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Full Length Research Paper

Conventional breeding for improving micronutritient density (Iron and Zinc) into seed of Common bean (Phaseolus vulgaris, L.)

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Food fortification and supplementation rarely benefit poor households. Since daily consumption of beans in Rwanda is one of the highest in the world, biofortified beans offer a unique opportunity for alleviating the prevalent micronutrient malnutrition. The objective of this research was to improve the micronutrient content of common beans consumed by most people in Rwanda using conventional plant breeding techniques. Biofortification breeding of Andean beans at RAB was done through single cross. Populations were developed in 2002 and advanced from F1 to F 7 following Pedigree advancement method. From the four successful crosses (CAB 2 X LAS 400, CAB 2 X BUBERUKA, NGWINURARE X CAB 2 and ANDx X UMWIZARAHENDA), promising lines were tested for different traits including seed iron (Fe) and zinc (Zn) content. XRF mineral analysis was used to select the most promising advanced lines for Fe and Zn content. From this breeding program, five resulting varieties (RWV 2361, RWV 2887, RWV 3006, RWV 3316 and RWV 3317) were released and are being produced and consumed across Rwanda and has been shared with some counties in the network as biofortified bean varieties. RWV 3317 and RWV3316 have had high Fe of 95 and 92 ppm respectively.

Keywords: Conventional breeding, Iron and Zinc, Common bean (Phaseolus vulgaris, L.).

INTRODUCTION

The common bean (Phaseolus vulgaris L.) is the leading and the priority pulse crop due to its prominent contribution to food, nutrition and income security in Rwanda. Consumed in a variety of dishes as green or dry grain, leafy or snap vegetables and as processed or blended products, beans have been described as a near-complete food; meat for the poor and green meat for the rich. This is due to their dietary contribution of quality proteins, energy, fiber and micronutrients: vitamin A, folic acid, iron, and zinc. The recent release and commercialization of high yielding and marketable climbing bean varieties

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in Rwanda has enhanced productivity, internal and regional trade and farmers' incomes. Rwanda is now a net exporter of beans (RAB 2015). Andean beans are more likely adopted than Mesoamerican. Andean beans have a tendency for higher average seed iron concentration, but significant lower seed zinc concentration than Mesoamerican beans (Blair et al., 2010). Varieties LAS400, Ngwinurare and Buberuka have been released as varieties in Rwanda before 1990 (Musoni 2008). Both of these bean varieties were considered valuable parents because of their wide adaptation and large-seeded types that is preferred in Rwanda. Unfortunately, these varieties are not among the ones high in iron and zinc content. Therefore the objective of this study was to perform Intra-specific crosses to introgress high iron from related Andean White bean CAB2 that appears to hold promise, especially for the other Andean beans where it has been difficult to reach levels of iron of 90+ ppm.

MATERIALS AND METHODS

Six parental materials were crossed following full Diallel mating design at the Rwerere station in Northern Agricultural Zone Division. These include CAB2, LAS 400, BUBERUKA, NGINURARE, Umwizarahenda and ANDx. The characteristics of these varieties are described in the table 1 below.

Table 1. Characteristics of the parental materials

Donor parent	Characteristics	Other parent	Characteristics	Improvements (Combinations) needed in addition to high Fe
LAS 400	Good colour (Red) Early maturity	CAB 2	White Late maturing High yield Resistant to root rot and anthracnose	Good colour High yield Early maturing Resistant to root rot and anthracnose
Buberuka	Local randrace Early maturing Good colour Susceptible to root rot and anthracnose	CAB 2	White Late maturing High yield Resistant to root rot and anthracnose	White Early maturing High yield Resistant to root rot and anthracnose
Ngwinurare	Susceptibility to root rot and anthracnose Good colour(Red) High yield	CAB 2	White Late maturing High yield Resistant to root rot and anthracnose	Red Early maturing High yield Resistant to root rot and anthracnose
ANDx	CIAT germplasm Resistant to root rot and anthracnose Early maturing High yield	Umwizarahenda	Local landrace Red Susceptible to root rot and anthracnose Early maturing High yield	Resistant to root rot and anthracnose Red Colour Early maturing High yield

