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Responses of Nodulation and Grain Yield to Fertilization with Phosphorus (P) of Outstanding Low P Tolerant *Phaseolus vulgaris* L. Genotypes in Highly Acidic and Phosphorus Deficient Soils Such of Rwanda

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Authors' contributions

This work was carried out in collaboration between both authors. Author JG designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author ON managed the literature searches and data analyses of the study. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Promoting the use of low phosphorus (P) and acidity tolerant bean genotypes in highly acidic and low P soils is a long term cost-effective strategy to increase bean production in sub-Saharan countries. Moreover, the performance of beans under the widespread soil related stresses like acidic and P deficient is not well documented. Thus, pot experiments were established in greenhouses in Rubona, Rwanda. Each pot was filled with 5 kg of highly acidic and P deficient soil collected from Nyamagabe district. The objective of the experiment was to identify superior grain yielding and nodulating bean genotypes suitable for such acidic and P deficient soils and their response to P inputs. Four low P tolerant genotypes used (G 2858, RWR 1873, RWV 1668; and

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RWV 1348) and 59/1-2 (local check) were subjected to three levels of P (0, 5, 10 mg kg^{-1}) and treatments were laid in completely randomized design with 6 replications. The climbing genotype RWV 1348 had the highest grain yield (22.9 g pot^{-1}), followed by two bush genotypes RWR 1873 and RWV 1668 with the same grain yield of 14.7 g pot^{-1}, while the local check (climbing) and GR 2548 (climbing) had low grain yield. Nodulation efficiency was 73, 54, 50% for RWR 1873, RWV 1348 and RWV 1668 respectively. Again, the local check and GR2548 had low effective nodulation (19 and 31% respectively). The input of P at 5 mg kg^{-1} soil increased grain yield of RWV 1348, RWR 1873 and RWV 1668 at 17, 21, and 33% respectively. On the other hand, doubling this rate has declined the yield of all genotypes. Genotype RWV 1348, a climber, is the best outstanding low P tolerant bean genotype under the highly acidic and low P deficient soils such of Rwanda and have a slight grain yield response when P fertilizer is applied. The two-bush bean genotype RWV 1873 and RWV 1688 are also outstanding low P tolerant bean genotypes compared to a climbing local check (59/1-2) and other low P tolerant climber (G2858). The genotypes RWV 1873 and RWV 1688 grain yield response to P fertilizer application has the same pattern as of RWV 1348. They are all outstanding low P tolerant genotypes and their nodulation efficiency is not responsive at P fertilizer application.

Keywords: Acidic and phosphorus deficient soils; nodulation response; low phosphorus tolerant bean genotypes; yield.

ABBREVIATIONS

ATP	:	*Adenosine Triphosphate*
AGRA	:	*Alliance for Green Revolution in Africa*
DAE	:	*Days after Emergency*
ECSA	:	*East, Central and South Africa Countries*
EN	:	*Effective Nodules*
IITA	:	*International Institute of Tropical Agriculture*
L	:	*Liter*
LPT	:	*Low Phosphorus Tolerant*
LSD	:	*Least Significant Difference*
RAB	:	*Rwanda Agriculture Board*
CRD	:	*Completely Randomized Design*
SSA	:	*Sub-Sahara Africa*
TSP :	:	*Triple Superphosphate*

1. INTRODUCTION

Bean (*Phaseolus vulgaris* L.) is important food legume for direct human consumption as cheap source proteins, and profitable source of income for populations in Sub-Saharan Africa (SSA) [1, 2]. However, bean yield has declined drastically over the past 10 years due to low soil fertility, which is estimated to be responsible for over 2.12 million tons of unrealized bean yield every year in SSA [3,4].

Phosphorus had been singled out as a limiting factor for bean production [5,6] and phosphorus deficiency is 65 to 80% in soils due to inherently low P content, its fixation by oxides of aluminium and iron in acidic soils and soil erosion [4]. In SSA, average bean yield is estimated to about 678 kg ha^{-1} and this yield is far inferior to the potential yield which is estimated at 2.5 t ha^{-1} for bush bean [7] and 4 t ha^{-1} for climbing beans [2].

