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### **Phenotypic and genotypic screening for rust resistance in common bean germplasm in Uganda**

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1 Kapchorwa white, and NABE 2 as new sources of rust resistance. This study identified sources of  
2 rust resistance that would be useful in the bean breeding programmes in Uganda.

3 **Keywords:** *Uromyces appendiculatus*, *Phaseolus vulgaris*, SSR markers, broad spectrum rust  
4 resistance, AUDPC.

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## 16 **Introduction**

17 Common bean (*Phaseolus vulgaris* L.) is the most widely grown grain legume in Uganda where it  
18 serves as a readily available and popular food for both the urban and rural populations (Kilimo  
19 Trust 2012; Sibiko et al. 2013). It is a primary source of dietary protein for people in the lower  
20 income bracket and is sometimes referred to as “poor man’s meat” (Nedumaran et al. 2015).  
21 However, common bean production is significantly affected by a number of diseases that occur

1 naturally in bean growing areas of Uganda (Wortmann et al. 1998). Common bean rust caused by  
2 *Uromyces appendiculatus* (Pers., Pers.) Unger, is one of the major foliar fungal diseases becoming  
3 a serious threat to bean production in Uganda (Paparou et al. 2014a). Rust has been reported to  
4 cause yield losses as high as 100% in susceptible cultivars depending on the plant stage and the  
5 severity of infection (Singh and Schwartz 2010). This disease was first reported in Uganda by  
6 Atkins (1973) in the common bean white haricot genotype, but its economic importance (Paparou  
7 et al. 2014b) and the need for disease management has just been recently recognized (Paparou et al.  
8 2014a, Odogwu, et al. 2014). Small-holder farmers in Uganda also have a history of planting  
9 landraces which are low yielding and susceptible to other fungal diseases such as anthracnose and  
10 angular leaf spot (Nkalubo et al. 2007; Ddamulira et al. 2014). These same cultivars also appear  
11 to be susceptible to *U. appendiculatus* (Kelly et al. 2013). According to various research findings  
12 (Mmbaga et al. 1996; Souza et al. 2013) the use of host resistance is currently considered to be the  
13 most economical, practical and effective strategy to manage the rust problem in Uganda especially  
14 for resource poor farmers with restricted access to expensive fungicides (Lunze et al. 2002; Paparou  
15 et al. 2014b).

16 Screening of available germplasm is normally a prerequisite for identifying effective resistance  
17 sources in any breeding programme (Buruchara et al. 2011; Ddamulira et al. 2014). Screening for  
18 rust resistance can be accomplished either through the use of disease severity scores where plants  
19 are assessed periodically by visual estimation of the leaf area covered by pustules (Sillero et al.  
20 2006) or through calculations of the area under disease progression curve (AUDPC, Friesen et al.  
21 2014). These methods can be enhanced when deliberate efforts are made to situate fields in  
22 locations where the disease is endemic or where susceptible cultivars are deliberately planted  
23 (Sillero et al. 2006). However, the high virulence diversity of the *U. appendiculatus* pathogen

1 (Jochua et al. 2008) and fluctuation of infection pressure due to weather (Sillero et al. 2006) can  
2 lower the overall disease infection level and hinder the predictability of resistance patterns  
3 expressed by different cultivars (Atkins, 1973). The identification of germplasm resistant to  
4 common bean rust can be accomplished with the use of molecular markers tightly-linked to  
5 resistance genes (Namayanja et al. 2006; Mienie et al. 2005). The use of markers has assisted plant  
6 breeders in matching molecular profiles to the physical properties of varieties (Park et al. 2004).  
7 Unlike morphological markers, molecular markers are not affected by the environment thus  
8 authenticating the sources of disease resistance selected phenotypically (Jonah et al. 2011). The  
9 use of PCR-based markers such as Randomly Amplified Polymorphic DNA (RAPD), Sequence  
10 Characterized Amplified Region (SCAR) and Microsatellite or Simple Sequence Repeats (SSRs)  
11 markers linked to rust resistance genes have been reported for single or multiple genes that are  
12 used in the indirect selection of promising genes and to facilitate gene pyramiding for more durable  
13 rust resistance (Park et al. 2004; Miklas et al. 1993; Mienie et al. 2005; Shin et al. 2014). SSR  
14 markers offer an ideal marker system that creates complex banding patterns by simultaneously  
15 detecting multiple DNA loci (Muhamba et al., 2013). Genetic mapping of chromosome four  
16 (Pv04) in common bean has identified the three genes, *Ur-5*, *Ur-14* and *Ur-15* which confer broad-  
17 spectrum rust resistance (Pastor-Corrales and Steadman 2015). Shin et al. (2014) identified 22 SSR  
18 markers linked to *Ur-15* gene present in the bean cultivar PI 310762 while Pastor-Corrales and  
19 Steadman (2015) and Valentini et al. (2015) acknowledged the linkage of these markers to *Ur-5*  
20 and *Ur-14* genes found in the cultivars Mexico 309 and Ouro Negro respectively. Although these  
21 markers have limited application, their effectiveness in selecting resistant materials outside the  
22 original mapping populations needs to be determined (Namayanja et al. 2006).

1 Knowledge about the genetic background present in an existing germplasm provides  
2 complementary information that can be used to select promising parents (Singh 2001). Within the  
3 common bean germplasm available in Uganda, only 100 accessions have been genotyped to  
4 determine their genetic diversity and structure (Okii 2009). It was observed that common beans  
5 from the Mesoamerican and Andean gene pools, and the inter-gene pool introgression are present  
6 in the Ugandan germplasm ((Blair et al. 2009, Okii et al. 2014), however most of the farmers'  
7 preferred genotypes are of the Andean background (Kiwuka et al. 2012). Liebenberg and Pretorius  
8 (2010) had recommended that Mesoamerican germplasm is better for rust resistance and is more  
9 suitable for use in Africa since most of the farmers' preferred genotypes in Eastern and South  
10 Africa regions are of the Andean origin which are susceptible to rust, whereas Pastor-Corrales and  
11 Steadman (2015) suggested that combining resistance sources of Andean and Mesoamerican origin  
12 should provide broad resistance to bean rust disease.

