67.136	61.748	60.058	59.994	3.131	2.512	2.914	7.527	6.002	6.431
PH	125.825	121.614	135.400	177.134	190.722	190.487	153.068	161.508	164.996	9.029	11.644	10.460	10.702	12.423	11.753
EH	54.899	38.900	59.295	99.475	112.218	120.024	74.575	79.688	83.142	7.381	9.712	9.582	17.862	20.546	18.201
LL	55.013	57.272	60.357	84.160	89.612	90.289	77.177	78.277	78.742	4.385	4.874	4.612	8.900	10.931	9.661
WL	6.312	6.515	6.483	9.520	9.217	8.883	7.746	7.726	7.771	0.481	0.464	0.440	19.932	39.638	16.852
PBN	10.004	10.482	12.833	18.019	19.723	27.550	13.634	14.733	19.332	1.659	1.722	2.515	21.575	19.701	24.535
PL	28.594	25.688	28.871	38.746	40.434	37.904	32.994	33.321	33.438	1.958	2.324	1.871	10.808	11.624	13.678
LPP	18.387	14.768	15.270	24.263	25.748	24.164	20.710	19.549	19.013	1.252	1.641	1.553	14.518	14.878	13.513
DPR	6.226	5.845	9.049	14.943	15.741	15.418	10.827	11.552	12.252	1.186	1.358	1.287	18.052	18.219	24.574
HEW	24.096	50.890	25.774	163.619	181.520	173.564	95.485	103.899	111.829	26.389	23.523	25.211	27.021	24.547	23.495
KNR	12.195	18.552	17.322	32.362	31.588	29.354	22.221	24.437	23.932	3.222	2.780	2.442	17.420	13.286	13.411
RN	10.197	11.371	11.107	14.889	15.894	15.835	12.763	13.184	13.250	0.906	0.798	0.812	10.017	9.016	9.747
EL	8.082	9.599	10.303	15.491	16.607	16.210	12.415	12.913	13.209	1.096	1.204	1.179	12.998	12.774	11.965
ED	21.843	29.939	30.317	42.320	43.669	42.749	36.203	37.017	37.768	3.145	2.452	2.723	10.322	9.113	10.063
GY	19.493	43.288	24.654	104.208	119.257	145.731	61.959	73.703	81.883	17.068	16.532	17.845	27.976	25.893	24.074
100-KW	17.090	15.094	10.342	33.306	33.236	32.778	24.040	25.216	25.709	3.498	3.435	3.149	16.276	15.613	14.384

DMF, Days to 50% male flowering; DFF, days to 50% female flowering; PH, plant height (cm), EH, ear height (cm); LL, length of leaf (cm); WL, width of leaf (cm); PBN, panicle branch number; PL, panicle length (cm); LPP, length of panicle peduncle (cm); DPR, distance of panicle ramification (cm); HEW, harvest ear weight (g); KNR, kernel number per row; RN, row number; EL, ear length (cm); ED, ear diameter (mm); GY, grain yield (g) and 100-KW,100 kernels weight (g); CV, coefficient de variation (%); SD, Standard deviation; P0, 0kg/ha of P; P1, 50kg/ha of P; P2, 100kg/ha of P; P, phosphorus

Table 3: Phenotypic correlation (upper matrix) and genotypic (lower matrix) between traits at 0 Kg/ha P

Traits	DMF	DFF	PH	EH	LL	WL	PBN	PL	LPP	DRP	HEW	KNR	RN	EL	ED	GY	100-KW
DMF		0.92***	0.41***	0.42***	0.28***	0.08	-0.06	0.24**	-0.15	-0.05	0.14	0.01	0.04	0.12	0.01	-0.05	-0.01
DFF	0.96***		0.40***	0.40***	0.19*	0.07	-0.05	0.14	-0.12	-0.09	0.12	0.00	0.04	0.09	-0.01	-0.07	-0.04
PH	0.80***	0.69***		0.82***	0.50***	0.12	0.22**	0.35***	0.12	0.29***	0.31***	0.27**	0.06	0.23**	0.00	0.13	0.15
EH	0.71***	0.59***	0.93***		0.24**	0.11	0.34***	0.18*	-0.07	0.30***	0.17*	0.17*	0.04	0.14	-0.11	-0.02	-0.02
LL	0.72***	0.41***	0.69***	0.43***		0.20*	0.11	0.58***	0.18*	0.27**	0.36***	0.18*	0.15	0.28**	0.27**	0.28***	0.31***
WL	0.36***	0.42***	0.58***	0.60***	0.92***		0.05	0.19*	-0.01	0.07	0.04	0.16	0.08	0.20*	0.13	0.18*	0.07
PBN	0.14	-0.02	0.50***	0.65***	0.33***	0.47***		0.21*	-0.14	0.68***	-0.02	0.01	0.09	0.01	-0.10	-0.03	0.01
PL	0.41***	0.25**	0.50***	0.29***	0.76***	0.55***	0.31***		0.14	0.49***	0.28***	0.12	0.25**	0.26**	0.19*	0.22*	0.26**
LPP	-0.12	-0.06	0.01	-0.10	-0.07	-0.07	-0.07	0.04		0.14	0.09	-0.06	0.05	0.05	0.09	0.24**	0.10
DPR	0.11	-0.14	0.59***	0.63***	0.51***	0.18*	0.90***	0.48***	0.14		0.07	0.00	0.12	0.07	-0.01	0.08	0.13
HEW	0.24**	0.17*	0.47***	0.22*	0.80***	0.20*	0.09	0.42***	-0.02	0.21*		0.45***	0.20*	0.64***	0.58***	0.60***	0.58***
KNR	0.00	-0.05	0.21*	0.07	0.25**	0.46***	-0.03	0.06	0.02	0.14	0.58***		0.15	0.58***	0.23**	0.38***	0.29***
RN	0.04	0.17*	-0.27**	-0.28**	-0.16	-0.36***	-0.23**	0.17	-0.07	-0.12	0.08	0.13		0.14	0.29***	0.02	0.01
EL	0.90***	0.68***	0.08	0.18*	-0.09	0.99***	-0.19*	-0.09	0.01	-0.52***	0.99***	0.99***	-0.11		0.41***	0.53***	0.47***
ED	0.14	0.02	-0.02	-0.08	0.44***	-0.09	0.03	0.07	0.04	0.08	0.80***	0.21*	0.06	0.70***		0.53***	0.51***
GY	-0.14	-0.15	0.08	-0.13	0.30***	-0.36***	-0.12	0.17*	0.07	-0.10	0.61***	0.54***	0.09	0.99***	0.54***		0.52***
100-KW	0.04	0.03	0.19*	-0.07	0.30***	-0.56***	-0.04	0.11	0.04	-0.01	0.72***	0.34***	-0.02	0.80***	0.58***	0.57***	