Population development and evaluation

Populations were developed in 2002 and advanced from F1 to F 7 following Pedigree advancement method. In 2004, 344 lines selected from four successful crosses (CAB 2 X LAS 400, CAB 2 X BUBERUKA, NGWINURARE X CAB 2 and ANDx X UMWIZARAHENDA) were evaluated in AYT in Rwerere. Promising ones were selected and evaluated in multilocational yield trial 1 (MYT 1) test, multilocational yield trial 2 (MYT 2) test and adaptability test in different environments based on their potential yield along with the improved check (G2331). All agricultural practices were respected. In 2012, these lines varieties were evaluated in Participatory variety selection and tested for seed iron and zinc content. XRF analysis was used to select the most promising lines for Fe and Zn content. Care was taken in preparing samples for mineral analysis (HarvestPlus, 2008) and cleaned thoroughly (Paltridge, *et al*., 2011) to reduce aluminium (Al) contamination. Ground samples were used (Stangoulis, 2010) to evaluate Fe and Zn make up of the promising lines (Oxford Instruments, 2009).

RESULTS AND DISCUSSION

Five promising lines (coded RWV) (Table2) were found to be accepted by farmers for their best colour, high yield, resistance to diseases and early maturing traits in addition to their high seed iron and zinc content.

Table 2. Cross combination and their respective varietal codes

Combinations done	Varietal code
CAB 2 X LAS 400	RWV 3316 + RWV 2887
CAB 2 X BUBERUKA	RWV 3006
NGWINURARE X CAB 2	RWV 3317
ANDx X UMWIZARAHENDA	RWV 2361

In 2007 A RWV 3006 was evaluated in AYT trial along 25 entries, including local and improved checks in plots of 2.4m^2 in RCBD designs with six replications where significant differences were observed at P<0.001 and LSD of 900. The variety RWV 3006 performed better with the mean yield of 4583 kg/ha. In 2007B RWV 3006 was evaluated in ECMI trial along 15 varieties, including local and improved (G2331) varieties. It performed well with the mean yield of 4200kg/ha where local check yielded 3300kg/ha and improved variety (G2331) 2778 kg/ha at P< 0.05 and LSD of 814 kg. Though RWV2413 had higher yield and was not selected for its susceptibility to anthracnose and BCMV. In 2008 A RWV 3006 was evaluated in MYT 2 in 7 sites along eight varieties, including local and improved varieties where it performed better with mean yield of 4100kg/ka while local and improved varieties had yielded 2700 and 3700 kg/ha respectively. The differences were significant at p<0.01 and LSD of 800 kg/ha. In 2008B RWV 3006 was evaluated in adaptability trial in 8 sites along with 4 varieties, including local and improved varieties with mean yield of 2660kg/ha while local and improved check had 1900 and 2489 kg/ha respectively. RWV 2409 and RWV 2345 were left because of their susceptibility to BCMV. The table 3 summarizes the significance of the variety RWV 3006 evaluated along with other varieties, locally grown and improved checks from 2007 to 2008

Table 3. Mean squares for the promising variety RWV 3006 evaluated along with other varieties, locally grown and improved checks in different trials and different sites.