In most African countries, bean is mainly produced by small-scale farmers with little capacity to use chemical fertilizers to replenish soil fertility and adopt the use of recycled organic material as P source, which unfortunately, cannot guarantee increases in bean production [4,8]. However, there are bean genotypes developed to grow reasonably well under low soil P conditions. Moreover, there is lack of information on their performance in highly acidic and P deficient soils such as those of Rwanda (pH= 3.5 - 5.1; P= 0- 5 ppm), where the crop is of the most importance, with per capita consumption in the range of 50-60 kg per year [1]. The identification of high yielding low P tolerant bean genotypes and evaluation of their performance under modest to high P levels offers an opportunity to enhance bean production on such otherwise poor soils.

The study aimed at determining superior outstanding low P tolerant bean (LPT) genotypes with high grain yield and nodulation efficiency in highly acidic and low P soil conditions such of Rwanda.

2. MATERIALS AND METHODS

A greenhouse experiment was conducted at Rwanda Agriculture Board- Rubona research site during November 2011 and July 2012. Soil was collected from Ruhunga cell, Kibirizi sector of Nyamagabe district located in Crete Congo Nile agro-ecological zone [9]. The soil used is a member of MUNINI series which is characterized as a 'Clayey-skeletal, kaolinitic, isothermic Ultic Tropudalfs' family [10]. The series comprises highly developed and altered soils originating from granitic rock, clay soil mixed with some quartz's, which is yellow in color and well drained. The depths of these soils range between 50 and 100 m, developed under isothermic–udic pedoclimatic regime [10]. Specific biophysical and chemical characteristic of soil used in pot is presented in Table 1.

2.1 Bean Genotypes Characterization

Four low LPT bean genotypes (RWR 1873, RWV 1343, RWV 1668, G 2858) and one local check (59/1-2) (Table 2) were screened for relative performance (yield and nodulation efficiency) on highly acidic and phosphorus deficient soil such of Rwanda.

2.2 Soil Sampling

Soil samples for pre-study characterization were collected randomly in a field at Ruhunga, in Nyamagabe district of Rwanda, from 0 to 20 cm depth, using a soil auger. The field had been subjected to cultivation for a long time (more than 20 years), but without fertilizer use for a period of one year.

The sub-samples were bulked into a plastic basin for quarter sampling. Approximately 500 g of the thoroughly mixed composite sample was taken into a polythene bag and labeled. Three such composite samples were taken from the same field. Concurrently, six other core samples were collected for bulk density determination.

2.3 Experimental Design

The experiment consisted in evaluating the genotypes for grain yielding capacity under the highly acidic and P deficient soil conditions and then the outstanding grain yielding and effective nodulating bean genotypes were subjected to different levels of P (0, 5, 10 mg kg^{-1}) into completely randomized design (CRD) with 6 replications. In this experiment, 5 kg of soil were filled in pots of 6 litre-capacity; after perforating the pots in the sides and bottoms to help in aeration and drainage of excess water. Drained leached soil solutions during watering were trapped using pot covers laid underneath the pots and poured back into the respective pots. Six seeds were planted per pot at a depth of about 3 cm. The pots were spaced at about 50 cm from each other to minimize competition for solar radiation.

Table 1. Characteristics of the soil used in a pot study with LPT bean genotypes in Rwanda

Parameter	Values	Methods
pH H_20	3.82	pH meter in 1:2.5 soils [11]
†OM (%)	0.95	Walkey and Black method [11]
Total N (%)	0.63	Semi-micro Kjeldahl procedure [11]
Bray I P (mg kg^{-1})	1.60	Bray-1 method [11]
††CEC (cmol kg^{-1})	9.23	Ammonium acetate saturation method [11]
†††† Exch. Al^{3+} (cmol kg^{-1})	6.25	Atomic absorption
Textural class	Clay loamy	Ascorbic acid [12] and textural classes using USDA textural class triangle method [13]
Bulk density (g/cm^3)	1.28	Core method [11]
†††WFC (%)	31.00	Gravimetric method [11]