DMF, Days to 50% male flowering; DFF, days to 50% female flowering; PH, plant height (cm), EH, ear height (cm); LL, length of leaf (cm); WL, width of leaf (cm); PBN, panicle branch number; PL, panicle length (cm); LPP, length of panicle peduncle (cm); DPR, distance of panicle ramification (cm); HEW, harvest ear weight (g); KNR, kernel number per row; RN, row number; EL, ear length (cm); ED, ear diameter (mm); GY, grain yield (g) and 100-KW,100 kernels weight (g); *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

Table 4: Phenotypic correlation (upper matrix) and genotypic (lower matrix) between traits at50 Kg/ha P

Traits	DMF	DFF	PH	EH	LL	WL	PBN	PL	LPP	DRP	HEW	KNR	RN	EL	ED	GY	100-KW
DMF		0.92***	0.36***	0.31***	0.32***	0.09	-0.13	0.22*	-0.22*	-0.05	0.26**	0.08	0.13	0.21*	0.29***	0.22*	0.13
DFF	0.93***		0.33***	0.28***	0.24**	0.05	-0.11	0.17*	-0.20*	-0.10	0.18*	0.06	0.13	0.14	0.20*	0.17*	0.07
PH	0.59***	0.37***		0.89***	0.37***	-0.09	0.20*	0.42***	-0.09	0.33***	0.42***	0.23**	-0.11	0.34***	0.33***	0.37***	0.31***
EH	0.52***	0.28***	0.98***		0.19*	-0.12	0.23**	0.30***	-0.20*	0.31***	0.32***	0.17*	-0.18*	0.23**	0.23**	0.29***	0.19*
LL	0.58***	0.29***	0.74***	0.63***		0.46***	0.17	0.53***	0.02	0.32***	0.46***	0.20*	0.03	0.42***	0.46***	0.37***	0.29***
WL	0.50***	0.16	0.24**	0.19*	0.72***		0.24**	0.15	-0.06	0.27**	0.23**	0.15	0.06	0.25**	0.17	0.18*	0.02
PBN	-0.01	-0.15	0.48***	0.54***	0.41***	0.99***		0.25**	-0.07	0.69***	0.03	0.11	-0.19*	0.08	0.04	0.12	0.01
PL	0.37***	0.31***	0.60***	0.45***	0.64***	0.56***	0.24**		0.11	0.51***	0.54***	0.40***	-0.02	0.50***	0.46***	0.50***	0.32***
LPP	-0.54***	-0.11	-0.82***	-0.89***	-0.50***	-0.26**	-0.44***	-0.05		0.12	0.02	0.05	-0.05	0.05	-0.03	0.07	0.20*
DPR	0.11	-0.06	0.64***	0.64***	0.61***	0.99***	0.89***	0.55***	-0.31***		0.24**	0.18*	-0.18*	0.30***	0.16	0.27**	0.25**
HEW	0.35***	0.17	0.54***	0.42***	0.62***	0.69***	0.11	0.62***	-0.26**	0.34***		0.61***	0.16*	0.79***	0.72***	0.83***	0.54***
KNR	0.13	0.08	0.26**	0.19*	0.24**	0.06	0.11	0.46***	-0.10	0.21*	0.65***		0.07	0.69****	0.24**	0.58***	0.21*
RN	0.18*	0.40***	-0.39***	-0.51***	-0.43***	-0.46***	-0.59***	-0.14	0.08	-0.59***	0.03	-0.03		0.12	0.29***	0.11	-0.14
EL	0.42***	0.26**	0.36***	0.29***	0.40***	0.99***	-0.01	0.63***	-0.23**	0.32***	0.99***	0.81***	0.04		0.45***	0.69***	0.40***
ED	0.51***	0.28***	0.56***	0.46***	0.72***	0.35***	0.17*	0.56***	-0.58***	0.30***	0.91***	0.31***	-0.08	0.53***		0.57***	0.49***
GY	0.30***	0.27**	0.33***	0.24**	0.42***	0.56***	0.03	0.68***	0.08	0.30***	0.83***	0.61***	0.19*	0.80***	0.56***		0.51***
100-KW	0.20*	0.09	0.32***	0.17*	0.39***	0.01	0.02	0.37***	0.20*	0.30***	0.58***	0.24**	-0.26**	0.48***	0.63***	0.53***	

DMF, Days to 50% male flowering; DFF, days to 50% female flowering; PH, plant height (cm), EH, ear height (cm); LL, length of leaf (cm); WL, width of leaf (cm); PBN, panicle branch number; PL, panicle length (cm); LPP, length of panicle peduncle (cm); DPR, distance of panicle ramification (cm); HEW, harvest ear weight (g); KNR, kernel number per row; RN, row number; EL, ear length (cm); ED, ear diameter (mm); GY, grain yield (g) and 100-KW,100 kernels weight (g); *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

Table 5: Phenotypic correlation (upper matrix) and genotypic (lower matrix) between traits at100 Kg/ha P

