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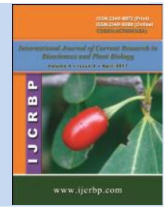

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Screening of 134 Maize Genotypes Collected in Benin for Tolerance to Phosphorus Deficiency

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Abstract

Maize (*Zea mays* L.) constitutes the basis of food security in the poorest zones of Africa. One of the limiting factors of its production is soil phosphorus deficiency. In this study, 134 maize genotypes collected in different localities of Benin were assessed with three phosphorus doses (0, 50 and 100 kg/ha) along with known tolerant (Mo17) and susceptible (B73) checks using 17 agromorphological traits. The selected traits showed heritability between 0.33 and 0.81 hence indicating that observed phenotypic differences are strongly linked to gene expression and therefore a selection can be made on the basis of those traits. Furthermore, the phosphorus application led to significant increase of traits apart from the width of the leaf. Genotype × treatment interaction was significant only for yield and its components. With 0 and 100 kg/ha of phosphorus, no resistant (grouped with Mo17) accessions were obtained while with 50 kg/ha 15 were identified and selected. The dose of 100 kg/ha showed that the accessions' tolerance is not at the same level with the one of the check Mo17. Finally, when comparing treatment 0 kg/ha to the one of 100 kg/ha, all the accessions are closer to B73 than Mo17 for the traits used. The study revealed that for the tolerant genotypes identified, dose of 50 kg/ha is sufficient for a maximum grain yield.

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Keywords

Maize genotypes
Phosphorus deficiency
Screening
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Introduction

With the growth of world population, it is imperative that the scientific community uses all the available ways to allow the producers to have an increase in necessary food resource (Park et al., 2015). Maize (*Zea mays* L.), is an important agricultural resource used not only as a source for human and animal food but also serves as raw material in certain industries (Boone et al., 2008; Xu et al., 2009; Shiferaw et al., 2011; N'da et al., 2014; Park et al., 2015). It establishes the base of the food security in poorest regions in Africa, Asia and Latin America, where the yields are often extremely low with, an average, nearly 1.5 tons/ha (Shiferaw et al., 2011). The global maize production was 843 million tons in 2013-2014 against 860 million tons in 2011-2012 (Planetoscope, 2014). In Benin, maize is produced in all agro-ecological zones and plays an important role food security and income generation.

Maize yields in pluvial zones with poor productivity are severely limited by a set of biotic and abiotic factors among with, aluminum, manganese, calcium, magnesium, molybdenum and iron toxicity decrease in the soil fertility, genotypes low adaptability in climates, rains irregularity, climate change, diseases, soil acidity (Mapiemfu-Lamaré et al., 2011) and particularly phosphorus deficiency (Mafouasson et al., 2006; Shiferaw et al., 2011). Phosphorus (P), key nutrient for crop production, plays an important role in several processes of plant development (Lambers et al., 2008; Ulrich and Schnug, 2013) including photosynthesis, glycolysis, metabolism of the carbohydrate, activation and/or inactivation of enzymes, redox reactions and nitrogen fixation (Vance et al., 2003). It is also involved in the production of energy, nucleic acids synthesis, cellular membrane constitution (Rausch and Bucher, 2002; Vance et al., 2003; Brunel-Muguet et al., 2014). This nutrient is so unavailable to the plant unless it is hydrolyzed to release the inorganic phosphate (Wang et al., 2010). It is one of the fertility components, the most important for crop growth in acid soil (Parentoni et al., 2010; Vance et al., 2003) and its deficiency is considered as a major limiting factor for crop productivity in tropical regions (Ramaekers et al., 2010; Wang et al., 2010). Soil acidity can cause about 67% yield loss in maize (Mapiemfu-Lamaré et al., 2011) and soil phosphorus deficiency can causes up to 50% yield losses. The use of maize genotypes tolerant to soil phosphorus deficiency is indeed an alternative to increase the yields on soils deficient in phosphorus. For most agronomic traits, phosphorus deficiency in plants has continuous phenotypic distributions, justifying that the characters are controlled by polygenes or quantitative trait loci (QTL) (Li et al., 2005). In addition to the study of the QTLs controlling the plant height and ear height (Cai et al., 2012) in case of nitrogen and phosphorus supply, several QTLs leading to the expression of yield and its components have been mapped in maize (Li et al., 2010).

The objective of this study is two folds:

- Assess the maize genotypes produced in Benin for tolerance to soil phosphorus deficiency.
- Test tolerant genotypes for different phosphorus doses to be applied to the soil to identify the requirement for a maximum grain yield.

Materials and methods

Plant material

The plant material is constituted of 136 maize genotypes among which 134 collected during survey in different agro-ecological zones (AEZ) of Benin and two checks(tolerant Mo17 and susceptible B73) obtained from CIMMYT (International Maize and Wheat Improvement Center) in Mexico.

Study area and experimental design

The study was conducted at IITA station located at Abomey-Calavi district in southern Benin. The climate is of Soudano-Guinean type characterized by two rainy seasons with an average rainfall oscillating between 800 and 1200 mm/year.

The experiment was established on a split-plot design with 3 replicates including 3 phosphorus levels (0, 50, and 100 kg/ha) respectively named P0, P1 and P2 and 136 genotypes (2 checks plus 134 recently collected). The whole plot factor was phosphorus level, the subplot factor was genotype and the replicate was time. A treatment against insects' attack and soil nematodes was made with carbofuran®. Each treatment was separated by a distance of 1.75 m whereas 3 m was kept between replication. Each genotype was sowed on a line of 3 m with 0.20 m between holes and 0.5 m between elementary plots. Three seeds were sown per hole and two weeks after sowing, seedlings were thinned to 1 plant/hole to obtain 15 plants per elementary plot. Seventy two hours after the plants thinning, nutrients such as nitrogen (N), phosphorus (P), and potassium (K) were apply through fertilizers urea, triple superphosphate (TSP) and potassium chloride (KCl) respectively according to treatments (+/-). The applications of N and K are uniform for all the replications and is respectively 180 and 63 kg/ha according to Li et al. (2010). The plant insect attack was treated with furandan®.

Soil collection

The top soil (0-20 cm) was collected and 15 g was put into each nylon bag. The top soil was also collected randomly from the field and taken to the laboratory for physiochemical analysis. Available phosphorus and total nitrogen have been determined respectively according to Bray1 and micro-Kjeldahl method

(Anderson and Ingram, 1993). The exchangeable bases (K^+, Mg^{2+}, 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 report soil/water of 1:2.5 while the pH KCl has been identified by the same method after addition of KCl in soil/water solution.