† Organic matter, ††Cation exchange capacity, ††† Water at field capacity, †††† Exchangeable aluminum

Table 2. Bean genotypes used in the study

Line code	Seed size	Seed colour	Growth type	Local name (Rwanda)	Source
RWR 1873	Medium	Carima	Bush	-	Kawanda-Uganda
RWV 1668	Large	Dark	Bush	Rwandarushya	Rubona –Rwanda
†59/1-2	Small	Red	Climbing	Garukurare	Rubona –Rwanda
G 2858	Small	Tan	Climbing	-	Kawanda-Uganda
RWV 1348	Small	Red	Climbing	-	Rubona –Rwanda

Source: Bean book chapter [4]; †Local check

Triple superphosphate (TSP) was used as the source of P during experimentation. Three rates of P (0, 5, 10 mg kg^{-1}) were used and all dosages were spot applied at planting. Rhizobium (CIAT 899) and muriate of potash were blanket applied to all treatments, the latter at rates of 5 mg kg^{-1} soil.

Planting was done using six bean seeds per pot and subsequently thinned to three seedlings after two weeks. Weeding was by hand whenever weeds appeared in the pots. Water requirement in one pot was determined as a product of water at field capacity (31%) (Table 1) and weight of soil in each pot (5 kg). One and half litres (1.5 l) of water was used in each pot at the beginning of pot watering every two days until water start draining on basket covers. Watering continued by using water harvested on covers under pots and additional water was supplied of as required. For climbers (RWV 1348, GR2848 and 59/1), one bamboo stake per seedling (3 stakes per pot) was plugged into each pot three weeks after emergence.

2.4 Data Collection

Data collected were seedling emergence, nodulation efficiency and grain yield. Seedling emergence was determined at ten days after planting. This was subsequently computed as the proportion of emerged plants out of the six originally sown seeds in each pot. Thirty days after emergence (DAM), one plant was gently removed to determine nodulation efficiency, which was done by computing first the number of effective nodules per plant, based on nodules possessing pink cross-sectional pigment following Peoples [14], by viewing cross sections of all nodules per plant.

$$\text{Nodulation efficiente} = EN/\ Nt \qquad (1)$$

Where EN is effective Nodule and Nt total number of Nodules per plant.

Grain yield was assessed by harvesting pods from the two remaining plants per pot at about two weeks after physiological maturity of each genotype.

2.5 Statistical Analysis

The data obtained from the soil and crop samples, were statistically analyzed using ANOVA function of COSTAT - Cohort software of 2005 - 6 .111 version. Means were separated using the Least Significant Difference (LSD) at a probability level of 5%.

3. RESULTS

3.1 Seedling Emergence

There was no significant difference ($p>0.05$) among the low P tolerant genotypes in terms of seedling emergence (Fig. 1). The emergence rates varied between 92.9% for RWV 1668, a bush type and 61.9% for GR2858, which is a climber.

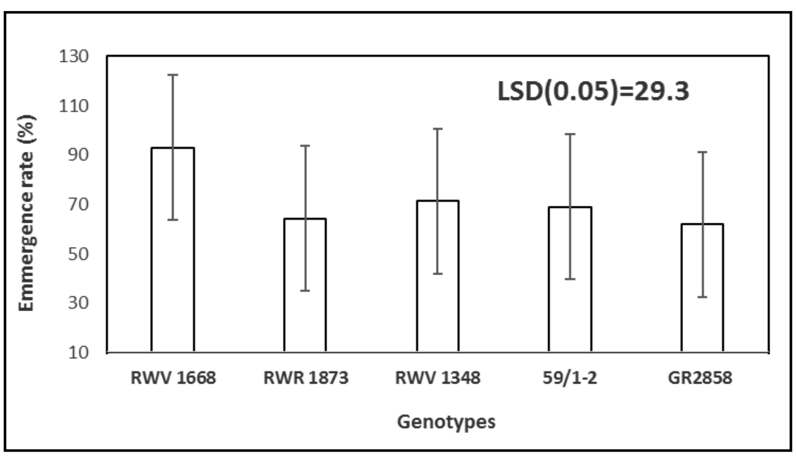

Fig. 1. Seedling emergence of LPT bean genotypes and a local check evaluated for performance under high acid soil conditions in Rwanda