Traits	DMF	DFF	PH	EH	LL	WL	PBN	PL	LPP	DRP	HEW	KNR	RN	EL	ED	GY	100-KW
DMF		0.92***	0.46***	0.43***	0.33***	-0.03	-0.13	0.19*	-0.11	0.02	0.26**	0.10	0.11	0.20*	0.18*	0.18*	0.17*
DFF	0.91***		0.42***	0.42***	0.23**	-0.07	-0.03	0.11	-0.11	0.01	0.17	0.04	0.07	0.15	0.06	0.11	0.11
PH	0.68***	0.45***		0.86***	0.47***	-0.02	0.34***	0.23**	0.07	0.39***	0.23**	0.19*	0.05	0.22*	0.18*	0.20*	0.22**
EH	0.59***	0.43***	0.93***		0.28***	-0.09	0.42***	0.16	-0.06	0.32***	0.10	0.17	0.03	0.16	0.09	0.10	0.13
LL	0.65***	0.32***	0.67***	0.44***		0.33***	0.05	0.46***	-0.05	0.34***	0.39***	0.21*	0.02	0.37***	0.40***	0.17*	0.21*
WL	-0.03	-0.22*	-0.16	-0.24**	0.99***		-0.07	0.15	0.02	0.09	0.21*	0.16	0.06	0.22**	0.27**	0.21*	0.12
PBN	-0.03	-0.10	0.64***	0.66***	0.29***	-0.33***		-0.04	-0.04	0.43***	-0.08	0.04	-0.12	0.06	-0.09	-0.02	0.06
PL	0.99***	0.81***	0.72***	0.57***	0.99***	0.42***	-0.41***		-0.02	0.25**	0.26**	0.25**	-0.08	0.34***	0.29***	0.15	0.14
LPP	-0.30***	0.00	-0.75***	-0.71***	-0.79***	-0.85***	-0.40***	-0.86***		0.11	-0.03	0.09	0.03	-0.02	0.06	0.03	0.02
DPR	0.12	-0.06	0.84***	0.66***	0.80***	0.54***	0.67***	0.76***	-0.49***		0.07	0.14	0.03	0.21	0.11	0.01	-0.03
HEW	0.33***	0.14	0.40***	0.21*	0.68***	0.61***	0.03	0.85***	-0.44***	0.19*		0.44***	0.00	0.68***	0.60***	0.69***	0.57***
KNR	0.21*	0.03	0.31***	0.27**	0.23**	0.58***	0.16	0.81***	0.17*	0.27**	0.54***		0.10	0.44***	0.24**	0.45***	0.22*
RN	0.14	0.24**	-0.34***	-0.26**	-0.52***	-0.40***	-0.49***	-0.70***	0.57***	-0.25**	-0.17*	-0.02		0.10	0.25**	0.05	-0.22*
EL	0.43***	0.25**	0.35***	0.28***	0.54***	0.72***	0.20*	0.99***	-0.29***	0.55***	0.84***	0.43***	0.01		0.45***	0.40***	0.36***
ED	0.31***	0.01	0.50***	0.35***	0.77***	0.61***	0.10	0.98***	-0.44***	0.43***	0.77***	0.34***	-0.15	0.61***		0.40***	0.47***
GY	0.18*	0.10	0.20*	0.02	0.28**	0.57***	-0.11	0.34***	0.22**	-0.04	0.76***	0.57***	0.08	0.45***	0.42***		0.64***
100-KW	0.23**	0.07	0.39***	0.23**	0.52***	0.28***	0.16	0.57***	-0.02	0.08	0.73***	0.27**	-0.33***	0.49***	0.65***	0.71***	

DMF, Days to 50% male flowering; DFF, days to 50% female flowering; PH, plant height (cm), EH, ear height (cm); LL, length of leaf (cm); WL, width of leaf (cm); PBN, panicle branch number; PL, panicle length (cm); LPP, length of panicle peduncle (cm); DPR, distance of panicle ramification (cm); HEW, harvest ear weight (g); KNR, kernel number per row; RN, row number; EL, ear length (cm); ED, ear diameter (mm); GY, grain yield (g) and 100-KW,100 kernels weight (g); *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

Table 6: Phenotypic correlation (upper matrix) and genotypic (lower matrix) between traits for combined treatments

Traits	DMF	DFF	PH	EH	LL	WL	PBN	PL	LPP	DRP	HEW	KNR	RN	EL	ED	GY	100-KW
DMF		0.95***	0.58***	0.56***	0.47***	0.10	-0.10	0.33**	-0.17*	0.06	0.44	0.17*	0.19*	0.35***	0.28***	0.24**	0.14
DFF	0.93***		0.57***	0.55***	0.38***	0.04	-0.05	0.25**	-0.13	0.01	0.38***	0.16	0.17*	0.30***	0.22**	0.18*	0.09
PH	0.70***	0.54***		0.89***	0.54***	0.07	0.27**	0.44***	-0.03	0.44***	0.47***	0.31**	0.02	0.42***	0.26**	0.38***	0.30***
EH	0.70***	0.54***	0.95***		0.33***	0.01	0.37***	0.32***	-0.18*	0.43***	0.29***	0.24**	-0.02	0.30**	0.11	0.25**	0.16
LL	0.62***	0.36***	0.76***	0.62***		0.48***	0.01	0.66***	0.05	0.34***	0.61***	0.31***	0.06	0.57****	0.57***	0.51***	0.42***
WL	0.32***	0.06	0.42***	0.32***	1.00***		0.15	0.27**	0.04	0.34***	0.23**	0.17	0.08	0.32***	0.32***	0.28**	0.14
PBN	0.03	-0.10	0.50***	0.59***	0.25**	0.48***		0.09	-0.10	0.62***	-0.09	0.06	-0.14	0.01	-0.07	0.03	0.08
PL	0.47***	0.35***	0.55***	0.46***	0.76***	0.74***	0.17*		0.03	0.44***	0.51***	0.33***	0.04	0.50***	0.44***	0.51***	0.38***
LPP	-0.29***	-0.04	-0.45***	-0.55***	-0.33***	-0.01	-0.40***	-0.12		0.10	-0.05	0.05	0.04	0.01	-0.02	0.01	0.03
DPR	0.19*	-0.03	0.72***	0.72***	0.59***	1.00***	0.82***	0.51***	-0.29***		0.17*	0.18*	-0.06	0.24**	0.15	0.21	0.24**
HEW	0.76***	0.56***	0.67***	0.48***	0.97***	0.50***	0.15	0.67***	-0.42***	0.47***		0.51	0.05	0.73***	0.69***	0.78***	0.69***
KNR	0.38***	0.28**	0.47***	0.35***	0.55***	0.15	0.22**	0.47***	0.04	0.42***	0.56***		0.12	0.61***	0.24**	0.55***	0.23**
RN	0.12	0.30***	-0.39***	-0.42***	-0.32***	-0.19*	-0.49***	-0.07	0.55***	-0.51***	-0.39***	-0.13		0.06	0.26**	0.01	-0.27**
EL	0.73***	0.56***	0.62***	0.46***	0.95***	0.91***	0.10	0.79***	-0.24**	0.43***	0.90***	0.91***	-0.32***		0.51***	0.62***	0.49***
ED	0.44***	0.19*	0.53***	0.40***	0.93***	0.89***	0.33***	0.55***	-0.51***	0.50***	0.88***	0.36***	-0.39***	0.74***		0.56***	0.57***
GY	0.49***	0.51***	0.32***	0.19*	0.89***	0.68***	-0.01	1.00***	-0.01	0.35***	0.80***	0.73***	0.14	0.84***	0.39***		0.62***
100-KW	0.20*	0.11	0.36***	0.23**	0.69***	0.48***	0.30***	0.58***	-0.19*	0.52***	0.96***	0.32***	-0.55***	0.84***	0.69***	0.75***	