13 It is thus envisaged that the identification of new sources of resistance is important for maintaining  
14 and further developing host resistance to diseases in common bean (Miklas et al. 2006). In addition,  
15 the selection of sources of resistance using molecular markers to compliment phenotyping is  
16 pertinent in the process of developing host resistance to bean rust. Therefore, this study was  
17 designed to analyze the reactions of several cultivars of dry beans to the predominant  
18 pathotypes of *U. appendiculatus* prevalent in Uganda and permit selection of broad spectrum  
19 rust resistance from available germplasm. This would provide initial information in  
20 establishing a breeding programme for improving common bean for rust resistant in  
21 Uganda.

22

## **MATERIAL AND METHODS**

1 **Plant materials:** The common bean germplasm used in this study was obtained from germplasm  
2 collections maintained by National Crop Resources Research Institute (NaCRRI), Uganda The  
3 Center for Tropical Agriculture (CIAT), Uganda, and Dr. J. Steadman, University of Nebraska,  
4 Lincoln, USA. The germplasm consisted of 57 landraces, 20 commercial and 61 introduced  
5 genotypes (Table 1). The landraces had been collected from the major bean growing regions in  
6 Uganda (Okii et al., 2014). The commercial genotypes with prefixes NABE and K are released  
7 commercial varieties from Uganda with either the Andean or Mesoamerican background. The  
8 introduced genotypes with the prefixes SCR, SEN, SCN, DAB and DOR were developed at CIAT  
9 with tolerance to drought (Beebe et al. 2008), while some were differentials possessing resistance  
10 genes for rust, anthracnose, and angular leaf spot (Miklas et al. 2006). The resistant check used  
11 was Mexico 309 which has the Mesoamerican resistance gene *Ur-5* (Pastor-Corrales and Steadman  
12 2015), and the susceptible check was NABE 16, a popular commercial cultivar that is tolerant to  
13 anthracnose but is susceptible to rust in the field in Uganda (Nkalubo 2014 personal  
14 communication).

### 15 **Phenotypic screening of Ugandan germplasm**

16 **Field experiment:** The field experiment was conducted on-station at NaCRRI (latitude: 0.3910  
17 N; longitude: 32.4270 E; altitude: 1,160 m above sea level). This site has been used in previous  
18 studies on bean rust (Paparú et al. 2014b). Two field trials were conducted from March to June  
19 known as the first planting seasons in 2014 and 2015 because of the high rainfall and moderate  
20 temperature suitable for increased rust infection (Nsubuga 2000). The trials were laid out in an  
21 alpha lattice design with three replicates. Each cultivar was planted in a plot sown with 22 seeds  
22 in 2 rows of 1m with inter- and intra-row spacing of 30cm and 10cm respectively. There was 1m  
23 spacing between plots to avoid inter-plot interference. The susceptible cultivar, NABE 16 was

1 planted after every 2 rows at relatively high plant density to ensure uniformity of natural inoculum  
2 and increased disease pressure (Maphosa et al., 2013).

3 **Field data collection and analysis:** Three visual assessments and scoring of rust severity and  
4 incidence were carried out when 50% of all cultivars were at the first trifoliolate leaf stage designated  
5 as V3; pre-flowering designated as R5 and pod formation designated as R7 plant developmental  
6 stages (Van Schoonhoven and Pastor-Corrales 1991; Paparu et al. 2014a). Disease incidence was  
7 estimated as the percentage of the number of infected plants per plot while the disease severity  
8 was rated using the CIAT 1 to 9 scale by Van Schoonhoven and Pastor-Corrales (1991), where 1-  
9 3 = resistant (no visible pustules to few pustules covering 2% of foliar area), 4-6=intermediate  
10 (small pustules covering 5% foliar area to large pustules often surrounded by chlorotic halos  
11 covering 10% foliar area) and 7-9 = susceptible (large to very large pustules covering 25% foliar  
12 area). For each genotype, the area under the disease curve (AUDPC) was calculated from the  
13 disease severity using the midpoint rule (Campbell and Madden 1990) equation:

$$14 \quad AUDPC = \sum_{i=1}^{n-1} \frac{y_i + y_{i+1}}{2} \cdot (t_{i+1} - t_i).$$

15 Where  $y_i$ , is the assessment of the disease at the  $i$ th observation,  $t_i$  is time at the  $i$ th observation,  
16 and  $n$  is the total number of observations. Each bean cultivar was considered resistant (R) when  
17 AUDPC value symptom score < 50, intermediate (I) and susceptible (S) when AUDPC value of  
18 50 to 100 and 100 to 150 respectively. Data on yield was recorded and seed yield (kg/ha) was  
19 estimated for yield per plot using the Microsoft Excel 2013.

20 Analyses of variance for disease AUDPC, disease incidence and grain yield were performed using  
21 GenStat discovery 12<sup>th</sup> edition. The AUDPC of both years (2014 and 2015) was used for selection  
22 of promising parental cultivars that would be used in the rust resistance breeding programme. A



1 scattered plot analysis was performed using *plot (xy)* function of the R statistical package for  
2 windows v.3.1.2.