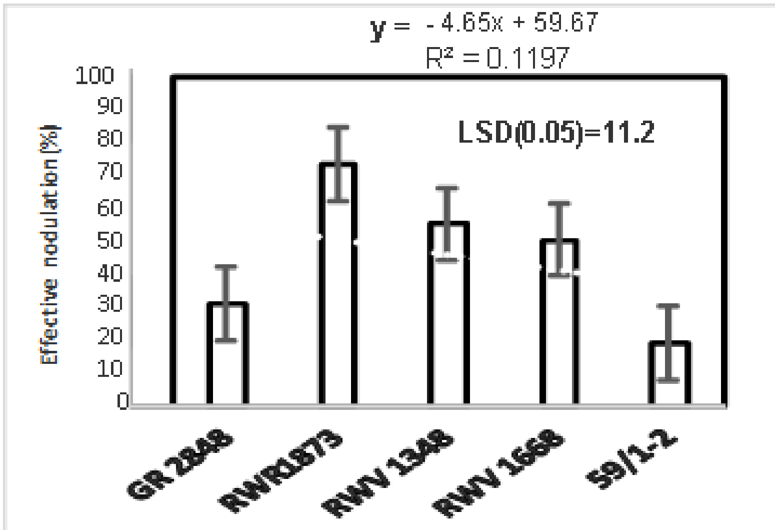

Fig. 2. Nodulation under low P and acidic conditions

3.2 Nodulation Efficiency under High Acidity and Phosphorus Deficiency Conditions

The genetic effect on nodulation efficiency was highly significant (*P*= .01) (Fig. 2). The variation in nodulation efficiency of LPT bean genotypes ranged from 29 to 73%. Genotype RWV 1873 again had the highest proportion of effective nodules (EN) of 73% and a local check 59/1-2 had the lowest (19%) (Fig. 2).

Table 3. Grain yield of low P tolerant bean genotypes under p deficiency and acidic conditions

Genotype	Yield (g pot^{-1})
RWV 1668	14.7
RWR 187	14.5
RWV 1348*	22.9
GR 2848*	04.5
†59/1-2*	10.2
LSD (0.05)	4.3

**Climbing bean genotypes, † Local check*

3.3 Grain Yield of Low P Tolerant Bean Genotypes under Highly Acidic and P Deficient Soils

Grain yield was significantly different among the genotypes (*P*= .05) (Above Table 3). Grain yield varied between 22.9 to 4.5 g pot^{-1}; with genotype RWV1348 bearing the highest grain yield among the climbers.

3.4 Response to Phosphorus Fertilization of Outstanding Low P Tolerant Bean Genotypes

3.4.1 Response of nodulation efficiency

There was a significant difference in nodulation efficiency of outstanding low P tolerant bean genotypes when P fertilizer was applied. The application of P reduced noduration efficiency of RWR 1873 and RWV 1668 (Figs. 3 and 5), while the RWV 1348 was less respondent to P input (Fig. 4).

3.4.2 Response of grain yield

There was a significant difference in grain yield of all selected outstanding low P tolerant plants (Table 4). All genotype had a significant increase of grain yield at a level of 5 mg kg^{-1}. Application of P at a rate of 10 mg kg^{-1} reduced grain yield of all genotypes.

4. DISCUSSION

4.1 Emergence

The lack of differences among the low P tolerant bean genotypes in this respect, presupposes that seed handling at the gene banks of Kawanda in Uganda and the Rubona (RAB) in Rwanda for a period of 5 years, was adequate to maintain high seed quality (Fig. 1). It is also clear that the acidity and P deficiency of the soil did not impair the germination of the seeds of the genotypes studied.