DMF, Days to 50% male flowering; DFF, days to 50% female flowering; PH, plant height (cm), EH, ear height (cm); LL, length of leaf (cm); WL, width of leaf (cm); PBN, panicle branch number; PL, panicle length (cm); LPP, length of panicle peduncle (cm); DPR, distance of panicle ramification (cm); HEW, harvest ear weight (g); KNR, kernel number per row; RN, row number; EL, ear length (cm); ED, ear diameter (mm); GY, grain yield (g) and 100-KW,100 kernels weight (g); *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

Phenotypic and genotypic coefficients of variation of the trait under different phosphorus levels

The selection of traits for crop breeding programs depends on the dominance of their coefficient of variation (CV), and more specifically, of the genotypic coefficients of variation (GCV). The phenotypic coefficients of variation (PCV) in this study ranged from 1.98% (LPP) to 27.38% (PER) with the treatment P0 at 0 kg/ha (Tables 7); 3.45% (DFF) to 24.88% (PER) with P1 at 50 kg/ha (Table 8); and from 3.46% (WL) to 21.86% (PER) with P2 at 100kg/ha (Table 9). The genotypic coefficients of variation were also evaluated and varied as 4.82% (FLM), 4.43% (FLF), and 5.10% (FLF) to 27.54%; 25.00%; and 22.44% in the respective treatment P0, P1 and P2. All maximum values were obtained with harvest ear weight (HEW) on the three treatments. Analyses of combined data from all treatments exhibited a strong difference between genotypic (GCV) and phenotypic (PCV) coefficients of variation. Also, the analysis of the variance estimated for genotype by environment was mostly weak for all traits (Table 10). As described in each specific treatment, the maximum values of both genotypic and phenotypic coefficients of variation were obtained with harvest ear weight.

Table 7: Estimation of traits variability at 0 kg/ha P

Traits	Vg	Vp	X	GCV (%)	PCV (%)	H^2	GA	GG (%)
DMF	4.12	7.47	56.73	3.58	4.82	0.55	3.10	5.47
DFF	4.95	10.15	61.74	3.60	5.16	0.49	3.20	5.19
PH	59.75	115.05	152.89	5.06	7.02	0.52	11.48	7.51
EH	41.25	77.23	74.07	8.67	11.86	0.53	9.67	13.05
LL	8.64	18.93	77.18	3.81	5.64	0.46	4.09	5.30
WL	0.09	0.87	7.74	3.80	12.08	0.10	0.19	2.46
PBN	2.87	4.61	13.51	12.53	15.89	0.62	2.75	20.35
PL	3.17	6.08	32.85	5.42	7.50	0.52	2.65	8.06
LPP	0.17	3.09	20.73	1.98	8.48	0.05	0.20	0.95
DPR	1.00	1.86	10.78	9.28	12.63	0.54	1.51	14.05
HEW	683.11	691.08	95.46	27.38	27.54	0.99	53.53	56.08
KNR	9.32	11.24	22.28	13.70	15.04	0.83	5.73	25.69
RN	0.47	0.84	12.83	5.33	7.16	0.55	1.05	8.16
EL	0.30	0.99	12.44	4.43	8.00	0.31	0.63	5.04
ED	7.20	9.33	36.15	7.42	8.45	0.77	4.86	13.44
GY	278.25	288.68	61.92	26.94	27.44	0.96	33.74	54.48
100-KW	10.38	12.05	23.88	13.49	14.54	0.86	6.16	25.80

DMF, Days to 50% male flowering; DFF, days to 50% female flowering; PH, plant height (cm), EH, ear height (cm); LL, length of leaf (cm); WL, width of leaf (cm); PBN, panicle branch number; PL, panicle length (cm); LPP, length of panicle peduncle (cm); DPR, distance of panicle ramification (cm); HEW, harvest ear weight (g); KNR, kernel number per row; RN, row number; EL, ear length (cm); ED, ear diameter (mm); GY, grain yield (g) and 100-KW, 100 kernels weight (g); CV, coefficient de variation (%); Vg: genotypic variance, Vp: phenotypic variance, x: general mean, GA: genetic advance, GG: genetic gains, GCV: genotypic coefficient of variation, PCV: phenotypic coefficient of variation, H^2: broad sense heritability