Data collection and analysis

The data was taken on a total of 5 randomly selected plants per accession in each treatment of replication making a total of 45 plants per genotype. Seventeen traits (10 reproductive and 7 post-harvest) selected among maize descriptors (IBPGR, 1991) were scored. Reproductive 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 branch (PBN), distance of panicle ramification (DPR). Post-harvest traits included harvest ear weight (HEW), ear length (EL), ear diameter (ED), row number (RN), number of kernel per row (KNR), grain yield (GY) and 100 kernels weight (100-KW).

The recorded data were firstly submitted to descriptive analysis. The mean, minimum, maximum, standard deviations, coefficient of variation and the standard deviation for the set of traits were determined. Morphological evaluation was made by a principal component analysis (PCA) and an ascending hierarchical classification (AHC). The correlations between different traits measured and the estimated genotypes were assessed using Pearson method (Lawrence, 1989). The analysis of variance (ANOVA), genotype × phosphorus interaction and traits heritability was also performed. The XLSTAT software version 2016 and Breeding View version 1.9 (Murray et al., 2014) were used for the various analyses.

Results

Soil analysis

The soil is ferrallitic type characterized by a high acidity [pH (H_2O) of 4.924; pH (KCl) of 4.188] and low organic compound (0.578%). The available phosphorus per plant at this experimental site is 24.796 ppm. Moreover, the concentration of the exchangeable bases K^+, Mg^{2+}, Ca^{2+} are respectively 0.472, 2.189 and 0.569 Cmol/kg of soil.

Variability of the traits recorded

The mean, minimal, maximal, standard deviations and coefficients of variation of traits for the three phosphorus levels are summarized in Table 1. An important gap is obtained between minima and maxima for almost all traits considered in the context of the study. The smallest mean of all traits is obtained for phosphorus level P0 with the exception of days to 50% female flowering (DFF) and length of panicle peduncle (LPP). Thus, phosphorus application led to the increase of these traits.DFF and WL decrease with the application of phosphorus (P). With P1 the coefficient of variation (CV) range from 6.002% (DFF) to 39.638% (WL) hence indicating a large variation between traits with regard to phosphorus level.

Analysis of variance and effects of phosphorus application on different traits

According to the analysis of variance (ANOVA), there was a significant difference between genotype of each trait apart from WL for which the difference recorded was not significant (Table 2). The highest significant difference were obtained with 10 traits which are DFF, PH, EH, HEW, EL, ED, RN, KNR, GY and 100-KW. Furthermore, interaction genotype x phosphorus level shows no significant difference during the reproductive stage. Nevertheless, this difference is highly significant at threshold of 0.0001 with grain yield and its components. An exception was noted with RN which is significant at threshold of 0.01. P deficiency therefore resulted in a negative effect on grain yield and its components which are justified by a loss of grain yield in 25% compared with the treatment that received a maximal P dose (Fig. 1).

Correlation between measured traits in the three phosphorus levels

The importance of the use of these agro-morphological traits is shown by the high heritability (0.33 to 0.81) obtained for each trait. The Pearson correlation between the various traits varies from one treatment to another (Table 3). Thus, for all traits studied, no significant correlation exists between LPP and other reproductive trait with phosphorus levels P0 (Table 3a) and P2 (Table 3c); but it is negatively correlated to DMF with phosphorus level P1 (Table 3b). The highest significant correlations for P0 and P2 are obtained between DMF and DFF for values of 0.848 and 0.833 respectively.

Table 1. Descriptive statistics of the 17 traits considered.

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 of variation (%); SD, Standard deviation; P0, 0kg/ha of P; P1, 50kg/ha of P; P2, 100kg/ha of P; P, phosphorus.

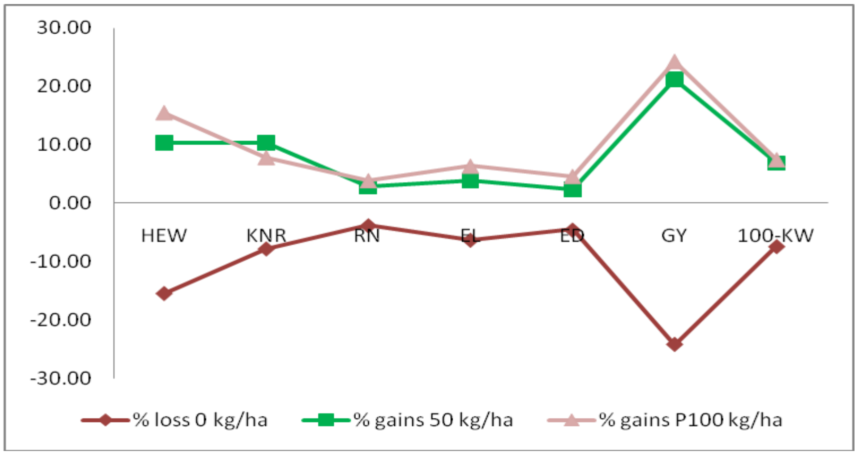

Fig. 1: Effect of phosphorus on yield and its components.

Table 2. ANOVA of the traits recorded.

Source		Model	Genotype	Phosphorus	Genotype × Phosphorus
DF		146	135	2	270
DMF	MS	49.678	51.091	76.973	9.407
	F	5.62***	5.780***	8.707**	1.066ns
DFF	MS	60.642	57.612	373.623	12.030
	F	5.399***	5.129***	33.263***	1.061ns
PH	MS	1444.341	1006.154	13540.020	241.902
	F	6.498***	4.526***	60.913***	0.905ns
EH	MS	906.202	659.244	7938.757	166.767
	F	6.266***	4.558***	54.891***	1.008ns
LL	MS	204.934	157.850	293.918	32.833
	F	5.061***	3.898***	7.259**	0.632ns
WL	MS	5.790	5.521	3.878	4.718
	F	1.241**	1.183ns	0.831ns	1.003ns
PBN	MS	95.031	45.363	3745.014	10.444
	F	10.657**	5.087**	419.987**	1.215ns
PL	MS	41.897	37.302	40.969	12.177
	F	3.280**	2.921**	3.208*	0.877ns
LPP	MS	19.014	14.567	327.194	6.168
	F	2.641**	2.023**	45.441**	0.806ns
DPR	MS	18.911	15.086	283.281	4.652
	F	4.103**	3.273**	61.461**	0.982ns
HEW	MS	3022.487	2716.293	30731.066	1150.237
	F	7.191***	6.463***	73.115***	7.085***
KNR	MS	38.397	29.606	605.750	19.021
	F	4.062***	3.132***	64.088***	2.885***
RN	MS	5.166	4.774	28.473	1.592
	F	4.304***	3.977***	23.722***	1.416**
EL	MS	6.195	4.986	71.181	3.345
	F	2.777***	2.235***	31.903***	1.755***
ED	MS	50.450	47.867	299.720	14.915
	F	5.555***	5.270***	33.000***	2.015***
GY	MS	1576.974	988.997	40633.599	630.689
	F	5.894***	3.697***	151.874***	4.084***
100-KW	MS	57.551	52.782	433.945	22.101
	F	5.672***	5.202***	42.768***	3.528***