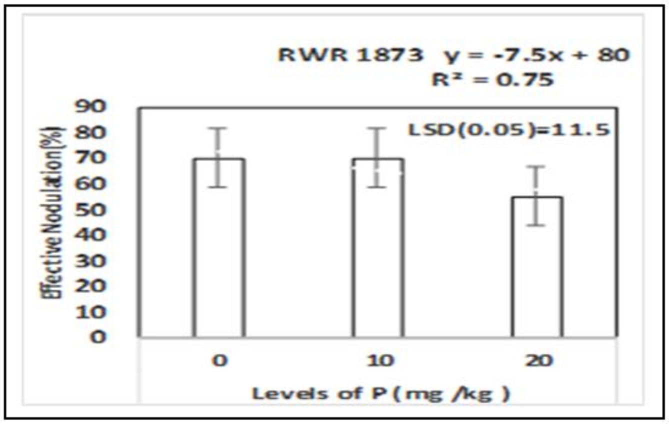

Fig. 3. Nodulation of an outstanding low P tolerant bean genotype at levels of P

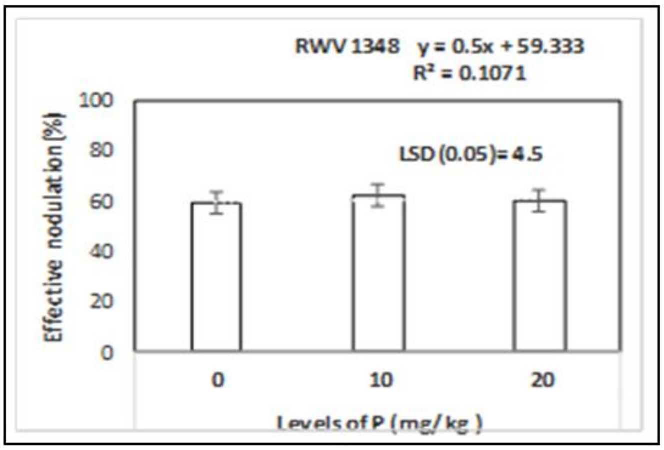

Fig. 4. Effect of levels of P on effective nodulation of RWV 1348

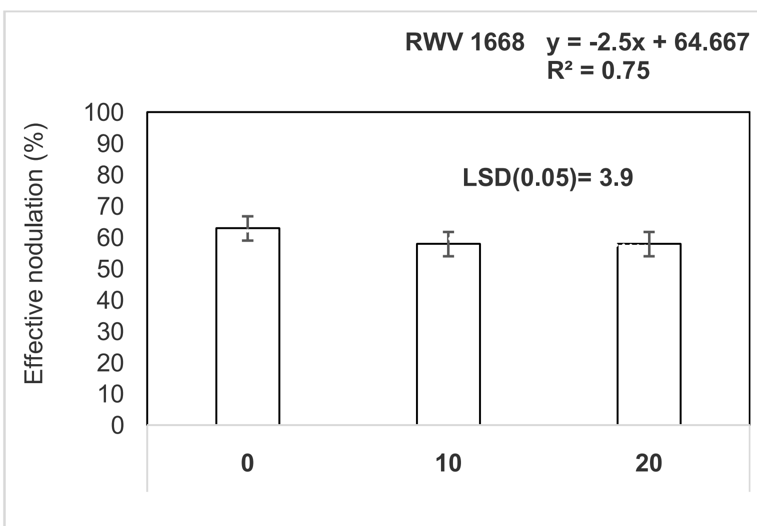

Fig. 5. Effect of levels of P on effective nodulation of RWV 1668

4.2 Nodulation Efficiency under High Acidity and Low P Conditions

Genotypes RWV 1348, RWR 1873, and RWV 1668 had higher nodulation efficiency under highly acidic and low P conditions than other genotypes (Fig. 2). A local check had the lowest nodulation efficiency. The genotypes with high number of effective nodules under P deficient conditions were classified as outstanding low P tolerant bean genotypes since they could nodulate much more than other genotypes in stressful environment. In normal soil conditions, effective nodulation occurs at a pH higher than 5.6 and at available soil phosphorus of 6 mg kg^{-1} [15]. The low P tolerant bean genotypes secrete organic substances that are realized in rhizosphere and solubilize phosphorous [16,17]. Phosphorus availability has been noted to affect the functioning of the biological Nitrogen fixation [16,18,19,20]. Thus, phosphorus solubilizing and root architecture change of LPT bean genotypes could have increased P availability and the nodulation efficiency in that way. This superior efficient nodulation under highly acidic conditions could have been due to the ability of low P tolerant bean genotypes to secrete organic substances that are released in rhizosphere and solubilize phosphorus, which is very important in biological N fixation. This in line with the results of research conducted on bean genotypes tolerance on low N and P in Uganda and Mozambique, which showed that some genotypes such as RWR1873 are outstanding tolerant to low P and N [4]. Under low P conditions genotypes that are low P tolerant greatly conserve efficient nodules [15,16]. This explains the possibility of LPT bean genotype nodulation under very stressful environment of high acidity and P deficiency.

4.3 Grain Yield of Low P Tolerant Bean Genotypes under Acidic and P Deficient Conditions