Table 8: Estimation of traits variability at 50 kg/ha P

Traits	Vg	Vp	X	GCV(%)	PCV(%)	H²	GA	GG(%)
DMF	4.19	6.65	56.04	3.65	4.60	0.63	3.35	5.97
DFF	4.29	7.11	60.11	3.45	4.43	0.60	3.32	5.52
PH	118.03	178.92	160.45	6.77	8.34	0.66	18.18	11.33
EH	85.64	129.27	79.20	11.68	14.35	0.66	15.52	19.59
LL	16.13	31.48	78.59	5.11	7.14	0.51	5.92	7.54
WL	0.24	3.51	8.04	6.15	23.32	0.07	0.27	3.34
PBN	3.45	4.90	14.69	12.64	15.08	0.70	3.21	21.85
PL	4.08	7.38	33.25	6.08	8.17	0.55	3.10	9.32
LPP	0.80	3.31	19.70	4.55	9.23	0.24	0.91	4.62
DPR	1.17	2.13	11.52	9.40	12.67	0.55	1.66	14.37
HEW	678.01	684.55	104.64	24.88	25.00	0.99	53.38	51.02
KNR	6.75	7.90	24.53	10.59	11.46	0.85	4.95	20.17
RN	0.41	0.73	13.21	4.84	6.47	0.56	0.99	7.47
EL	0.85	1.38	12.90	7.16	9.11	0.62	1.49	11.57
ED	5.10	7.12	36.97	6.11	7.22	0.72	3.93	10.64
GY	276.80	308.58	74.63	22.29	23.54	0.90	32.46	43.50
100-KW	12.85	13.84	25.40	14.12	14.65	0.93	7.12	28.02

DMF, Days to 50% male flowering; DFF, days to 50% female flowering; PH, plant height (cm), EH, ear height (cm); LL, length of leaf (cm); WL, width of leaf (cm); PBN, panicle branch number; PL, panicle length (cm); LPP, length of panicle peduncle (cm); DPR, distance of panicle ramification (cm); HEW, harvest ear weight (g); KNR, kernel number per row; RN, row number; EL, ear length (cm); ED, ear diameter (mm); GY, grain yield (g) and 100-KW,100 kernels weight (g); CV, coefficient de variation (%); Vg: genotypic variance, Vp: phenotypic variance, x: general mean, GA: genetic advance, GG: genetic gains, GCV: genotypic coefficient of variation, PCV: phenotypic coefficient of variation, H^2: broad sense heritability

Table 9: Estimation of traits variability at 100 kg/ha P

Traits	Vg	Vp	X	GCV(%)	PCV(%)	H²	GA	GG(%)
DMF	6.10	8.73	55.92	4.41	5.28	0.70	4.25	7.60
DFF	6.78	9.36	60.05	4.33	5.10	0.72	4.56	7.60
PH	104.97	170.41	164.20	6.24	7.95	0.62	16.57	10.09
EH	77.79	115.41	82.85	10.65	12.97	0.67	14.92	18.00
LL	10.28	23.07	78.70	4.07	6.10	0.45	4.41	5.60
WL	0.07	0.62	7.85	3.46	10.01	0.12	0.19	2.46
PBN	7.67	12.40	19.25	14.38	18.29	0.62	4.48	23.30
PL	0.50	6.98	33.48	2.11	7.89	0.07	0.39	1.17
LPP	0.44	2.42	18.95	3.48	8.20	0.18	0.58	3.05
DPR	1.33	3.97	12.45	9.28	16.02	0.34	1.38	11.08
HEW	597.43	629.89	111.83	21.86	22.44	0.95	49.04	43.85
KNR	4.32	6.15	24.00	8.66	10.33	0.70	3.59	14.95
RN	0.65	0.99	13.33	6.06	7.47	0.66	1.35	10.13
EL	0.90	1.43	13.22	7.18	9.03	0.63	1.56	11.76
ED	7.11	9.01	37.82	7.05	7.94	0.79	4.88	12.89
GY	273.30	310.54	81.87	20.19	21.52	0.88	31.95	39.02
100-KW	8.30	9.94	25.74	11.19	12.25	0.84	5.42	21.08

DMF, Days to 50% male flowering; DFF, days to 50% female flowering; PH, plant height (cm), EH, ear height (cm); LL, length of leaf (cm); WL, width of leaf (cm); PBN, panicle branch number; PL, panicle length (cm); LPP, length of panicle peduncle (cm); DPR, distance of panicle ramification (cm); HEW, harvest ear weight (g); KNR, kernel number per row; RN, row number; EL, ear length (cm); ED, ear diameter (mm); GY, grain yield (g) and 100-KW,100 kernels weight (g); CV, coefficient de variation (%); Vg: genotypic variance, Vp: phenotypic variance, x: general mean, GA: genetic advance, GG: genetic gains, GCV: genotypic coefficient of variation, PCV: phenotypic coefficient of variation, H^2: broad sense heritability

Table 10: Mean variability estimation of collected traits in the combined treatment

Traits	Vg	Vp	X	GCV (%)	PCV (%)	H^2	GA	GG (%)	Traits
DMF	4.63	5.63	0.21	56.23	0.82	3.83	4.22	4.02	7.14
DFF	5.06	6.33	0.29	60.63	0.80	3.71	4.15	4.14	6.83
PH	89.64	110.76	2.73	159.18	0.81	5.95	6.61	17.55	11.02
EH	57.77	74.07	9.85	78.71	0.78	9.66	10.93	13.83	17.57
LL	13.61	17.68	0.00	78.16	0.77	4.72	5.38	6.67	8.53
WL	0.10	0.62	0.04	7.93	0.15	3.90	9.93	0.25	3.15
PBN	3.93	5.05	0.72	15.82	0.78	12.54	14.21	3.60	22.79
PL	2.84	4.23	0.00	33.19	0.67	5.08	6.19	2.85	8.58
LPP	0.83	1.61	0.00	19.79	0.51	4.59	6.41	1.34	6.78
DPR	1.18	1.67	0.00	11.58	0.71	9.37	11.15	1.88	16.20
HEW	171.86	336.40	477.19	103.97	0.51	12.61	17.64	19.30	18.57
KNR	1.30	3.65	5.45	23.60	0.36	4.83	8.10	1.40	5.93
RN	0.34	0.52	0.18	13.13	0.66	4.45	5.48	0.98	7.45
EL	0.18	0.55	0.54	12.86	0.33	3.33	5.79	0.51	3.95
ED	3.51	5.17	2.98	36.95	0.68	5.07	6.15	3.18	8.60
GY	31.50	120.82	239.71	72.77	0.30	7.71	15.10	5.90	8.11
100-KW	3.69	6.46	6.88	25.00	0.57	7.69	10.16	2.99	11.97