### 3 **Genotypic screening of Ugandan germplasm**

4 The total genomic DNA of newly emerged trifoliolate leaflets of each of the 143 cultivars including  
5 the resistant and susceptible checks were isolated using the DNEASY 250 plant mini kit (Qiagen,  
6 CA) following the manufacturer's protocol at the CIAT molecular laboratory, Uganda. The DNA  
7 were quantified using the NanoDrop 8000 UV-Vis Spectrophotometer (Thermo Scientific) at  
8 260/280nm and genotyped using twenty-two (SSRs) primers linked to rust resistance currently  
9 used at the Bean Breeding and Genetics laboratory at Michigan State University, USA. The 22  
10 SSR markers obtained from the Soybean Genomics and Improvement Laboratory, USDA-ARS,  
11 Beltsville MD and used in this study: bean\_ssr\_2903, BARC\_PV\_SSR04719,  
12 BARC\_PV\_SSR04728, SSRbeanur36, bean\_ssr\_2895, BARC\_PV\_SSR04703,  
13 BARC\_PV\_SSR04722, bean\_ssr\_2904, bean\_ssr\_2892, BARC\_PV\_SSR04721, bean\_ssr\_1170,  
14 bean\_ssr\_1170, bean\_ssr\_2909, bean\_ssr\_2906, bean\_ssr\_1168, BARC\_PV\_SSR04725,  
15 bean\_ssr\_0669, bean\_ssr\_2898, BARC\_PV\_SSR04425, bean\_ssr\_2901, bean\_ssr\_0778 and  
16 bean\_ssr\_1167. Following the protocol of Shin et al. (2014), the resulting PCR products were  
17 analyzed using 3% agarose gel electrophoresis and visualized using the Gel Doc EZ Imager (Bio-  
18 Rad). Scoring was done using a binary system where 1 and 0 indicated presence and absence of  
19 bands respectively of the SSR markers (Duncan et al. 2013). Resistance of each cultivar was  
20 determined based on the presence and absence of SSRs amplification. For more stringent selection  
21 measure, cultivars who had 10 SSR markers and above were considered resistant. The  
22 polymorphism information content (PIC) was analyzed using the PowerMarker V3.25 software  
23 (Liu and Muse 2005). Correlation of the phenotypic data for disease severity and seed yield, and

1 genotypic data was done using the general linear model (GLM) option in the Trait Analysis by  
2 Association Evolution and Linkage (TASSEL) 5.2.21 software (Jamshidi and Mohebbalipour  
3 2014).

## 4 **RESULTS**

### 5 **Phenotypic variability and reaction of Ugandan common beans to rust.**

6 In this study a germplasm collection of 138 dry bean genotypes was screened to identify those  
7 genotypes which could be used as effective sources for rust resistance. The results of the analysis  
8 of variance for rust disease incidence, AUDPC and seed yield evaluated in 2014 and 2015 first  
9 planting seasons are presented in Table 2. The results indicated high significant differences ( $P <$   
10  $0.001$ ) among genotypes and interaction of the genotype and season for incidence, AUDPC, and  
11 seed yield in both years. Although there were high significant differences for yield in 2014 and  
12 2015, there was no difference for disease incidence and AUDPC for both years. The results of  
13 correlation analysis among the traits studied are presented in Table 3. Rust disease incidence was  
14 shown to be significantly correlated ( $P < 0.001$ ) with AUDPC (0.6) suggesting that the severity of  
15 rust disease increased with disease incidence.

16

17 In general, rust disease incidence, severity and AUDPC were more severe in 2015 than in 2014.  
18 In 2014, the AUDPC values ranged from 38.5-87.5 with 13% of the genotypes showing resistant  
19 response while 87% had intermediate response. However, in 2015 the AUDPC values ranged from 19.4-  
20 130 with 41% of the genotypes showing resistance, 43% with intermediate response and 17% showing  
21 susceptible response. The scattered plot of the AUDPC estimates in 2014 and 2015 is presented in Fig.1.  
22 The scatterplot does not show any strong association between the AUDPC of both years. This lack  
23 of association is supported by a correlation of 0.109. However, three main groups of genotypes

1 were observed to have the same response in both years. The first group fell within the AUDPC  
2 value range of 1-50, which had nine genotypes that consistently had resistant response in 2014 and  
3 2015. These genotypes and their AUDPC values for 2014 and 2015 were as follows, Aurora (49.0;  
4 19.0), KW814 (50.0; 33.4), CNCPI181996 (43.2; 25.0), Kapchorwa white (48.0; 37.0), G2333  
5 (50.0; 43.0), SEN 80 (48.0; 38.0), SEN 46 (47.0; 43.0), DOR 500 (43.2; 25.0) and NABE 2 (42.0;  
6 38.1). The resistant check, Mexico 309 (65.3, 36) was found in the second group with 78 genotypes  
7 that fell within the intermediate response with the AUDPC value range of 50-100 while the  
8 susceptible check, NABE 16 (56.0; 96.8) was found in the third group with over 21 genotypes that  
9 had the susceptible response with the AUDPC values of 100-130 for the years 2014 and 2015.

#### 10 **Genotypic selection of cultivars with broad-spectrum rust resistance**

11 In this study, 138 alleles were detected ranging from 144 to 288 base pairs and the polymorphism  
12 information content (PIC) ranged from 0.1 for the marker bean\_ssr\_2903 to 0.4 for bean\_ssr\_1167  
13 (Table 4). The frequency of the major alleles ranged from 0.5 to 0.9 with the mean of 0.8 which is  
14 below 1. This indicated that all markers were polymorphic and highly informative. The  
15 correlation of the plants' reaction to rust indicated by the mean disease severity and the SSRs  
16 markers for broad-spectrum resistance were analyzed using the GLM. In this study, three SSR  
17 markers, BARC\_PV\_SSR04725, bean\_ssr\_0778 and bean\_ssr\_2892 were observed to be strongly  
18 associated ( $P$ -values  $\leq 0.05$ ) with the mean disease severity (Table 5). There was no significant  
19 correlation observed with the molecular markers and yield.