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); DF: degree of freedom; MS: mean square; F: value of Fisher; ns: non-significant; * : significant at 0.05 level; ** : significant at 0.01 level; *** highly significant at 0.01 level.

Table 3. Correlations between traits at different phosphorus levels, P0, P1 and P2.

Table 3(a). Correlations between traits at P0 phosphorus level.

Traits	DMF	DFF	PH	EH	LL	WL	PBN	PL	LPP	DPR	HEW	KNR	RN	EL	ED	GY	100-KW
DMF	**1**	**0.848**	**0.382**	**0.377**	**0.323**	0.158	-0.031	**0.332**	-0.084	-0.028	0.115	-0.016	0.007	0.050	0.009	-0.139	-0.012
DFF		**1**	**0.463**	**0.420**	**0.224**	0.121	-0.023	**0.268**	-0.021	-0.077	0.150	0.017	0.008	0.082	0.016	-0.041	0.069
PH			**1**	**0.727**	**0.453**	**0.280**	0.165	**0.241**	0.080	0.177	**0.283**	**0.253**	0.018	0.203	0.066	0.128	**0.237**
EH				**1**	0.190	0.106	**0.330**	0.075	-0.092	**0.249**	0.165	0.164	0.011	0.108	-0.095	-0.047	0.003
LL					**1**	**0.485**	-0.034	**0.506**	0.081	**0.225**	**0.409**	0.171	0.120	**0.302**	**0.394**	0.195	**0.251**
WL						**1**	0.015	**0.378**	0.011	0.192	**0.360**	**0.229**	0.077	**0.328**	**0.356**	**0.294**	**0.271**
PBN							**1**	-0.040	-0.072	**0.536**	-0.101	-0.041	0.023	-0.049	-0.187	-0.151	-0.164
PL								**1**	0.144	**0.311**	**0.258**	0.088	0.144	**0.279**	**0.232**	0.151	0.135
LPP									**1**	0.185	0.059	-0.067	0.052	-0.029	-0.025	0.175	0.032
DPR										**1**	0.109	-0.002	0.148	0.082	-0.009	0.078	-0.009
HEW											**1**	**0.430**	0.202	**0.654**	**0.623**	**0.556**	**0.544**
KNR												**1**	0.157	**0.580**	0.215	**0.354**	**0.269**
RN													**1**	0.171	**0.305**	0.041	-0.053
EL														**1**	**0.445**	**0.490**	**0.427**
ED															**1**	**0.440**	**0.491**
GY																**1**	**0.454**
100-KW																	**1**

Table 3(b). Correlations between traits at P1 phosphorus level.

Traits	DMF	DFF	PH	EH	LL	WL	PBN	PL	LPP	DPR	HEW	KNR	RN	EL	ED	GY	100-KW
DMF	**1**	**0.852**	**0.368**	**0.339**	**0.348**	0.108	-0.083	**0.257**	**-0.234**	-0.033	**0.246**	0.087	0.112	**0.229**	**0.283**	0.178	0.157
DFF		**1**	**0.361**	**0.297**	0.207	-0.027	-0.073	0.201	-0.120	-0.061	0.156	0.046	0.095	0.129	0.200	0.154	0.043
PH			**1**	**0.860**	**0.550**	**0.234**	0.117	**0.308**	-0.046	**0.352**	**0.427**	0.215	-0.137	**0.335**	**0.316**	**0.227**	**0.312**
EH				**1**	**0.357**	**0.231**	0.177	**0.223**	-0.148	**0.388**	**0.324**	0.195	-0.208	**0.251**	**0.222**	0.193	0.196
LL					**1**	**0.400**	0.020	**0.588**	-0.091	**0.335**	**0.529**	**0.291**	0.013	**0.509**	**0.499**	**0.261**	**0.420**
WL						**1**	**0.240**	**0.326**	-0.093	**0.400**	**0.429**	**0.224**	-0.061	**0.436**	**0.372**	**0.234**	**0.333**
PBN							**1**	0.130	0.019	**0.569**	-0.047	0.063	-0.093	0.105	-0.045	-0.069	-0.095
PL								**1**	0.078	**0.397**	**0.513**	**0.347**	0.120	**0.468**	**0.415**	**0.375**	**0.280**
LPP									**1**	0.142	0.012	-0.025	0.009	0.011	-0.036	0.101	0.064
DPR										**1**	**0.231**	0.153	-0.197	**0.276**	0.158	0.162	**0.275**
HEW											**1**	**0.548**	0.102	**0.703**	**0.657**	**0.731**	**0.541**
KNR												**1**	0.056	**0.664**	**0.225**	**0.522**	0.186
RN													**1**	0.064	**0.284**	0.177	-0.137
EL														**1**	**0.397**	**0.517**	**0.359**
ED															**1**	**0.485**	**0.523**
GY																**1**	**0.480**
100-KW																	**1**

Table 3(c). Correlations between traits at P2 phosphorus level.