	AYT 2007A		MYT I 2007B		MYT II 2008A		Adaptability 2008B	
Source of variation	DF	MS	DF	MS	DF	MS	DF	MS
Rep	5	1835185	2	961283	6	2250295	7	2222444
Genotype	24	3861039 ***	15	600798 *	8	1701878 **	4	820161 *
Residual	120	620269	30	238414	48	515448	28	242742
GM		3514		3616		3253		2406
CV (%)		22		14		22		20
SED		322		282		271		174
SEM		455		399		384		246
T crit		2		2		2		2
LSD		900		814		772		505

In 2007B the varieties RWV 2361, RWV 2872 and RWV 3317 were evaluated in PYT along with 22 varieties, including local and improved checks where the two firs performed well for yield with the means of 4790 and 4250 kg/ha respectively. Though RWV 3317 with the mean of 3730 which is less than the grand mean (3790kg/ha) but higher that local and improved checks which yielded 2986 and 3230 kg/ha respectively was selected for its resistance to major diseases and good colour (sugar) making it highly marketable. RWV 2360 and RWV 2350 were not selected because of their susceptibility to Halo bright. RWV 3317 despite moderate yield, it was selected for its high resistance to multiple diseases. In 2008A the varieties RWV 2361, RWV 2872 and RWV 3317 were evaluated in MYT 1 along with13 varieties including local and improved checks. The mean yield of these varieties was 3958, 4062, 3715 respectively, while the local and improved checks were 3056 and 3854 kg/ha respectively. The grand mean yield was 3793. The selection of these varieties was based on combination of different traits including high yield, good resistance to major diseases and good colour (sugar) making the variety highly marketable. Ngwinurare X CAB2/5/1/3/1 was left because of its Halo bright, RWV2875 by BCMV, Ngwinurare X RWV377/1/1/1 by Anthracnose and RWV 3213 by BCMV. In 2008B the varieties RWV 2361, RWV 2872 and RWV 3317 were evaluated along 6 genotypes including local and improved checks. These varieties were not scientifically different. The genotype by environment interactions had a strong and significant effects on yield of these varieties with the mean yield 3405, 3265, 3119 kg/ha for RWV 2361 RWV 2872 and RWV 3317 respectively while the local check and improved check had 2236 and 3381kg/ha respectively. The grand mean yield was 2953kg/ha across 4 environments. In 2009A, the varieties RWV 2361, RWV 2872 and RWV 3317 were evaluated in adaptability test along with six varieties including local and improved varieties in six different environments. Significant differences were observed among varieties and these varieties (RWV 2361, RWV 2872 and RWV 3317) performed better with mean yield of 3824, 3610 and 3327 kg/ha respectively while the local and improved checks had 2201 and 3144 kg/ha respectively. The grand mean yield was 3193 kg/ha. Ngwinurare X RWV377/1/1/1 was left because of its high susceptibility to Anthracnose. The table 4 summarizes the significance of these varieties evaluated along with other varieties, locally grown and improved checks from 2007 to 2009.

Table 4. Mean squares for the promising varieties RWV 3317, RWV 2361 and RWV 2872 evaluated along with other varieties, locally grown and improved checks in different trials and different sites.

	AYT 2007B		MTY I 2008A		MYT II 2008B			Adaptability 2009A		
SOV	DF	MS	DF	MS	SOV	DF	MS	SOV	DF	MS
Rep	5	4826345	2	1281286	Site	3	15249124	Site	5	2245817
genotype	24	1721119 ***	15	270785 *	Site/Rep	4	1037967	entry	5	1918087 ***
Residual	120	629206	30	130630	genotype	8	1417107 ns	Residual	25	184203
					G X E	24	1115739 ***			
GM	3790		3793		Pooled error	32	244522	GM	3193	
CV (%)	21		10		GM	2953		CV (%)	13	
SED	324		209		CV (%)	17		SED	175	
SEM	458		295		SED	175		SEM	248	
T crit	2		2		SEM	247		T crit	2	
LSD	907		603		T crit	2		LSD	510	
					LSD	510				