20

21 The number of genotypes per each amplified marker ranged from 9 to 69 (Table 3) while the  
22 number of amplified markers per cultivar ranged from 1 to 14 (Table 6). The marker with the  
23 highest number of occurrence among the cultivars was bean\_ssr\_2898 with 69 cultivars while

1 bean\_ssr\_2903 (9 cultivars) had the least number of occurrence among the cultivars studied. The  
2 SSR markers BARC\_PV\_SSR04725, bean\_ssr\_0778 and bean\_ssr\_2892 occurred in 51, 46 and  
3 44 cultivars respectively. The cultivar Ouro Negro (14 markers) had the highest number of markers  
4 while NABE 3 (1 marker) had the least number of markers. Amongst the landraces the cultivar  
5 with highest number of markers was Nabufumbo (11 markers) while the cultivars Kitinda and  
6 Wakiso brown had 2 markers each. For the commercial cultivars, NABE 2 (11 markers) had the  
7 highest number of markers while NABE 3 (1 markers) had the lowest occurrence of the markers.  
8 Amongst the introduced drought tolerant cultivars, SEN 92 (11 markers) and SCN-1 (10 markers)  
9 had the highest number of markers while SCN-12 (2 markers) had the lowest occurrence of the  
10 markers. It was observed that among the anthracnose resistant materials, 6 markers occurred in  
11 G2333 while Widusa had 2 markers. For the rust resistant materials, Ouro Negro (14 markers) had  
12 the highest number of markers, followed by PI181996 (11 markers) and Mexico 309 (10 markers)  
13 while PC50 had three markers. Seven cultivars were identified to have the highest number of  
14 occurrence of the broad-spectrum rust resistance SSR markers.

15

16

## **DISCUSSION**

17 The field screening of the Ugandan germplasm in the first planting seasons of 2014 and 2015 was  
18 useful in establishing the response of genotypes to rust from one season to another. The result of  
19 the analysis of variance showed significant differences for disease AUDPC, disease incidence and  
20 grain yield that indicated high variability among the genotypes' response to rust in both years and  
21 the possibility of obtaining genotypes with different genes for rust resistance among the different  
22 genotypes screened. The variation of the cultivars' response to rust complimented the work of Okii

1 et al. (2014), who had recommended that the high genetic variability among Ugandan common  
2 bean germplasm would be useful in selecting materials for breeding for biotic constraints.  
3  
4 The inconsistent responses of the genotypes in 2014 and 2015 could be attributed to either the  
5 changes in bean rust races structure or weather conditions. *U. appendiculatus* is known to be highly  
6 variable (Jochua et al., 2008; Liebenberg and Pretorius 2011). This variability may have  
7 contributed to the inconsistent response of the resistant check, Mexico 309 in 2014 and 2015. This  
8 cultivar has been reported to show 77.3% resistance when challenged with 88 rust races (Pastor-  
9 Corrales and Steadman, 2015). Since Mexico 309 showed some level of resistance (AUDPC=36)  
10 in 2015, it could be a useful source of resistance to rust in Uganda for some specific rust races.  
11 However, identification of the rust races within and between fields would need to be further  
12 investigated. The weather conditions at the NaCRRI research station at Namulonge varied in 2014  
13 and 2015. For instance, the average rainfall at NaCRRI in 2015 was higher (150 mm) than in 2014  
14 (119 mm). Nsubuga et al. (2011) had reported similar annual increase in rainfall within Namulonge  
15 where the research station is located. The increased rainfall and moisture in 2015 may have  
16 contributed to increase in rust disease pressure in field. Rust disease incidence and severity have  
17 been reported to be influenced by moist conditions such as prolonged periods of water on leaf  
18 surfaces (Harveson 2013). Under favorable conditions, rust disease cycles may repeat every 10 to  
19 14 days due to the macrocyclic nature of *U. appendiculatus* in which the urediniospore stage often  
20 called the “repeating” stage, which increase the amount of inoculum produced by the pathogen  
21 and thus increase the disease intensity and subsequently the disease severity (Pastor-Corrales and  
22 Liebenberg 2010). The strong association of rust disease incidence and AUDPC in 2014 and 2015  
23 suggested that the severity of rust disease increased with disease incidence in both years. This

1 association was similar to the report by Atkins (1973) in which rust disease levels increased with  
2 the disease intensity from season to season.

3

4 The use of the AUDPC as a disease severity measure and as a tool for plant resistance evaluation helps  
5 to reflect disease progress throughout the whole growing season (Campbell and Madden 1990). It can be  
6 used to determine and select promising parental genotypes that are useful in disease resistance  
7 breeding programmes (Ferreira et al. 2014). In this study, the rust differentials cultivars Aurora  
8 with the *Ur-3* and KW184 with the *Ur-4* had a consistent resistant response for the years 2014 and  
9 2015. This finding suggest that these genotypes could be used as source of resistance to rust in  
10 Uganda. These genotypes are among the sources of rust resistance reported to be suitable for East  
11 Africa by Kimani et al. (2001). The cultivars CNCPI181996, Kapchorwa white, G2333, SEN 80,  
12 SEN 46, DOR 500 and NABE 2 were also observed as resistant. The genotype CNCPI181996 was  
13 developed from the cross of two rust differentials with different resistant genes *CNC (Ur-CNC)*  
14 and *PI181996 (Ur-II)*. Genotypes with multiple rust resistant genes have been recommended as  
15 effective genetic resistance strategy to manage bean rust to provide a broader and longer lasting or  
16 more durable resistance (Wasonga and Porch 2010). Only one anthracnose resistant and three  
17 drought tolerant genotypes showed resistance. These genotypes would be excellent materials for  
18 breeding multiple stress resistant dry bean genotypes since the disease pathogens and weather  
19 patterns are becoming more variable and unpredictable (PABRA, 2015). The only commercial  
20 genotype and landrace to show resistance were NABE 2 and Kapchorwa white respectively. The  
21 genotype, NABE 2, is known to be drought tolerant, resistant to bean common mosaic virus  
22 (BCMV) and is commonly grown in the Northern region of Uganda (Ugen et al. 2014) while the  
23 landrace Kapchorwa white was collected from Kapchorwa district in the eastern region of Uganda.