Traits	DMF	DFF	PH	EH	LL	WL	PBN	PL	LPP	DPR	HEW	KNR	RN	EL	ED	GY	100-KW
DMF	**1**	**0.833**	**0.428**	**0.370**	**0.321**	-0.045	-0.035	0.154	-0.117	-0.098	**0.258**	0.127	0.084	0.181	0.207	0.150	0.186
DFF		**1**	**0.363**	**0.349**	0.154	-0.117	-0.002	0.056	-0.042	-0.135	0.115	0.031	0.061	0.092	0.131	0.107	0.056
PH			**1**	**0.812**	**0.396**	0.086	**0.314**	0.197	-0.025	**0.433**	**0.252**	**0.236**	0.141	**0.249**	0.182	**0.274**	**0.247**
EH				**1**	0.213	-0.127	**0.409**	0.039	-0.095	**0.359**	0.086	0.178	0.135	0.086	0.051	0.083	0.113
LL					**1**	**0.492**	-0.033	**0.552**	-0.139	**0.285**	**0.448**	0.211	0.074	**0.354**	**0.382**	0.137	**0.247**
WL						**1**	0.027	**0.378**	-0.061	**0.261**	**0.319**	0.151	0.061	**0.386**	**0.330**	**0.224**	0.213
PBN							**1**	-0.051	-0.073	**0.592**	-0.163	-0.074	0.085	-0.038	-0.133	-0.159	-0.140
PL								**1**	0.012	**0.375**	**0.320**	0.201	0.103	**0.457**	**0.312**	0.099	0.044
LPP									**1**	0.120	0.017	0.023	-0.047	0.058	0.085	0.090	0.053
DPR										**1**	0.070	0.089	0.085	**0.233**	0.062	0.004	-0.004
HEW											**1**	**0.429**	0.081	**0.683**	**0.487**	**0.647**	**0.587**
KNR												**1**	0.173	**0.430**	0.206	**0.380**	0.218
RN													**1**	0.123	**0.300**	0.151	-0.050
EL														**1**	**0.374**	**0.395**	**0.364**
ED															**1**	**0.350**	**0.432**
GY																**1**	**0.622**
100-KW																	**1**

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); the bold values indicate a significant correlation at the threshold of 0.01.

In P1, the highest value (0.860) was obtained between PH and EH. The lowest correlations values are highly variable from one P application to another. Apart from DMF, DFF, PH, EH, the best significant correlations are essentially obtained between the harvest ear weigh, the grain yield and all other yield components except of row number which is only correlated to ED (Table 3).

Principal component analysis

Five (Table 4a) to six (Tables 4b and 4c) axes having Eigen value of 1 allowed to explain 67.163%, 75.967%, and 74.207% of the variance presents in traits during treatments P0, P1, and P2 respectively. The variance accumulation test for all treatments shows that the first four axes are the most relevant and will be used to describe the total variability with genotypes. The description of each axis is presented in Table 4. For all P level, all components are defined by the positive side of each trait. Traits such as LL, HEW, EL, ED, GY and 100-KW are represented on the first axis for different P level. In addition to these, PH and WL (P0 and P1); KNR (P0); and PL (P1 and P2) are represented on the same axis which describe 26.050%, 32.335%, and 26.575 for P0, P1 and P2 respectively. Axis 2 defined by DMF and DFF at P0 and P1 to which are added DPR (P1); EH in both P1 and P2; and PH and PBN at P2. This axis describes 15.759%, 12.294% and 14.655% in P0, P1 and P2 respectively. Axis 3 of P0 is represented by PBN and DPR, while P1 is only represented by EH. The same traits represented on the axis 2 in P1 are on the axis 3 in P2. One trait (LPP) contributes significantly to the formation of axis 4 describing 8.397% of the total variability in P0. Finally, RN and PBN are on this component at P1 describing 6.991% of total variability. No character is associated with this axis in P2 level.

Table 4. Eigen value and percentage of variation expressed by axes from all traits.

Table 4(a). At P0 level.

Principal component	Axe 1	Axe 2	Axe 3	Axe 4	Axe 5
Eigen value	4.428	2.679	1.751	1.428	1.132
Variability (%)	26.050	15.759	10.300	8.397	6.657
% cumulated	26.050	41.809	52.109	60.507	67.163
DMF	0.119	**0.478**	0.210	0.022	0.013
DFF	0.137	**0.439**	0.226	0.001	0.000
PH	**0.327**	0.307	0.002	0.062	0.056
EH	0.118	**0.454**	0.023	0.195	0.003
LL	**0.455**	0.032	0.001	0.124	0.000
WL	**0.369**	0.000	0.012	0.048	0.000
PBN	0.002	0.148	**0.502**	0.057	0.009
PL	0.285	0.039	0.006	**0.310**	0.002
LPP	0.006	0.004	0.048	0.188	0.296
DPR	0.053	0.056	**0.646**	0.030	0.001
HEW	**0.644**	0.073	0.000	0.015	0.000
KNR	**0.273**	0.046	0.001	0.225	0.021
RN	0.056	0.008	0.041	0.037	**0.488**
EL	**0.511**	0.085	0.001	0.061	0.016
ED	**0.400**	0.178	0.010	0.022	0.051
GY	**0.327**	0.217	0.003	0.009	0.084
100-KW	**0.345**	0.114	0.020	0.022	0.092

Table 4(b). At P1 level.

Principal component	**Axe 1**	**Axe 2**	**Axe 3**	**Axe 4**	**Axe 5**	**Axe 6**
Eigen value	5.497	2.090	2.004	1.189	1.082	1.053
Variability (%)	32.335	12.294	11.787	6.991	6.365	6.194
% cumulated	32.335	44.629	56.417	63.408	69.773	75.967
DMF	0.207	**0.573**	0.022	0.030	0.004	0.000
DFF	0.120	**0.605**	0.028	0.038	0.004	0.047
PH	**0.440**	0.038	0.249	0.049	0.002	0.030
EH	0.318	0.027	**0.359**	0.038	0.031	0.017
LL	**0.551**	0.002	0.009	0.000	0.033	0.034
WL	**0.305**	0.098	0.011	0.007	0.009	0.211
PBN	0.015	0.200	0.268	**0.281**	0.000	0.000
PL	**0.451**	0.009	0.002	0.087	0.044	0.006
LPP	0.002	0.123	0.024	0.004	0.141	**0.580**
DPR	0.213	**0.268**	0.241	0.030	0.038	0.008
HEW	**0.718**	0.006	0.100	0.012	0.008	0.000
KNR	0.318	0.033	0.074	0.024	**0.384**	0.021
RN	0.003	0.051	0.268	**0.302**	0.053	0.000
EL	**0.568**	0.028	0.041	0.019	0.104	0.000
ED	**0.471**	0.006	0.088	0.002	0.131	0.041
GY	**0.435**	0.005	0.197	0.006	0.016	0.049
100-KW	**0.362**	0.018	0.023	0.258	0.079	0.008

Table 4(c). At P2 level.