The genotype RWV1348 doubled the yield of a local check (59/1-2) and had eight times more yield than another low P tolerant bean genotype which was a climbing (GR 258) (Table 3). The bush low P tolerant bean genotypes, RWR 1873 and RWV 1668, had higher yield compared to a climbing local check and a climbing low P tolerant bean genotype. High yield of the bean genotypes in this study was due to increased phosphorus use efficiency under acidic and soil P deficient conditions. Phosphorus use efficiency implies an increased grain yield under low P conditions which is related to a common response to P deficiency by improving acquisition and utilization of P.

Research conducted by Kajumula and Muhamba [20] showed that under low P conditions, some bean genotypes increases low P tolerance mechanisms to improved P acquisition, uptake and utilization and thus increase their grain yield. This explains that low P tolerant bean genotypes RWV1348 (climbing) and genotypes RWR 1873 and RWV 1668 (bush) have used the acquired P efficiently.

This is in line with research results of LPT bean genotypes yield under P deficiency soil that has been correlated to modification of root architecture for phosphorous acquisition [21] and utilization efficiency [16,22] under constraints environment.

4.4 Responses to Phosphorus Application of Outstanding Low P Tolerant Bean Genotypes

4.4.1 Nodulation

Application of P has reduced nodulation efficiency in outstanding low P tolerant bean genotypes especially genotype RWR 1873 (Figs. 3, 4, 5), and the genotype RWV1348 did not show any nodulation response to P application. However, the uses of phosphorus fertilizer on a no responsive genotype such as RWV1348 is an economical loss. Phosphorus is not only essential for plant growth; but its availability has also been noted to affect the functioning of biological nitrogen fixation, by improving nodule number and size [18,19]. The application of phosphorus fertilizer could have increased the number of effective nodules but the results of this study have shown a negative response.

It was reported that high P application reduces synthesis of some organic acid complexes which are partly responsible for P-induced Zn deficiency and partly due to reduction of unit absorption rate of Zn by roots [23] putting restraint on the functional requirement of Zn on nodulation by leguminous [24,25]. In this study, the reduction of nodulation efficiency especially to RWR 1873 due to the application of high level of P, could have induced reduction of Zn uptake. This is in line with results of research conducted by Ali [25] which showed a positive correlation between number of effective nodules and amount of Zn absorbed, and a decrease of Zn uptake at high levels of P.