In adaptability test, a site represented a replication

In 2008 A the varieties RWV 2887 and RWV 3316 were evaluated in AYT in 6 replications along with 23 genotypes including local and improved varieties. Significant differences were observed ($p<0.001$) where these varieties performed well with the mean yield of 3889kg/ha and 3629 kg/ha for RWV 2887 and RWV 3316 respectively. The local and improved checks had 2118 and 3108 kg/ha respectively. The grand mean yield was 2845 kg/ha. In 2008 B the varieties RWV 2887 and RWV 3316 were evaluated in MYT 1 along with local and improved checks in 3 replications. Significant differences ($p<0.001$) were observed among varieties were the above mentioned varieties performed best with the mean yield of 3854and 3576 kg/ha. The local and improved checks had 1801 and 2431 kg/ha respectively. The grand mean yield was 2801 kg/ha. RWV 2985 was left because of its high susceptibility to Halo blight. In 2009 A the varieties RWV3316 and RWV 2887 were being evaluated in MYT 2 in 4 sites and 2 replications along with 7 varieties including local and improved varieties. The significant differences ($p<0.01$) were observed among varieties where RWV3316 and RWV 2887 ranked first and second class respectively with the respective means yield of 3740 and 3635 kg/ha. The local and improved checks yielded 2343 and 2859 kg/ha respectively. The grand mean was 3015 kg/ha. In 2009 B the varieties RWV 3316 and RWV 2887 were being evaluated in adaptability trial in 6 different sites along 5 varieties including local and improved checks. The varieties were significantly different ($p<0.05$) and the varieties RWV 3316 and RWV 2887 performed better than the rest with the mean yield of 4139 and 3662 kg/ha respectively. The local and improved checks were averaging the yield of 2739 and 3486 kg/ha respectively with the grand mean yield of 3363 kg/ha. In 2010 B the same varieties were replanted in 7 sites where significant differences were observed among site at p<0.001 and no significant differences among genotypes. Though the grand mean yield for this experiment was 2164.571 kg/ha, the varieties RWV 3316 and RWV 2887 yielded respectively the means of 2392kg/ha and 2071 kg/ha which is statistically not different from the improved check yield. The local check mean was 1663kg/ha while the improved check had the average of 2429 kg/ha. The table 6 summarizes the significance of these varieties evaluated along with other varieties, locally grown and improved checks from 2007 to 2010.

Table 5. Mean squares for the promising varieties RWV 2887 and RWV 3316 evaluated along with other varieties, locally grown and improved checks in different trials and different sites.

									Adaptability			
	ECV 2008 A		MYT I 2008B		MYT II 2009A				2009B		2010B	
SOV	D.F.	M.S.	D.F.	M.S.	SOV	D.F.	M.S.	SOV	D.F.	M.S.	D.F.	M.S.
rep	5	3949352	2	1922683	site	3	7355904	Site	5	2474504 **	6	5964040 ***
genotype	24	1237576 ***	11	1813929 ***	site.rep	4	1142408	genotype	6	1335327 *	6	465149 ns
Residual	120	306406	22	300997	genotype	8	1724681 ***	Residual	30	449527	32	424103
					G X E	24	489988 ns					
GM	2845		2801		Pooled error	32	563268	GM	3363		2164.571	
CV (%)	19		20					CV (%)	20		30	
SED	226		317		GM	3015		SED	274		246	
SEM	320		448		CV (%)	25		SEM	387		348	
T crit	2		2		SED	265		T crit	2		2	
LSD	633		929		SEM	375		LSD	791		709	
					T crit	2						
					LSD	764						

Adaptability test in 2010B has 44df for total instead of 48 due to 4 missing data points

In 2009 the yield performance of the selected varieties across seasons and locations was not significantly different from the improved check, but significantly different from the local check grown by farmers. (Figure1)