Principal component	**Axe 1**	**Axe 2**	**Axe 3**	**Axe 4**	**Axe 5**	**Axe 6**
Eigen value	4.518	2.491	2.037	1.441	1.094	1.034
Variability (%)	26.575	14.655	11.982	8.476	6.434	6.085
% cumulated	26.575	41.230	53.212	61.688	68.122	74.207
DMF	0.229	0.152	**0.385**	0.096	0.002	0.013
DFF	0.110	0.193	**0.429**	0.071	0.000	0.045
PH	0.383	**0.389**	0.000	0.036	0.008	0.001
EH	0.165	**0.581**	0.004	0.062	0.000	0.008
LL	**0.449**	0.002	0.037	0.225	0.023	0.006
WL	0.220	0.081	0.199	0.088	0.010	0.023
PBN	0.000	**0.414**	0.213	0.058	0.000	0.012
PL	**0.288**	0.004	0.135	0.237	0.000	0.065
LPP	0.000	0.024	0.006	0.127	0.003	**0.771**
DPR	0.111	0.174	**0.509**	0.017	0.008	0.021
HEW	**0.619**	0.126	0.009	0.017	0.008	0.006
KNR	0.266	0.018	0.000	0.055	0.058	0.004
RN	0.059	0.008	0.002	0.000	**0.823**	0.001
EL	**0.515**	0.057	0.020	0.000	0.000	0.007
ED	**0.381**	0.068	0.001	0.006	0.068	0.013
GY	**0.379**	0.106	0.043	0.205	0.000	0.012
100-KW	**0.344**	0.095	0.044	0.140	0.083	0.027

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).

Analysis of the variability within genotypes in different phosphorus application: Hierarchical ascending classification

At P0 level

The diversity analysis of genotypes by aggregation method based on the Pearson correlation coefficient (similarity) allows structuring the genotypes into 7 classes (Fig. 2). At this level, Mo17 is more distant from other classes including the B73. The one-dimensional test of Wilks carried out on the threshold of 0.01 on the different classes obtained indicated that only seven traits discriminate each class (Table 5). These are the grains yield (GY), and its components (HEW, KNR, ED, EL and 100-KW) except RN. The only character separating at threshold 0.05 is the width of leaf. This test is confirmed by Fisher value which is very high for these traits. The genotypes of classes 5 and 6, have characteristics close to the tolerant check, single genotype in class 1. These classes are the ones having the greatest agronomic performance. A very large significant difference in other discriminating components, especially with 100-KW which is higher with classes 5 and 6 compared with class 1. Furthermore, the class 2 containing the sensitive checks B73 with seven other genotype (VA21, VA63, VA65, VA89, VA109, VA128, and VA129) present most low grains yields (31.53 g/plant). Genotype performance of classes 3, 4 and 7 leads classify in the sensitive class.

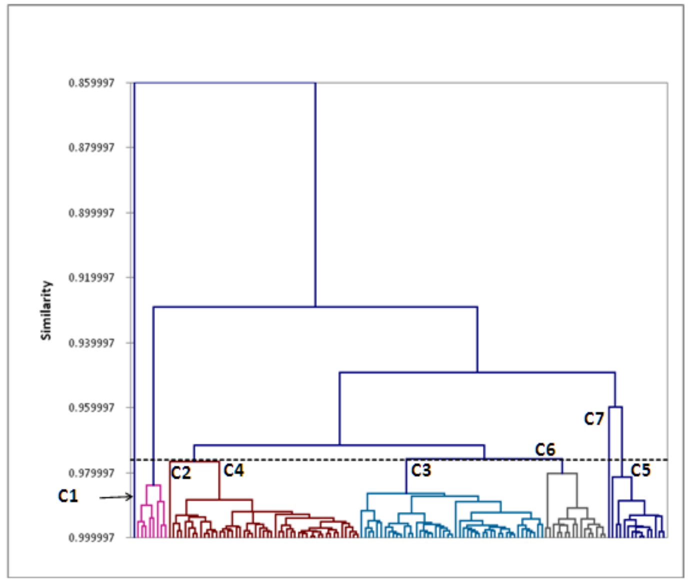

Fig. 2: Hierarchical ascending classification of maize genotypes subjected to P0.

Table 5. Main characteristics of different groups and their significance at P0.

Class	1 (n=1)	2 (n=8)	3 (n=47)	4 (n=49)	5 (n=14)	6 (n=16)	7 (n=1)	Lambda	F	*p*-value
DMF	51.74	58.64	57.09	56.76	55.93	56.57	56.58	0.96	1.39	0.242
DFF	62.59	62.19	62.00	61.61	60.73	61.97	63.25	0.98	0.52	0.723
PH	153.18	151.32	155.56	152.26	152.09	149.89	153.82	0.95	1.61	0.175
EH	74.43	77.14	76.22	74.37	72.27	70.74	80.63	0.93	2.32	0.061
LL	55.01	74.49	78.10	76.87	78.35	77.01	78.73	0.94	1.93	0.109
WL	6.78	7.37	7.83	7.65	7.89	7.85	7.51	0.92	2.75	0.031
PBN	13.64	13.99	13.60	14.02	13.21	12.74	13.71	0.94	2.21	0.072
PL	30.25	32.67	33.38	32.64	33.03	33.54	30.25	0.96	1.20	0.312
LPP	24.08	20.66	20.69	20.58	21.09	20.71	19.40	0.99	0.47	0.757
DPR	10.82	10.23	10.94	10.89	10.97	10.54	9.71	0.97	0.92	0.454
HEW	24.10	53.48	108.69	76.50	138.99	99.52	138.93	0.23	106.03	< 0.0001
KNR	20.40	20.29	22.83	21.35	25.02	22.00	17.87	0.86	5.30	0.001
RN	12.78	12.10	12.94	12.72	12.96	12.45	14.60	0.93	2.31	0.061
EL	10.68	10.96	12.71	11.93	13.63	12.77	12.97	0.66	16.97	< 0.0001
ED	21.84	31.77	37.27	34.81	38.73	37.93	41.27	0.60	21.63	< 0.0001
GY	88.16	31.53	61.05	53.07	85.17	85.81	38.48	0.27	87.65	< 0.0001
100-KW	19.34	19.99	25.19	22.28	26.42	26.00	28.49	0.70	13.92	< 0.0001

At P1 level

With 50 kg/ha P, genotype similarity range from 0.97 and 0.98 and a restructuring of the various classes was noticed by obtaining 6 classes of genotypes (Fig. 3) according to the previously method used. At this level, the Lambda of Wilks test at the threshold of 0.01 shows only four trait such as DMF, DFF, PBN, and LPP don't allow to separate the classes from the HAC (Table 6).