Table 4. Grain yield of outstanding low P genotypes at different levels of P

Genotypes	P levels (mg kg $^{-1}$)			LSD (0.05)
	0	+5	+10	
RWR 1873	14.5	17.5	12.0	4.4
RWV 1668	14.7	19.5	12.3	3.7
RWV 1348	22.9	26.5	22.4	3.0
LSD (0.05)	3.6	1.6	3.9	
Genotype effect .00***				
P level effect .00***				
GXP Ns				

****= *Significant at P (= .05), NS = Not significant*

4.4.2 Grain yield

The application of phosphorus at 5 mg kg^{-1} soil had increased yields of all selected outstanding low P tolerant bean genotypes RWV1348, 1873 and RWV 1668 at 17, 21, and 33% respectively (Table 4). Yet, this rate is still too low to achieve bean optimum yields in other beans genotypes [26]. Low P tolerant bean genotypes have been genetically manufactured to perform well under low phosphorus condition [27,28]. However, some low p tolerant bean genotypes are responsive to P inputs application [20]. A slight but significant increment of yield in outstanding LPT bean genotypes implies that they are P efficient and less responsive at low rate of P inputs. According to Kajumula [20], higher response to P application implies the lower tolerance to low P conditions. The genotype RWV 1668 tolerance to low P was lower than RWV 1348 and RWR 1873. The last two low P tolerant bean genotypes should be tested in field conditions and recommended to farmers under low acidity and P deficiency.

Doubling this rate of 5 mg kg^{-1} has reduced yield of all these genotypes. In other study,an application rate of more than 10 kg ha^{-1}, (corresponding to 25 mg kg^{-1} in this study), has showed a significant increase in biomass production and a decreased of grain yield [26]. This shows that the outstanding LPT selected requires less P inputs to achieve optimum grain yield. In most cases, grain yield is the goal of the growers; therefore, it is an important criterion in adopting a genotype for low soil fertility situations that request less or no inputs.

In the present study, the addition of greater than 5 mg P kg^{-1} soil caused a decline in grain yield (Table 4). According to Araujo [29], high level of phosphorus in soil declines phosphorus use efficiency of low p tolerant bean genotypes by reducing low P tolerance mechanisms of absorption and utilization of absorbed P. Yield reduction at high P levels (10 mg kg^{-1}) could be coupled with phosphorus and Zn uptake antagonism that reduce yield and nodulation efficiency when high phosphorus is applied without applying zn [25]. On the other hand, a research conducted on ultisols in Ethiopia showed application of high P level a delayed maturity and yield reduction of bean [26]. This is line with other researches confirming that that the application of P in high amounts leads to excessive vegetative growth and grain yield reduction [24,25].

5. CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Genotype RWV 1348, a climber, has the highest yield under the highly acidic and low P deficient soils such of Rwanda compared to other tested genotypes. The two-bush bean genotype RWV 1873 and RWV 1688 have the same yield and higher grain yield compared to 59/1-2 (a local check) and GR2848 (climbing). Low P tolerant bean genotypes have different nodulation efficiency, with RWR 1873 bearing the highest nodulation efficiency. A local check and GR 2848 have low nodulation efficiency.

The response of grain yield to different application levels of low P tolerant bean genotypes has the same pattern. They are all high yielding at a moderate rate (5 mg kg^{-1}) and a supply of high rate reduces yield potentials of the genotypes.

The response of nodulation to phosphorus fertilization of LPT bean genotypes has different pattern. Supply of phosphorus do not change the nodulation efficiency of the genotype RWV 1348 while it reduces considerably the nodulation efficiency of RWR 1873. High response of P supply of the genotype RWV 1668 shows that it is less tolerant to low P than RWR 1873.

5.2 Recommendations

The climbing bean genotype RWV 1348 should be tested in field condition for use by farmers in acidic and P deficient soils such of study area, as a high yielding bean genotype. The two-bush low P tolerant bean genotypes should be tested for their performance in field for use in acidic and low P deficient soils of regions where farmers still have challenges of obtaining staking materials for climbing beans.

The findings of this study suggest the following: The fertilizer recommendation rate for low P tolerant bean genotypes should be investigated to minimize the losses due to high application rates, in field conditions since, during this study, the application of more than 5 mg kg^{-1} soil suppressed yield of the outstanding low P tolerant bean genotypes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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