1 The low number of indigenous cultivars from Uganda showing disease resistance have been  
2 reported by Atkins (1973) and Ddamulira et al. (2014). Nonetheless, the resistant cultivars  
3 identified could be indigenous sources of resistance, especially if the gene conferring rust  
4 resistance is identified (Kelly et al. 2003), which can supplement other existing exotic resistance  
5 sources to develop durable rust resistance in Uganda.

6  
7 The correlation of molecular markers and plant (such as leaf, pod and seed) reactions have been  
8 reported in common bean (Arnaud-Santana et al. 1994). Although all 22 markers were informative  
9 in this study, only three markers BARC\_PV\_SSR04725, bean\_ssr\_0778, bean\_ssr\_2892 were  
10 observed to be strongly associated with the mean disease severity. The marker, bean\_ssr\_0778 has  
11 been reported to be closely linked to the rust resistance gene *Ur-15* in PI 310762 (Shin et al. 2014).  
12 Since this marker is among the SSR markers closely associated with rust resistance among the  
13 Ugandan common bean germplasm, it would be suitable for use in marker assisted selection in a  
14 rust resistance breeding programme, Among the Ugandan common bean germplasm genotyped,  
15 seven cultivars with the highest number of markers were selected as sources of broad-spectrum  
16 rust resistance. The highest occurrence of the SSR markers for broad spectrum rust resistance  
17 where found in the introduced materials especially the rust differential materials. Similar  
18 observation was made by Bokosi et al. (1994). The cultivars Ouro Negro with the *Ur-14* gene,  
19 PI181996 with the *Ur-11* gene and Mexico 309 with the *Ur-5* gene had the highest presence of the  
20 broad-spectrum rust resistance and previously reported to have 98.9% and 77.3% resistance to 88  
21 rust races respectively (Pastor-Corrales and Steadman 2015). The landrace Nabufumbo, the  
22 commercial variety NABE 2 from the Andean background, and the drought tolerant cultivars SCN-  
23 92 and SCN-1 both from the Mesoamerican background, need to be further explored to ascertain

1 their rust resistance potential. The cultivar NABE 2 which was selected by the phenotypic and  
2 SSR marker screening methods can be considered a new source of rust resistance.

3 A comparison of the genetic background of the 15 cultivars selected as potential sources for rust  
4 resistance by the phenotypic and genotypic screening, indicated that cultivars from the small-  
5 seeded Mesoamerican (76.7%) and large-seeded Andean (23.3%) background were resistant to  
6 rust. However, most of the resistance materials were of the Mesoamerican background. In Uganda,  
7 farmer preferred common bean cultivars derived from the large-seeded Andean background  
8 (Kiwuka et al. 2012; Okii et al. 2014). Still broad based resistance to bean rust can be best achieved  
9 by combining different sources of resistance from both the Andean and Mesoamerican gene pools  
10 as suggested by Pastor-Corrales and Steadman (2015) to provide durable resistance and more  
11 effectively manage the bean rust disease.

12

13

### **CONCLUSION**

14 In this study, 15 different sources of resistance to bean rust from the Andean (26.7%) and  
15 Mesoamerican (73.3%) genetic background were identified. The phenotypic screening identified  
16 nine cultivars with different rust resistance response which provided a range of promising sources  
17 for rust resistance. However, genotypic screening identified seven cultivars with 10 to 14 amplified  
18 SSR markers associated with resistance. The Andean cultivar NABE 2 was selected by the both  
19 phenotypic and SSR marker screening methods can be considered a new source of rust resistance  
20 in Uganda. This study provided a range of SSR markers and sources of resistance that would be  
21 useful in breeding for resistance to bean rust in Uganda and in other breeding programmes  
22 worldwide.

23

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6

7 **Conflict of interest**

8 The authors declare that they have no conflict of interest.

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**Table1: Description of the 138 Ugandan common bean collection screened for resistance to rust disease**

S/N	‡Genotype	Genotype Type	Stress response	Gene pool
1	NABE 1	Commercial	Susceptible to multiple constraints	Andean
2	NABE 2	Commercial	BCMV resistant/ Drought tolerant	Mesoamerican
3	NABE 3	Commercial	Bean common mosaic virus BCMV resistant	Mesoamerican
4	NABE 4	Commercial	CBB resistant/ALS	Andean
5	NABE 5	Commercial	CBB resistant	Andean
6	NABE 6	Commercial	Unknown	Mesoamerican
7	NABE 11	Commercial	CBB resistant/ALS	Andean
8	NABE 13	Commercial	Root rot/low soil fertility tolerant	Andean
9	NABE 14	Commercial	Root rot/low soil fertility tolerant	Andean
10	NABE 15	Commercial	Anthracnose tolerant	Andean
11	NABE 16	Commercial	Anthracnose tolerant	Andean
12	NABE 17	Commercial	Anthracnose, BCMV, ALS tolerant	Andean
13	NABE 18	Commercial	Anthracnose, BCMV, ALS tolerant	Andean
14	NABE 19	Commercial	Anthracnose, BCMV, ALS tolerant	Andean
15	NABE 20	Commercial	Anthracnose, BCMV, ALS tolerant	Andean
16	NABE 21	Commercial	Anthracnose, BCMV, ALS tolerant	Andean
17	NABE 22	Commercial	Anthracnose, BCMV, ALS tolerant	Andean
18	NABE 23	Commercial	Anthracnose, BCMV, ALS tolerant	Andean
19	K131	Commercial	BCMV/black root/ anthracnose resistant	Mesoamerican
20	K132	Commercial	Unknown	Andean
21	DAB 474	Introduced	Drought tolerant	Andean
22	DAB 475	Introduced	Drought tolerant	Andean
23	DAB 478	Introduced	Drought tolerant	Andean
24	DAB 479	Introduced	Drought tolerant	Andean
25	DAB 480	Introduced	Drought tolerant	Andean
26	DAB 482	Introduced	Drought tolerant	Andean
27	TU	Introduced	Anthracnose differential	Mesoamerican