A low value of Fisher comprise between 0.584 (DFF) and 2.526 (PBN) is noticed for these trait. Class 1 consists of 50 genotype including Mo17 differs from other classes except class 5 (1 genotype) with high value in GY (89.404 g/plant), 100-KW (27.081 g), EL (13.695 cm), RN (25.909) and HEW (125.868 g). Thus, class 5 is closer to that class 1 by maximal values to these traits compared with all other classes. The sensitive check B73 is the one genotype in the class 2 with values clearly lower than those obtained of the Mo17 class. Classes 4 and 6 with very low grains yield are furthermore close to the sensitive. The greater class 3 constitute of 82 genotypes, is the intermediary of the class 1 and the class 2 in comparison to their characteristic.

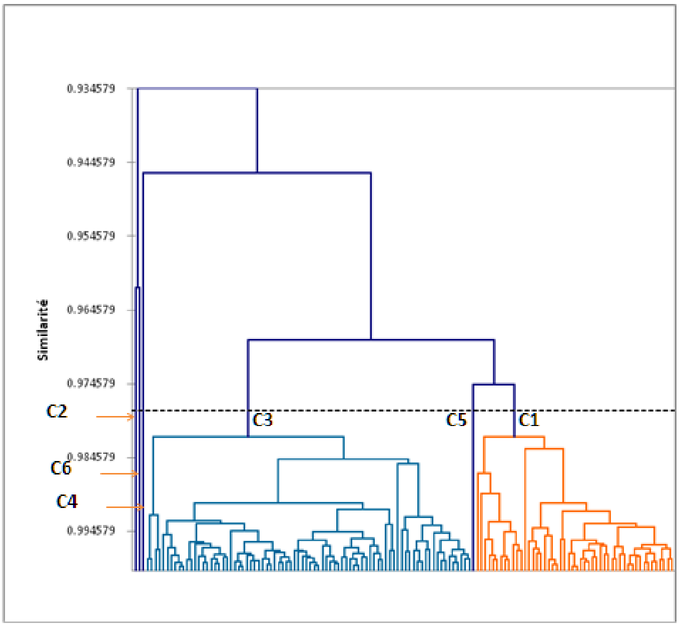

Fig. 3: Hierarchical ascending classification of maize genotypes subjected to P1.

Table 6. Main characteristics of different groups and their significance at P1.

Classe	1 (n=50)	2 (n=1)	3 (n=82)	4 (n=1)	5 (n=1)	6 (n=1)	Lambda	F	*p*-value
DMF	56.367	58	55.949	59.333	57	49.333	0.98	1.334	0.267
DFF	60.295	62.711	59.87	63.408	62.388	55.263	0.991	0.584	0.559
PH	163.11	121.61	161.102	190.722	155.028	131.993	0.863	10.55	< 0.0001
EH	80.79	48.782	79.492	112.218	79.85	38.9	0.793	17.34	< 0.0001
LL	79.606	63.792	77.805	73.504	80.507	67.559	0.865	10.36	< 0.0001
WL	7.875	6.75	7.667	7.653	7.382	6.515	0.875	9.528	0.000
PBN	14.492	14.735	14.899	17.85	14	10.712	0.963	2.526	0.084
PL	34.494	33.323	32.726	29.554	34.03	26.494	0.824	14.2	< 0.0001
LPP	19.741	21.308	19.447	16.949	20.573	18.127	0.99	0.689	0.504
DPR	11.681	5.845	11.589	12.051	11.775	7.02	0.785	18.19	< 0.0001
HEW	125.87	50.89	92.137	73.707	103.899	53.1	0.442	83.99	< 0.0001
KNR	25.909	20.576	23.582	21.549	28.172	23.974	0.817	14.87	< 0.0001
RN	13.294	15.894	13.063	13.15	13.923	14.136	0.899	7.447	0.001
EL	13.695	12.052	12.492	11.3	13.024	10.697	0.741	23.18	< 0.0001
ED	38.486	29.939	36.257	34.393	41.788	30.734	0.68	31.34	< 0.0001
GY	89.404	73.706	64.192	54.676	115.59	45.693	0.433	87.12	< 0.0001
100-KW	27.081	15.094	24.169	25.385	30.842	22.178	0.769	20.01	< 0.0001

At P2 level

Genotypes are subdivided into five classes as indicated in Fig. 4. A characterization of these classes allowed to notice that in addition to discriminant trait in P0, there is the panicle branch number at threshold of 0.05 (Table 7). As at P level P0, Mo17 is alone in its class with upper grains yield of 90.200 g/plant compared to class 2 with lower GY of 24.654 g/plant that is constituted also only B73. All genotype are thus distributed in three remaining classes which have 100-KW upper to that both checks. The classes 3 and 5 with respectively 54 and 74 genotypes have low performance except 100-KW upper than Mo17. Comprise 6 genotypes; the class 4 possesses higher performances compared to Mo17 with GY of 116.708 g/plant and a 100-KW equal to 29.109 g.

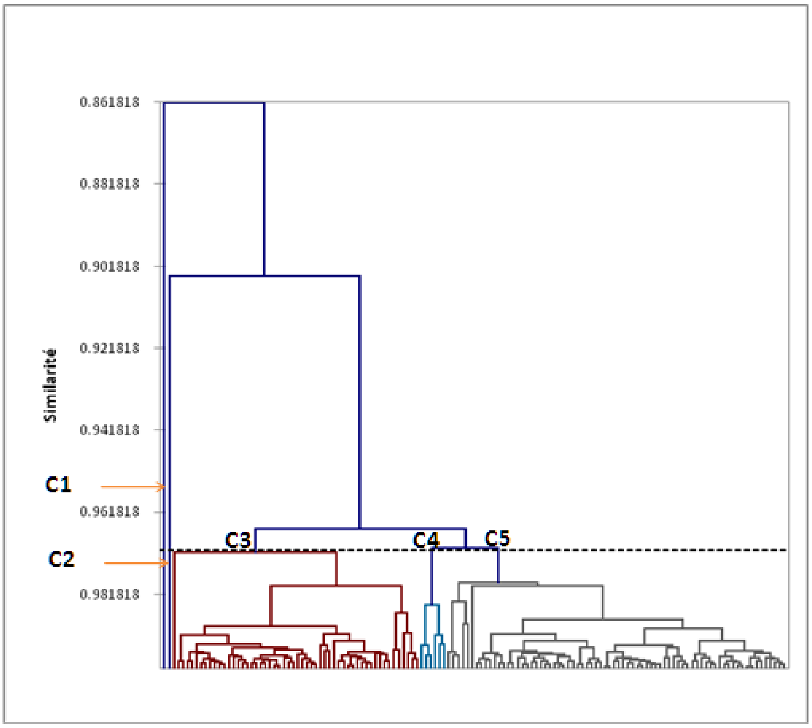

Fig. 4: Hierarchical ascending classification of genotypes subjected to P2.