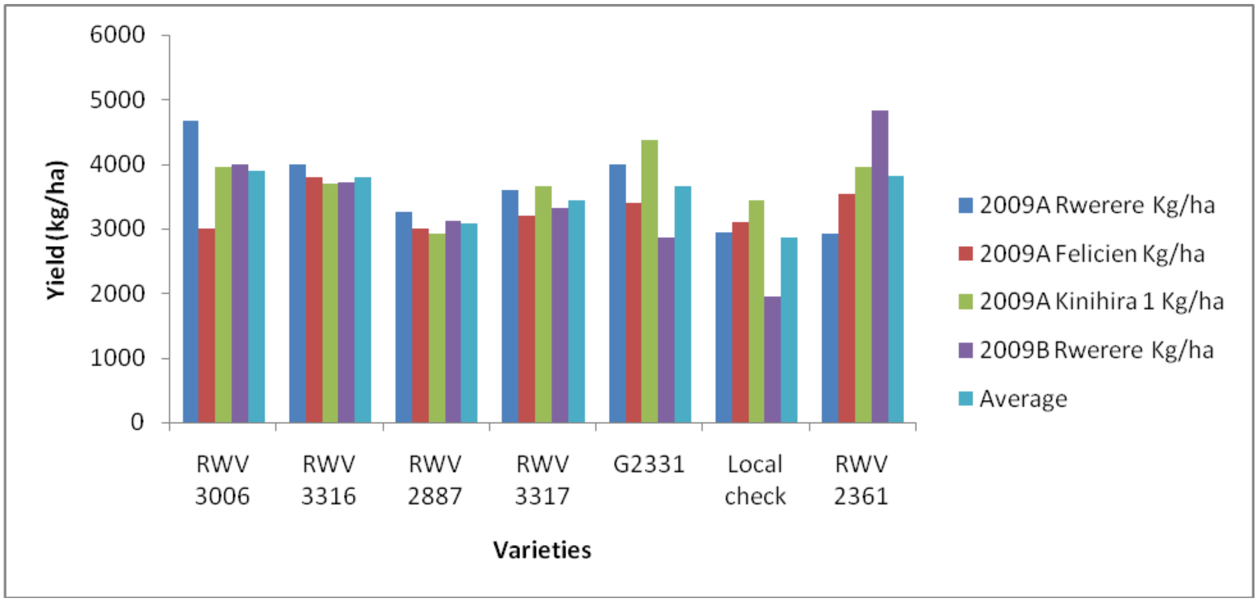

Figure 1Averages of the promising varieties planted in 3 sites (4 environments) along with local and improved checks in 2009

These varieties were evaluated in multilocational yield trial in 5 environments during 2012 and 2013 along the improved check (G2331) for their yield and other traits stability in addition to their high iron and zinc content. Significant differences were observed among experiments (Seasons) and varieties at p<0.001. Genotype by environment interactions had no significant effects on yield of varieties under investigation

Table 6. Means square of the promising varieties in 5 environments along with improved check and Gitanga

Source of variation	DF	Yield Mean square
Experiment	4	19234632 ***
Experiment/replication	10	788006 *
Genotype	6	2606425 ***
G X E	24	469828 ns
Residual	59	356251
Total	103	
GM	2725	
CV(%)	21.9	
L.S.D.	516.6	

One missing plot making residual df=59 instead of 60 and 103 for total instead of 104

Table 7. mean yield and ranks of the promising varieties in 5 environments along with improved check and Gitanga

Genotype	Mean yield	Rank
G2331	3383	A

RWV2361	3089	Ab
RWV3317	2878	Abc
RWV3006	2617	Bcd
RWV3316	2542	Cd
RWV2887	2386	Cd
Gitanga	2183	D