DMF, Days to 50% male flowering; DFF, days to 50% female flowering; PH, plant height (cm), EH, ear height (cm); LL, length of leaf (cm); WL, width of leaf (cm); PBN, panicle branch number; PL, panicle length (cm); LPP, length of panicle peduncle (cm); DPR, distance of panicle ramification (cm); HEW, harvest ear weight (g); KNR, kernel number per row; RN, row number; EL, ear length (cm); ED, ear diameter (mm); GY, grain yield (g) and 100-KW,100 kernels weight (g); CV, coefficient de variation (%); Vg: genotypic variance, Vp: phenotypic variance, x: general mean, GA: genetic advance, GG: genetic gains, GCV: genotypic coefficient of variation, PCV: phenotypic coefficient of variation, H^2: broad sense heritability

Broad-sense heritability and genetic gain of traits

According to specific treatment, huge variations were observed between heritability and genetic gains in the studied traits. Descriptive analysis exhibited 0.10 as the minimum for P0 (Table 7), and 0.07 in P1 and P2(Tables 8 and 9) while the maximum values were 0.99 in P0 and P1 and 0.95 for P2.The genetic gains ranged from 2.46 to 56.08 in P0, 3.34 to 51.02 in P1, and 1.17 to 43.85 in P2. Heritability and genetic gain evaluated in mean percentage exhibited minimum values0.10 and 2.46% at the P0 level for the WL against0.07 and 3.34% at the P1 level for the same trait. Heritability and genetic gain values were estimated at 0.07 and 1.17% for PL at P2. For these parameters, only harvest ear weight exhibited the maximum value along with the three treatments (Tables 7, 8, and 9).Traits such as grain yield (GY), plant height (PH), harvest ear weight (HEW), kernel number per row (KNR), ear diameter (ED), 100-kernels weight (100-KW), days at 50% male flowering, and days at 50% female flowering showed high values of heritability with slight variations between treatments. To better understand the contribution of the genetic gains to yield increases, their values were split into three groups. Its value was higher than 20% for PBN, HEW, GY, and 100-KW, along with all treatments. This gain varied between 10% and 20% for some traits such as plant height (PH), distance of panicle ramification (DPR), and ear diameter (ED), while being less than 10% for certain morphological traits like flowering date, length and width of leaf, and panicle length.

Identification of the best genotypes

A combination of different genetic parameters, including the genotypic coefficient of variation, heritability, and genetic gain, was used to select the best-performing genotypes. The harvested ear

weight and grain yield per plant with high values for those genetic parameters are characteristics used to select the best-performing genotypes in each treatment.

With a selection index applied at 5% to the total assessed genotype, eight genotypes could be selected per treatment, ie VA13 (104.21 g), VA97 (102.16 g), VA105 (98.31 g), VA99 (97.09g), VA103 (94.62g), VA60 (93.96g), VA22 (92.37g), and VA98 (91.28g) at P0; VA8 (119.26 g), VA37 (115.59 g), VA38 (114.87 g), VA134 (114.59 g), VA40 (114.49 g), VA36 (113.67 g), VA100 (106.42 g), and VA76 (101.12 g) at P1; and VA29 (145.73 g), VA100 (137.13 g), VA104 (121.01 g), VA108 (119.17 g), VA79 (118.33 g), VA93 (118.24 g), VA131 (117.34 g), and VA76 (113,51 g) at P2.

SSR marker variation among maize genotypes

In Figure 1, sample 1 exhibited a profile identical to that of the tolerant T1 accession, which implies the presence of a phosphorus deficiency tolerance gene at this locus. No tolerant check genotype profile for phosphorus deficiency tolerance was identified with markers umc1298 (chromosome 1), umc1587, umc1264, and umc1792 (chromosome 5). Based on the markers used, 0.75% to 14.93% of the screened accessions exhibited a tolerance to phosphorus deficiency.

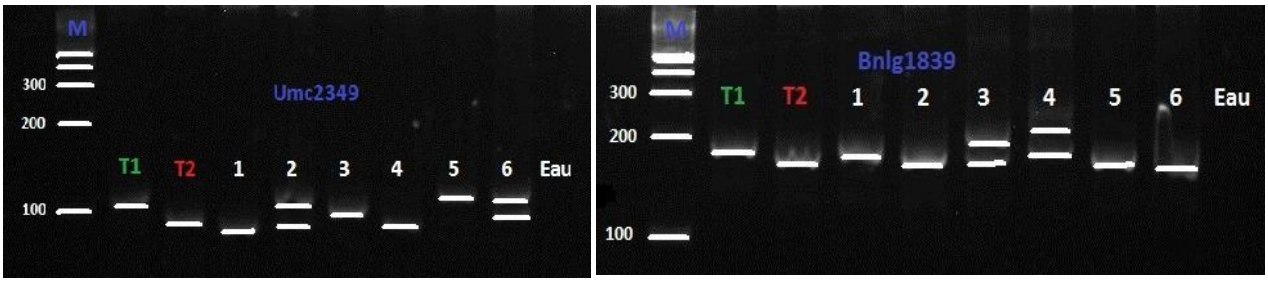

Figure 1: Profiles of band obtained with two used markers

M: Marker of molecular weight 100bp; T1: tolerate check (Mo17); T2: sensitive check (B73); 1 - 6: samples from local maize collection; Eau: negative control (water).