Table 7. Main characteristics of different groups and their significance at P2.

Class	1 (n=1)	2 (n=1)	3 (n=54)	4 (n=6)	5 (n=74)	Lambda	F	*p*-value
DMF	52.025	57.015	55.743	56.685	56.354	0.986	0.937	0.394
DFF	65.05	60.89	59.71	60.446	60.085	0.995	0.348	0.707
PH	165.02	135.4	165.609	163.249	165.089	0.998	0.152	0.859
EH	83.027	59.295	85.589	80.186	81.919	0.961	2.678	0.072
LL	60.357	67.035	78.089	81.248	79.422	0.963	2.508	0.085
WL	7.173	6.988	7.636	7.926	7.875	0.921	5.593	0.005
PBN	19.309	19.309	20.062	18.105	18.9	0.939	4.288	0.016
PL	31.738	33.443	33.252	35.002	33.47	0.964	2.424	0.092
LPP	22.695	19.905	18.838	19.48	19.04	0.991	0.609	0.546
DPR	12.253	9.461	12.473	12.028	12.146	0.983	1.16	0.317
HEW	25.774	58.309	92.399	161.83	123.84	0.391	102.1	< 0.0001
KNR	20.223	19.904	23.199	25.502	24.444	0.918	5.851	0.004
RN	13.257	13.257	13.232	13.608	13.235	0.996	0.25	0.779
EL	11.167	12.022	12.627	14.57	13.566	0.787	17.75	< 0.0001
ED	37.815	31.278	36.699	38.333	38.589	0.882	8.778	0
GY	90.2	24.654	67.75	116.708	90.034	0.421	90.24	< 0.0001
100-KW	19.558	10.342	24.182	29.109	26.839	0.73	24.18	< 0.0001

Discussion

Effect of phosphorus application on maize growth and yield traits collected during the study

The genetic variability is so essential in varietal selection. The aim of this study is to identify on the basis of the selected agro-morphological traits, genotypes either tolerant or sensitive to P deficiency included in the trial. The crop soil acidity and particular its phosphorus deficiency involves a genetic difference observed between traits collected. A study led by the team of Bayuelo-Jimenez on trait link to maize root had shown a genotypic difference in low or high phosphorus cropping conditions (Bayuelo-Jiménez et al., 2011). Thus, the lack of phosphorus application induces a decrease of reproductive except the days to 50% female flowering and length panicle peduncle for which an increase was noticed. It is the case for instance of plant height reported for most of crop by Balemi (2009). Contrary to certain authors with whom no significant difference is observed by P application, difference observed in this study is significant for almost all of the reproductive traits (Eltelib et al., 2006; Withers et al., 2000). This opposite result could give some explanation by the difference in soil characteristic like pH which differs highly. Furthermore, in spite of the significant difference between these traits, non-significant difference observed between genotype × phosphorus interactions is in compliance with the result obtained by Aslam-Khan and his collaborators who studied several of phosphorus application on maize cultivars in salt condition (Aslam-Khan et al., 2005).

In addition to the genetic variability, the environment effect could have a very important action in the expression of these genotypes for the reproductive traits measured in this work. Moreover, increase of phosphorus level leading a significant increase of grains yield per plant and its components opposite to reproductive variables showing a specific answer of the genotypes to the various P level. Similar to the results after different applications of nitrogen, an increase in grains weight was responsible for high yield (Silva et al., 2005) approved here by the high correlation between these two traits but also with HEW, ED and EL. Maize production is consequently limited by non-adequate P application applied during production (Plénet et al., 2000) and only the tolerant genotypes including the check Mo17 and the genotypes very stable VA39 and VA76 shows a good performance whatever is the variability of phosphorus level. According to Aslam-Khan et al. (2005), an increase of the leaf area due to an application of phosphorus increase the potential yield on most of the important traits contributing to the maize yield. So, while justifying the role of the reproductive trait in the yield improvement, these traits shows no significant difference for three P level and thus cannot be included in the traits occurring in the yield improvement. In addition, the strong correlation between grain yield and its components show that improvements of this component involve yield improvement. This result supports that of Aslam-Khan et al. (2005) which also noted that the variation of yield components have influence on the maize grain yield. Several authors indicated also an increase of the yield by the phosphorus application to the maize (Bayuelo-Jiménez et al., 2011; Li et al., 2010; Miraj et al., 2013), the wheat (Ali et al., 2008), the cowpea (Nkaa et al., 2014) and even the potato (Balemi, 2009). The most important trait identified during this screening are PH, EH, HEW, EL, ED, GY, and 100-KW. It is also important to note that the trait RN non-significant correlated to GY and especially have negative correlation with 100-KW provides no interest in trait choice for selection of efficient genotypes. A comparison of the different answers from phosphorus levels applications allows holding for this study 50 kg/ha of P application for a maximal 100-KW since no significant difference is obtained between P1 and P2. This finding suggests that P0 and P1 are ideal for the screening of these genotypes in the experimental site. However, the P2 level is important to confirm the tolerance level for those genotypes selected in P1 level.

Grouping of genotypes according to their tolerance level in the three phosphorus doses application

Belonging to different classes and distant each other whatever is the P level, the checks Mo17 and B73 allowed to classify the 134 maize genotypes. A high heritability for these traits indicates the recorded phenotypic differences are related to gene expression of genotypes. Significant interaction between genotype and phosphorus for grain yield and its components confirms the use of this traits identified by Wilks test in the grouping of genotypes. Indeed, phosphorus level P0 and P1 by highlighting sensitive and tolerant genotype respectively shows that the experimental site is well characterized by phosphorus deficient trough it strong acidity. Then, only average phosphorus intake allows an increase of performance leading to reorganization of the classes by HAC. Furthermore, considering two groups for these classifications, all genotypes are very closer to sensitive check B73 at P0 while they appear to be with the tolerant check Mo17 at P1 level. The value of applying P2 level is in the confirmation and selection of potentially tolerant genotype to phosphorus deficiency. This last result confirms that all the 134 maize genotypes produce in Benin agro-ecological zones are distant from Mo17 allowing to conclude that those genotypes selected at P1 possess a partial tolerance to P deficiency. Based on yield performance of tolerant check Mo17, it is advisable to retain as tolerant genotype VA2, VA15, VA22 for AEZ4; VA34, VA36 for AEZ3; VA60 for AEZ1; VA76, VA78 for AEZ6 and VA39, VA97, VA103 and VA105 for AEZ5. So the most successful in terms of GY in all P level are VA76 and VA39. Those potentially sensitive are VA63 (AEZ5), VA129 (AEZ6), VA65, VA89, and VA109 (AEZ7) because showing lowest yield regardless of the phosphorus level and compared also to sensitive checks.