<b>S/N</b>	<b>‡Genotype</b>	<b>Genotype Type</b>	<b>Stress response</b>	<b>Gene pool</b>
28	TO	Introduced	Anthracnose differential	Mesoamerican
29	Michigan dark red kidney	Introduced	Anthracnose differential	Andean
30	Michelite	Introduced	Anthracnose differential	Mesoamerican
31	Widusa	Introduced	Anthracnose differential	Mesoamerican
32	PI207262	Introduced	Anthracnose differential	Mesoamerican
33	AB 136	Introduced	Anthracnose differential	Mesoamerican
34	G2333	Introduced	Anthracnose differential	Mesoamerican
35	SCN-4	Introduced	Drought tolerant	Mesoamerican
36	SCN-6	Introduced	Drought tolerant	Mesoamerican
37	SEN-80	Introduced	Drought tolerant	Mesoamerican
38	SCN-5	Introduced	Drought tolerant	Mesoamerican
39	SEN-34	Introduced	Drought tolerant	Mesoamerican
40	DOR-500	Introduced	Drought tolerant	Andean
41	SCR-5	Introduced	Drought tolerant	Mesoamerican
42	SCR-35	Introduced	Drought tolerant	Mesoamerican
43	SCN-10	Introduced	Drought tolerant	Mesoamerican
44	SCN-12	Introduced	Drought tolerant	Mesoamerican
45	SCN-37	Introduced	Drought tolerant	Mesoamerican
46	DOR-364	Introduced	Drought tolerant	Andean
47	SEN-95	Introduced	Drought tolerant	Mesoamerican
48	SCN-8	Introduced	Drought tolerant	Mesoamerican
49	SEN-46	Introduced	Drought tolerant	Mesoamerican
50	SCN-1	Introduced	Drought tolerant	Mesoamerican
51	SCR-26	Introduced	Drought tolerant	Mesoamerican
52	SEN-56	Introduced	Drought tolerant	Mesoamerican
53	SCR-18	Introduced	Drought tolerant	Mesoamerican
54	SEN-92	Introduced	Drought tolerant	Mesoamerican

S/N	‡Genotype	Genotype Type	Stress response	Gene pool
55	SCN-3	Introduced	Drought tolerant	Mesoamerican
56	SCR-25	Introduced	Drought tolerant	Mesoamerican
57	SEN-90	Introduced	Drought tolerant	Mesoamerican
58	California small white (CSW)643	Introduced	Rust differential,1983	Mesoamerican
59	PC50	Introduced	Rust differential,2002	Andean
60	US#3	Introduced	Rust differential,1983	Andean
61	NEP 2 (G5693)	Introduced	Rust differential,1983	Mesoamerican
62	Redland Pioneer	Introduced	Rust differential,2002	Andean/Mesoamerican
63	GN1140	Introduced	Rust differential,2002	Mesoamerican
64	Early Gallatin	Introduced	Rust differential,2002	Andean
65	#Mexico 309	Introduced	Rust differential,2002	Mesoamerican
66	Compuesto Negro Chimaltenango(CNC)	Introduced	Rust differential,2002	Mesoamerican
67	Montcalm	Introduced	Rust differential,2002	Andean
68	DAB 476	Introduced	Drought tolerant	Andean
69	DAB 477	Introduced	Drought tolerant	Andean
70	Mexico 235	Introduced	Rust differential,2002	Mesoamerican
71	Ecuador 299	Introduced	Rust differential,1983	Mesoamerican
72	Kentucky Wonder (KW) 814	Introduced	Rust differential,1983	Mesoamerican
73	CIAT Aurora	Introduced	Unknown	Unknown
74	51051	Introduced	Rust differential,1983	Mesoamerican
75	CNCPI181996	Introduced	Unknown	Unknown
76	Aurora	Introduced	Rust differential, 2002	Mesoamerican
77	Teebus	Introduced	Rust resistant	Unknown
78	#Ouro Negro	Introduced	Rust resistant	Mesoamerican
79	PI 181996	Introduced	Rust differential,2002	Mesoamerican
80	PI 260418	Introduced	Rust differential,2002	Andean
81	Golden Gate Wax (GGW)	Introduced	Rust differential,2003	Andean



S/N	†Genotype	Genotype type	Gene pool	S/N	Genotype	Genotype type	Gene pool
82	Kamuli Yellow	††Landrace	Unknown	101	Kamuli black	Landrace	Unknown
83	Lira Yellow	Landrace	Unknown	102	Kamula	Landrace	Unknown
84	Kajeru	Landrace	Unknown	103	Kaborole red	Landrace	Unknown
85	Kinbwogegwa	Landrace	Unknown	104	Lira Pink	Landrace	Unknown
86	Kamwenge Purple	Land race	Unknown	105	Kaborole Purple	Landrace	Unknown
87	Mukono cream	Landrace	Unknown	106	Kitinda	Landrace	Unknown
88	Kamuli Purple	Landrace	Unknown	107	Obuhiumbaobukere	Landrace	Unknown
89	Mpigi Pink	Land race	Unknown	108	Wakiso cream	Landrace	Unknown
90	Masindi red	Land race	Unknown	109	Masindi Purple	Landrace	Unknown
91	Mutike	Landrace	Unknown	110	Nambale (U00143)	Landrace	Unknown
92	Kanyebwa long	Landrace	Unknown	111	Ndume (U00069)	Landrace	Unknown
93	Nkalyebawere	Landrace	Unknown	112	Mukono cream	Landrace	Unknown
94	Mbarara Purple	Landrace	Unknown	113	Kamwenge cream	Landrace	Unknown
95	Kapchorwa White	Landrace	Unknown	114	Zebra	Landrace	Unknown
96	Kaborole Maroon	Landrace	Unknown	115	Kanyebwa (U00271)	Landrace	Unknown
97	Nabufumbo	Landrace	Unknown	116	Wakiso brown	Landrace	Unknown
98	Bumwufu	Landrace	Unknown	117	Mpigi white	Landrace	Unknown
99	Lira White	Landrace	Unknown	118	Kamuli White	Landrace	Unknown
100	Kahura	Landrace	Unknown	119	Kaborole cream	Landrace	Unknown