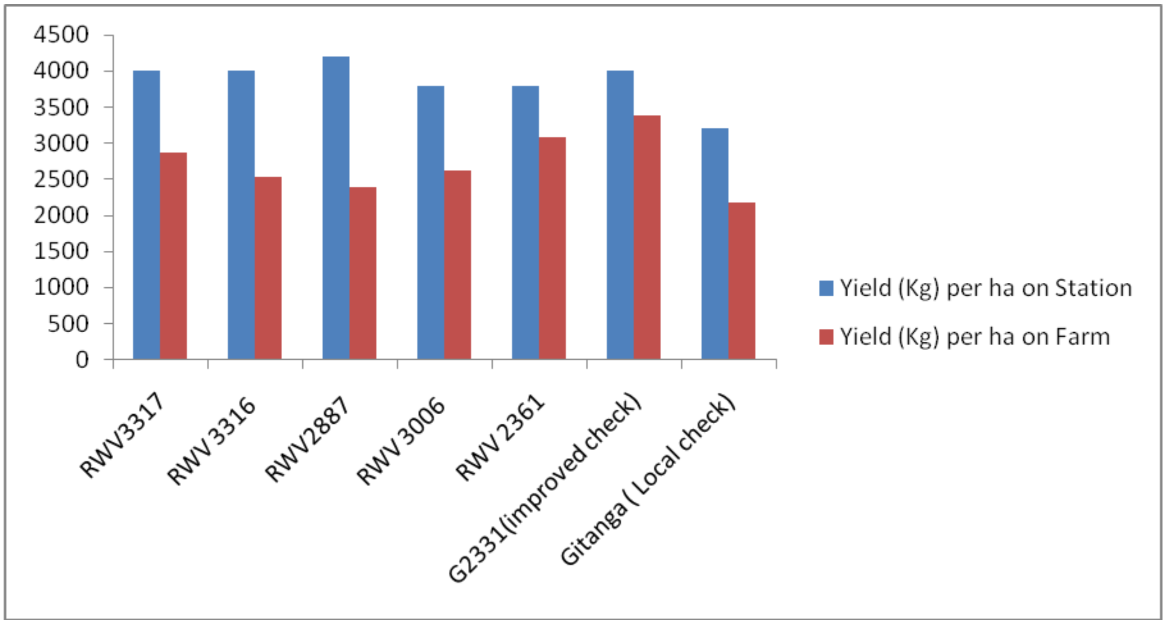

Figure 2. Comparison of the promising varieties on station and on farm

Table 8. Other characteristics of the promising and selected varieties on station and on farm along the checks

Variety	DF	DM	Seed color	Seed size	Fe content	Zn content	Yield (Kg/ha)
RWV3317	56	110	Red	L	95	28	4000
RWV 3316	58	110	Red	L	92	31	4000
RWV2872	56	108	Sugar	L	85	29	4200
RWV 3006	58	110	White	L	84	35	3800
RWV 2361	57	108	Sugar	M	78		3800
G2331(improved check)			Yellow				
Gitanga (Local check)			Cream				

Varieties with high iron also had long number of days to maturity implying that in high altitude, genotypes grow for a long time and get more chance to accumulate high contents of this micronutrient confirming results reported by Mukamuhirwa *et al.* (2014). High Fe and zinc content were also observed in large seeded genotypes confirming results in Blair *et al* (2010) which showed that Andean beans had a tendency for higher average seed iron concentration, but significant lower seed zinc concentration than Mesoamerican beans.

Participatory plant breeding and participatory variety selection

Participatory variety selection resulted in giving new and local names because of their characteristics and how farmers were excited about the new varieties. The table 8 below describes the local names given for each variety and the meaning. During adaptability test, farmers' selection criteria, including high yield, colour and marketability, seed size and early maturing were the main basis of selection. The varieties were officially released in 2012 (RAB, 2012). Then the foundation seed was given to the seed unity of RAB to multiply and get basic seed to be given to Seed companies and seed multipliers for certified seed category. The seed was then disseminated to farmers RAB through NGOs, seed companies, farmers' cooperatives, seed multipliers and individual farmers.

Table 9. Local names given for each variety and the meaning

Variety	Local name	Meaning
RWV 3006	Nshutinziza	Good friend
RWV 3316	Vuzimpundu	Be happy/
RWV 3317	Mpanguhe	Give me as I give you
RWV 2887	Girubuzima	Have good health
RWV 2361	Zanisuka	Bring the hoe

CONCLUSIONS

Conventional breeding hold promises in increasing iron and zinc content, especially in Andean beans where it has been difficult to reach levels of iron above 90 ppm.

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