Conclusion

This study allowed the identification and selection among the existing collection of the maize genotypes that respond well in phosphorus deficiency conditions. Harvest ear weight (HEW) grain yield (GY), ear length (EL), ear diameter (ED), kernels numbers per row (KNR), row number (RN), and 100 kernels weight (100-KW) were found very important for screening the best genotypes in a breeding program. To complete this study and also identify news QTLs linked to P deficiency, molecular characterization of the best genotypes found using existing markers linked to genes controlling this stress is recommended.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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Appendix – I: Local names of the maize genotypes included in the study.

N°	Code	Local name	N°	Code	Local name	N°	Code	Local name	N°	Code	Local name
1	T1	Mo17	36	VA13	Baiya bépipialéci	71	VA3b	Arana wètchéfounonb	106	VA70	Gbayé souaton
2	T2	B73	37	VA130	Gbadé souwoué adadé1	72	VA4	Pisaback	107	VA71	Massahoué
3	VA1	Manzo tchroitama	38	VA131	Gbadé souwoué adadé2	73	VA40	Bétérou1b	108	VA72	Gbayé souaton ton
4	VA10	Amomboyo ognilassa	39	VA132	Gbogbouin	74	VA41	Mancé monplana (1)	109	VA73	Agbayé souènè
5	VA100	Gbado sassakawa	40	VA133	Taala	75	VA42	Mancé monplana (2)	110	VA74	Goun-koun
6	VA101	Gbado ognibo	41	VA134	Shishi	76	VA43	Mancé tian	111	VA75	Agrique
7	VA102	Ebrydji	42	VA135	grégoire	77	VA44	Mancé mon aon	112	VA76	Gbadé vôvô
8	VA103	Ehrini	43	VA14	Baiya bépipialéci1	78	VA45	Macé tingnan	113	VA77	Gbadé vôvô 1
9	VA104	Kokou rôkpètè	44	VA15	Baiya bépipialéci2	79	VA46	Macé poua	114	VA78	Gbadé vôvô 2
10	VA105	Inconnu	45	VA16	Segyoudi wingnita	80	VA47	Mandésouan	115	VA78b	Gbadé vôvô 2b
11	VA106	Gbado kpikpa	46	VA17	Abadiyo yê	81	VA48	Mandésouan	116	VA79	Lingbo noukoun
12	VA107	DMR	47	VA18	Sègê yoladji (1)	82	VA49	Sourouirou	117	VA8	Amonboyo bankam
13	VA108	Gbado otchoublihoun	48	VA19	Sègê yoladji (2)	83	VA5	Artchéfounon ikinka	118	VA80	Lingbo noukounb
14	VA109	Gouvé	49	VA2	Manzo tchroitama (2)	84	VA50	DMR	119	VA81	Gbadé souaton
15	VA11	Baiya Bélaaci	50	VA20	Ségê yonika	85	VA51	Sourouita(1)	120	VA82	Fonkoun
16	VA110	DMR	51	VA21	Ségê yonika1	86	VA52	Sourouita(2)	121	VA83	Agrique
17	VA111	Enouatin	52	VA22	Ségê yonika2	87	VA53	mandésouan	122	VA84	Agor
18	VA112	Enouatin 2	53	VA23	Manan so kpodé	88	VA54	Coopérative	123	VA85	Kpoléhoun
19	VA113	Bogan	54	VA24	Manan so tèrè	89	VA55	mandé numbertia	124	VA86	Pozarica
20	VA114	Gbogbouin	55	VA25	Gbédé chouannou	90	VA57	Congo	125	VA87	DMR
21	VA115	Gbogbouin2	56	VA26	Gbadénou bacadou	91	VA58	Congo2	126	VA88	QPM
22	VA116	Gbogbouin3	57	VA27	Sèwaga djiomatin	92	VA59	Congo3	127	VA89	Tavèkoun
23	VA117	Kounado	58	VA28	Gbè kpikounou	93	VA6	Artchéfounon fonnon (2)	128	VA9	Amomboyo okano
24	VA118	DMR (Wlétchivékou)	59	VA29	Sourounin	94	VA60	Kotokoli kouaré	129	VA90	Gouvè
25	VA119	Yokouin sahouèton	60	VA3	Arana wètchéfounon	95	VA61	Kotokoli tchèrè	130	VA93	Agbadé ADF
26	VA12	Baiya Bétouinzi	61	VA30	Gbè souannou	96	VA62	Gbadé Agonlikpahoun	131	VA94	Agbadé carder
27	VA120	Yokouin sahouèton2	62	VA31	Gbè kpika	97	VA63	Gbadé Tchankpo	132	VA95	Ikpetchi
28	VA121	DMR	63	VA32	Yayi boni (1)	98	VA64	Gbadé souwoué adadé	133	VA96	Otchoukpamè
29	VA122	Awouévi	64	VA33	Yayi boni (2)	99	VA65	Ohouya	134	VA97	Otchoukpamèta
30	VA123	Akpayibô	65	VA34	Inconnu (3)	100	VA66	Tchankpo	135	VA98	QPM faaba
31	VA125	Djongbô	66	VA35	Sourouigou	101	VA67	Gbadé vè1	136	VA99	QPM
32	VA126	Inconnu (1)	67	VA36	Gbérénou	102	VA67b	Gbadé vè1b			
33	VA127	Carder bli héé	68	VA37	Sourounèmè	103	VA68	Gbadé vè2			
34	VA128	DMR	69	VA38	Alafiarou1	104	VA69	Gbayé-Koungli			
35	VA129	Gbadé souwoué adadé	70	VA39	Bétérou1	105	VA7	Paaza cri ita			