S/N	†Genotype	Genotype type	Gene pool	S/N	Genotype	Genotype type	Gene pool
120	Mukono cream	Landrace	Unknown	131	Mukono black	Landrace	Unknown
121	Kankulyembaluke	Landrace	Andean	131	U00236	Landrace	Unknown
122	Kanyawama	Landrace	Unknown	132	Kamuli pink	Landrace	Unknown
123	Roba	Landrace	Unknown	133	Apac cream	Landrace	Unknown
124	Masindi yellow	Landrace	Andean	134	Masindi cream	Landrace	Unknown
125	Kamuli red	Landrace	Unknown	135	Kamwenge Maroon	Landrace	Unknown
126	Kamwenge red	Landrace	Unknown	136	Masaka red	Landrace	Unknown
127	Apac pink	Landrace	Unknown	137	Masaka yellow	Landrace	Unknown
128	Kanyamunyo	Landrace	Unknown	138	Nyekera	Landrace	Unknown
129	Apac pink	Landrace	Unknown				

†source of materials and information from NaCRRI, Uganda; ‡ source of materials and information from CIAT, Uganda and University of Nebraska, Lincoln, USA; §susceptible check; #resistant check; BCMV, Bean common mosaic virus; CBB, Common bacterial blight; ALS, Angular leaf Spot; †† the stress responses of the Ugandan landraces are unknown.

**Table 2: Analysis of variance of the means of disease incidence, yield and AUDPC of 138 bean cultivars infected with bean rust in Uganda.**

Source of variation	DF	Incidence	AUDPC	Yield
			MS	
Genotype	137	3265.7***	1312.3***	496670***
Year	1	35452.7***	2680.7ns	205604560***
Genotype. Year	137	3206.2***	1297.2***	488368***
Error	548	580.2	467.6	309388

DF: degree of freedom, MS: Mean square, Values with \*, \*\* and \*\*\* implies significant at P = .05, P < .01 and P < .001 respectively; ns: not significant

**Table 3: Correlation of the means of disease incidence, yield and AUDPC of 138 bean cultivars infected with bean rust in Uganda.**

Traits	AUDPC	Yield
AUDPC	-	
Yield	-0.1083**	-
Incidence	0.5490***	0.0482ns

Values with \*\* and \*\*\* implies significant at P = 0.01 and 0.001 respectively; ns: not significant

**Table 4: 22 SSRs markers, allele size, frequency, allele number, gene diversity and PIC of 138 genotyped common beans in Uganda**

†Marker	Allele size (base pair)	Major. Allele. Frequency	Allele Number	Gene Diversity	PIC	Number of cultivar per markers
<b>bean_ssr_2903</b>	288	0.9	2	0.1	0.1	9
<b>BARC_PV_SSR04719</b>	192	0.9	2	0.2	0.1	13
<b>BARC_PV_SSR04728</b>	288	0.9	2	0.2	0.1	13
<b>SSRbeanur036</b>	285	0.9	2	0.2	0.2	15
<b>bean_ssr_2895</b>	257	0.9	2	0.2	0.2	19
<b>BARC_PV_SSR04703</b>	178	0.8	3	0.3	0.3	26
<b>bean_ssr_2909</b>	266	0.8	2	0.3	0.3	25
<b>bean_ssr_1168</b>	234	0.8	2	0.4	0.3	30
<b>bean_ssr_0669</b>	233	0.8	2	0.4	0.3	35
<b>BARC_PV_SSR04425</b>	234	0.7	2	0.4	0.3	36
<b>bean_ssr_0778</b>	276	0.7	2	0.4	0.3	46
<b>BARC_PV_SSR04722</b>	287	0.7	2	0.4	0.3	40
<b>bean_ssr_2904</b>	200	0.7	2	0.4	0.3	46
<b>bean_ssr_2892</b>	228	0.7	2	0.4	0.3	44
<b>BARC_PV_SSR04721</b>	227	0.7	2	0.4	0.3	69
<b>bean_ssr_1170</b>	278	0.7	2	0.5	0.3	55
<b>bean_ssr_0169</b>	297	0.6	2	0.5	0.4	11
<b>bean_ssr_2906</b>	206	0.6	2	0.5	0.4	53
<b>BARC_PV_SSR04725</b>	177	0.6	2	0.5	0.4	51
<b>bean_ssr_2898</b>	278	0.6	2	0.5	0.4	63
<b>bean_ssr_2901</b>	144	0.6	2	0.5	0.4	62
<b>bean_ssr_1167</b>	257	0.5	2	0.5	0.4	50
Mean		0.8	2.0	0.3	0.3	

PIC: polymorphism information content; † Shin et al. (2014)