Genotypic identification using similar profile of tolerance in phosphorus deficiency

Grain yield

According to Bonou-gbo et al. (2017), the grain yield after harvest is the second selection criterion, and its high expression in genotypes was estimated using the four microsatellite markers above mentioned. Two of the markers (Umc1298 and Umc1792) did not reveal any allele of tolerance. Therefore, the maize accession (VA26) was identified using the marker Umc1166 as tolerant. The marker Bnlg1331 allowed for the selection of 20 tolerant accessions, including VA26. This variety possessed two alleles of tolerance and could be used as a donor of grain yield QTL in Benin maize breeding. These 20 phosphorus deficiency tolerant genotypes are cultivated in five agro-ecological zones in Benin. The genotypes can be summarized as follows: VA26, VA27, VA28, VA33, and VA35 in AEZIII; VA2, VA8, VA12, VA13, VA14, VA17, VA18, VA19, and VA20 in AEZIV. In AEZV, genotypes VA5, VA6, VA64, and VA107 were identified, and VA134and VA84 were from AEZVII and AEZVIII, respectively.

Kernels and row number of maize grain

SSR markers were also used to assess the row number (RN) and kernel number (KN). Only three genotypes were identified as possessing the KN allele and being associated with three markers out of the four tested. The genotypes identified and their zones are VA3 in AEZV for the marker Phi059; VA13 in AEZIV for the marker

Bnlg1360; and VA55 in AEZII for the marker Umc2349. On the allele of the row number (RN), three markers allowed the identification of nine genotypes. Among those genotypes, seven had the allele of marker Umc1155. The remaining genotypes carried the allele of the marker Bnlg1429 or that of the marker Bnlg1839. These nine genotypes and their agro-ecological zones are summarized as follows: AEZIV (VA15, VA16, VA17, VA18, and VA20) and AEZV (VA3, VA5, and VA6). This result shows that genetic information on accession VA3 points out its best filling of grain on the ear.

Ear length and diameter

The previous allelic marker, Bnlg1360 used to identify the kernel number was also linked to ear diameter. The second marker Bnlg1429, which had been used to identify VA3, already possessed the allele with a high expression of row number. According to these last results, VA3 possesses three alleles linked to three yield components.

Hundred kernels weight (100-KW)

Kernel weight is prominent in determining the yield of maize. The three molecular markers Umc1587, Umc1792, and Umc1166 were used to investigate the genotypes with high expression of 100 kernel weight. Only the allele of marker Umc1166 also used for grain yield allowed selection of accession VA26 in AEZIII. This accession is then the only one with an allele linked to the expression of high kernel weight among the 134 screened but also identified for high expression of grain yield.

Discussion

The aim was to select, based on agro-morphological traits, the genotypes that show good agronomic performance and have a high yield under phosphorus deficiency conditions using SSR markers. As described by Wopereis et al., (2008), the average values of available phosphorus content were recorded in soil and were below 25 ppm. The checks Mo17 and B73 used in this study were well known and tested by researchers as parents or checks in maize breeding for phosphorus deficiency (Fan et al., 2007; Kaeppler et al., 2000; Trachsel et al., 2011; Zhu et al., 2005, 2006). The soil acidity and particularly its phosphorus deficiency imply a highly significant difference between local genotypes for most of the agro-morphological traits evaluated in this study, which suggests the presence of a high level of genetic diversity among them. Considering that fewer molecular studies leading to the identification of major genes linked to tolerance of phosphorus deficiency in maize had been completed, the SSR markers used were mainly selected based on research carried out during the mapping, QTLs for grain yield and its components in different localities (Li et al., 2010). Maize is produced in all agro-ecological zones in Benin Republic and prefers soil with a light structure, deep and easy to cultivate. For a suitable adaptation of improved crops to a specific agro-climatic condition, knowledge of gene expression is paramount. Studies conducted by the Bayuelo-Jiménez team on the effects of low and high phosphorus applications on traits related to maize roots revealed a genotypic difference between accessions (Bayuelo-Jiménez et al., 2011). The lack of phosphorus application would have led to a decrease in reproductive traits except days to 50% female flowering (FLF) and the length of panicle peduncle (LPP), for which an increase was noted. That was the case for the plant height reported for most crops by Balemi, (2009). This result could be due to other soil factors that affect the availability of phosphorus, including soil acidity (pH <7), which limits the availability and solubility of phosphorus. The strong genotypic and phenotypic correlation between the yield and its components implies that an improvement in one of those traits could enhance the yield. Our finding is consistent with that of Aslam-Khan et al. (2005), who also noted that the variation in different yield components affects maize grain yield. For other authors, lower phenotypic correlation could result from a change in the environmental effect in association with traits at the genetic level. In this context, selection for traits with a significant positive genotypic and phenotypic correlation would be very useful in indirect and direct selection for grain yield (Alake