**Table 5: Genotypic–phenotypic association indicating the trait, marker and p-values.**

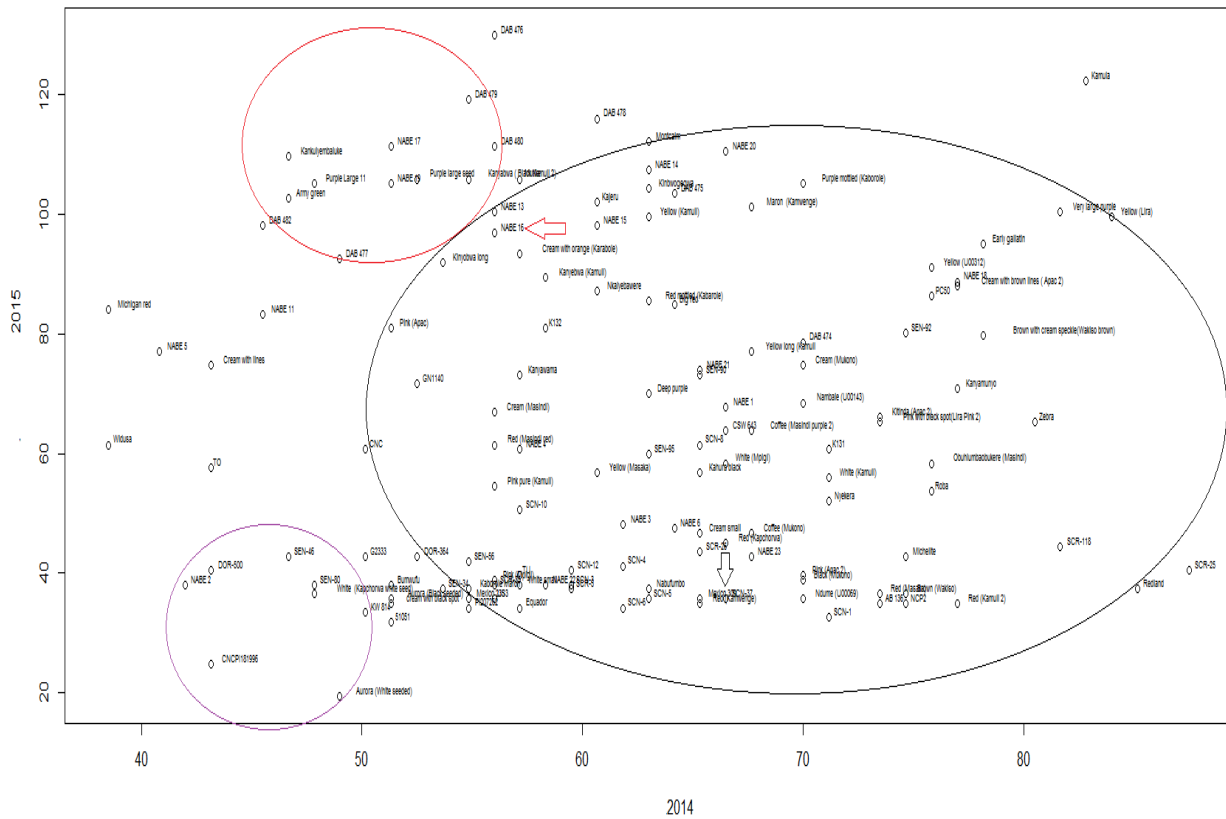
<b>Trait</b>	<b>Marker</b>	<b>Chromosome</b>	<b>Position</b>	<b>Marker_F</b>	<b>P</b>	<b>Estimate</b>
Rust severity	<b>bean_ssr_0778</b>	Pv04	6	15.12244	1.57E-04***	0.20566
Rust severity	<b>BARC_PV_SSR04725</b>	Pv04	20	12.78092	4.85E-04***	7.34887
Rust severity	<b>bean_ssr_2892</b>	Pv04	11	4.81404	0.02993*	2.06379
Seed yield	<b>bean_ssr_1167</b>	Pv04	0	3.81605	0.05282ns	-5.0077

p: p-value; Values with \*and \*\*\* implies significant at P = .05 and P < .001 respectively; ns: not significant

**Table 6: Selected common bean genotypes from the Ugandan germplasm evaluated for rust disease incidence, AUDPC, severity and seed yield in 2014 and 2015, the number of SSR markers present and their resistance response (HR)**

Genotype	Incidence (%)		AUDPC		Severity		Yield (kg/ha)		SSR	
	2014	2015	2014	2015	2014	2015	2014	2015	markers	HR
Aurora	0	0	49	19	1	1	1566	1300	9	R†
KW 814	0	0	26	33	1	1	1300	1476	7	R
CNCPI181996	0	0	30	25	1	1	1461	1598	5	R
G2333	0	0	28	58	2	3	1420	1042	6	R
SEN-80	0	0	30	37	1	1	1820	642	8	R
DOR-500	2	0	42	51	1	1	1733	320	6	R
SEN-46	7	0	37	35	2	1	1586	1434	3	R
NABE 2	11	0	49	44	2	1	1686	642	11	R
Kapchorwa white	0	0	40	35	1	1	2126	542	6	R
NABE 15	97	92	49	133	4	4	1826	308	3	I
NABE 16	96	87	26	123	4	3	1860	426	2	I
NABE 5	52	100	37	65	4	3	1846	308	6	I
Mexico 309	33	0	65	56	3	4	1760	358	10	I
CNC	67	0	50	61	4	3	1446	956	9	I
Mexico 235	0	0	55	46	3	3	1353	516	2	I
DAB 478	91	92	61	116	4	5	1473	1480	3	S
DAB 479	89	87	55	119	4	5	2973	400	6	S
Kamula	67	100	83	122	5	4	1466	856	3	S

† Genotype response; R= Resistant, I= Intermediate, S= Susceptible



**Fig. 1.** Comparison of the AUDPC values ( $r=0.109$ ) for Ugandan common bean germplasm screened in 2014 and 2015 at the Namulonge Research Station, Uganda. (Note: Black arrow=resistant cultivar, Mexico 309; Red arrow=susceptible cultivar, NABE 16)