et al., 2008). Therefore, a realistic selection cannot be only focused on the analysis of the coefficients of correlation (Abuali et al., 2014), since most traits (such as yield) are complex in transmission and are controlled by multiple genes. This fact also interacts with various environmental conditions. The evaluation of phenotypic and genotypic coefficients of variation was not only useful to compare the relative quantity of phenotypic and genotypic variation between different traits but also to estimate the probabilities of success through breeding (Abuali et al., 2014; Ahsan et al., 2015; Fayeun et al., 2012; Ghosh et al., 2014; Jat et al., 2014). For the second purpose, three groups of traits were identified according to Singh et al., (2011). For other researchers, a selection could be made with regards to previous traits to identify the most promising genotypes from each treatment (Ahsan et al., 2015; Ghosh et al., 2014). It is obvious that traits such as ear diameter, ear length, and row number have a low genotypic and phenotypic coefficients of variation, which will not impact the selection. Similar conclusions were reported by others in cucumber (Arunkumar et al., 2011; Jat et al., 2014; Veena et al., 2012) and rice (Singh et al., 2011). In order to reinforce this conclusion, the measure of broad-sense heritability according to Jat et al. (2014) gives details on the proportion of variability related to the genetic difference. According to Ghosh et al. (2014), the genetic gain evaluation is also helpful in understanding the role of different genes in expression of the different polygenic characters. For many authors, broad-sense heritability plays a large role in the relative value of selection. Then, efficient selection that combines strong heritability with high genetic gain is mandatory in the identification of reliable traits of genotype selection (Abuali et al., 2014; Ahsan et al., 2015; Ghosh et al., 2014; Johnson et al., 1955; Mahmood et al., 2004; Vashistha et al., 2013). As a result of genetic gain, three groups could be identified from the heritability of evaluated traits (Bello et al., 2012; Johnson et al., 1955). According to those researchers, except for the length of leaf and the panicle peduncle length, which showed a low heritability (<30%), the days to 50% female flowering, the length of leaf, and the ear length showed a moderate heritability (30% ≤ H2 < 60%), and all remaining characters exhibited a high heritability (H2 ≥ 60%) in P0. According to Abuali et al. (2014), traits with strong heritability are strong genetic control and less influenced by environmental factors. In the same treatment P0, genetic gains were moderate (10% ≤ GG < 20%) in ear height, distance between panicle ramifications, row number, and ear diameter. Heritability was high (GG > 20%) in panicle branch number, harvest ear weight, grain yield, and 100 kernel weights and low (GG <10%) for the other 8 characters included in the study. The high and low values of the genetic gain point out the effects of additive genes, and non-additive genes according to Ghosh et al. (2014). Many characters showed high heritability and high genetic gains in trials where phosphorus was applied. Results showed that the treatment, grain yield, and harvest ear weight have strong value for both genetic gains and heritability. Early research reported results during studies on the genetic variability of different plant species (Abuali et al., 2014; Ahsan et al., 2015; Fayeun et al., 2012; Ghosh et al., 2014; Ogunniyan and Olakojo, 2014). Plant characteristics with high heritability linked with high, moderate, or low genetic gain are governed by the action of additive genes, additive and non-additive genes with equal contributions, and non-additive genes, as reported by several researchers in maize (Bello et al., 2012; Ghosh et al., 2014; Ogunniyan and Olakojo, 2014; Sumathi et al., 2005), sweet potato (Shelby, 2000), and chilli (Ojo and Amanze, 2001).

Other studies on genetic variability in maize (Abuali et al., 2014; Ghosh et al., 2014) and in pepper (Sahao et al., 1990) revealed that a character with a high genotypic coefficient of variation associated with a high heritability and a high genetic gain could be used to improve plant productivity under stress conditions. The harvested ear weight and grain yield per plant are characteristics fulfilling those aforementioned conditions. These traits could be used to select the best performing genotypes for each treatment.

The grain yield under P deficiency depends on the amount of P taken up, and the efficient internal use of P dry matter, and subsequent grain production (Wissuwa and Ae, 2001). Often limited by low phosphorus (P) availability, there is a need in agricultural production to develop plants that are more efficient on low P soils (Richardson et al. 2011). Molecular markers are used to more accurately assess the genetic variability of varieties. Those SSR markers used in this study were chosen according to Li et al., (2010) for their codominant action and the stability of their expression in different environments. High variability was also noticed using these markers, which allows a specific selection according to each assessed agronomic character. The most relevant result of this molecular screening is that a small number of maize genotypes screened possess diverse tolerance genes compared to the used check Mo17 revealed by Zhu et al., (2005, 2006). Comparing agro-morphological and SSR marker analyses, the genotype VA13 is only high-performing one identified in P0 with the phosphorus deficiency tolerance genes linked specifically to grain yield, ear length, and ear diameter. Beside this genotype, two others (VA8 and VA134) had been identified in P1 at 50 kg/ha of phosphorus level, and the identification of genes associated with grain yield according to the SSR markers assisted screening. Our findings will help in the search for probable genes or QTLs that contribute to maize tolerance in Benin's phosphorus deficient soils. It might be important to choose other markers linked to this stress to completely cover the plant genome. As noticed by Barcaccia et al., (2003), molecular markers enabled us to ascertain the level of phosphorus deficiency tolerance in the landrace populations maintained by farmers, who mostly select plants by observing morphological traits. Molecular markers better showed the degree of genetic differentiation between the different assessed genotypes.

Conclusion

Infertility in agricultural soils is nowadays noticed in Benin's different maize agro-ecological production zones. This study allows us to identify and select, among the collection of existing genotypes, those that exhibited better performance in phosphorus deficiency soils and possessed at least one tolerance gene to phosphorus deficiency after the SSR analysis. The genetic improvement of complex traits such as grain yield can be achieved through different components involved in increasing yield. During the molecular and agro-morphological characterization, the ear length, the ear diameter, row number, kernel number per row, and 100 kernel weight are very important for an indirect improvement of grain yield under soil phosphorus deficiency conditions. To confirm the tolerance level of the genotypes identified in this study, other field trials could be implemented in each agro-ecological zone under soil phosphorus deficiency conditions supported by a large number of molecular markers well distributed on 10 pairs of maize chromosomes.

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Authors contribution

Bonou-Gbo Zaki, Djedatin Gustave and Dansi Alexandre contribute to the study conceptualization and designed the experiments. Bonou-Gbo Zaki, Sangare Jean Rodrigue, Faton M. Oscar Euloge and Djedatin Gustave contributed to the statistical analysis. Bonou-Gbo Zaki, wrote the manuscript. Djedatin Gustave and Dansi Alexandre supervised the entire study. Faton M. Oscar Euloge, Sow Mounirou, Joseph Adomako and Bocco Roland reviewed and edited the manuscript. All authors contributed to the manuscript.

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Declarations

Conflict of interest

The authors declare that they have no conflict of interest.

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