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APPLICATION OF MORPHO-ANATOMICAL TRAITS OF MAIZE PLANT TO QUALITY CONTROL AND QUALITY ASSURANCE IN MAIZE SEED SYSTEM

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ABSTRACT

Good seed quality can be obtained through control of the entire seed production process. There are emerging issues in the seed sector due to seed quality in market. In many countries seed and planting materials available to farmers are often of insufficient quality, thus undermining the potential performance of crops. The objective this study was to use morpho-anatomical traits to determine the levels of maize hybrid seed purity on the market in Uganda. A total of 120 samples of four popular commercial maize hybrid were collected from agro-dealers in 13 districts, representing 3 seed companies' distribution network. The samples were planted and evaluated based on their descriptors, using an Alpha Lattice Design, with two replications at the National Crops Resources Research Institute (NaCRRI), Namulonge. Data were collected for 33 morpho-anatomical traits. Results showed significant (P<0.05) differences within hybrids, and sources of seed among districts. Among the test materials, only Longe 9H had no significant (P<0.05) difference within traits but others had some levels of significant (P<0.05) differences. The principal component analysis of quantitative traits resulted in four components for each variety, with the explanation total of 53.3% for Longe 6H, 51.1% for Longe 7H, and 92.8% for Longe 9H and 39.1% for Longe 10%. There were varying levels of contamination of commercial hybrids based on morpho-anatomical traits diversity within hybrids, compared to their descriptors for the traits evaluated. Hence, there is need to strengthen the quality control sub-sector for improved productivity at farmer levels.

Key Words: Diversity, Longe, seed, Uganda, Zea mays

RÉSUMÉ

Des semences de bonne qualité peuvent être obtenues en exerçant un control systématique sur chacun des maillons du système de production semencière. Le secteur semencier connaît de plus en plus de difficultés, et ceci en raison de la qualité des semences sur le marché. Dans plusieurs pays, les semences disponibles pour les producteurs sont souvent de qualité non satisfaisante, reduisant ainsi la performance des cultures. L'objectif de l'étude était de se servir des caractères morpho-anatomiques afin de determiner le niveau purité des semences de maïs hybride sur le marché semencier en Ouganda. Au total 120 échantillons des quatre variétées commerciales populaires de maïs hybride ont été collectés chez les distributeurs de semences dans 13 districts, représentant les réseaux de distribution de 3 compagnies semencières. Les échantillons ont été semés et évalués en se basant sur leurs descripteurs. Le dispositive expérimental Alpha Lattice a été utilisé, avec deux répétitions à l'institut national de recherche des ressources culturales (NaCRRI), Namulonge. Les données de 33 caractères morpho-anatomiques ont été collectées. Les résultats ont montré des differences significatives (P<0,05) ont été observées parmi les hybrides, ainsi que les sources des semences au sein des districts. Parmi les matériels testés, seul Longe

9H n'avait exhibé aucune difference significative (P<0,05) des caractères évalués, mais certains ont des differences significatives à (P<0,05). l'analyse en composntes principales des traits quantitatifs a montré quatre composantes pour chaque variété, avec l'ensemble de ces composantes expliquant 53,3% de la variation totale pour Longe 6H, 51,1% pour Longe 7H, 92,8% pour Longe 9H et 39,1% pour Longe 10%. Les niveaux de contamination étaient variables, suggérant la nécessité de renforcer le sous-secteur de control de qualité en vue d'une meilleure productivité au niveau des producteurs.

Mots Clés: Diversité, Longe, semence, Ouganda, Zea mays

INTRODUCTION

Development of seed supply systems is a prerequisite for ensuring food security, especially in Sub-Saharan Africa (SSA) (FNSU-UDS, 2011). A regulated seed system is crucial for amplified production and productivity of maize, a highly demanded cereal crop in SSA. Formal and informal seed production and supply systems are recognised as the two systems of seed production and supply in SSA (Mubangizi *et al.*, 2012).

The formal seed production and supply system in countries like Uganda generate high-quality seeds of genetically improved hybrids (Barnett *et al.*, 2011). It aims at enabling farmers access seed of high quality, as well as being free from seed-borne pathogens. Production of high quality seeds is accomplished by regular and rigorous monitoring and supervision of the seed crop in the field, and after harvest during processing and packaging, as well as confirmation of quality through laboratory tests before sale to farmers. Thus, seeds are expected to have better yield potential than seeds from the informal seed sector (farmer-saved and community-based seed systems).

Good quality seed is the cheapest input in crop production chain, and the key to agriculture development. The status of a crop largely depends on the seed materials used for sowing, provided other conditions of weather and nutrients are kept optimum (Mrutu, 2015). It is estimated that good quality seeds of improved hybrids can contribute about 20 to 25% increase in yield (Beshir, 2011, Mrutu, 2015). Poor access to quality seeds by farmers is a major crop production constraint in Uganda.

Despite all efforts to ensure that high quality seeds are available to farmers, the Uganda seed sector is characterised by counterfeit seeds, which leads to an inability of farmers to exploit the full potential of improved hybrids (Mubangizi et al., 2012). Barnett et al. (2011) reported that 40% of seed available on the market in Uganda is counterfeit. The presence of counterfeit seeds on the market reduces farmers' confidence in the seed supply chain, and in turn, affects the adoption of improved hybrids. In order for farmers to obtain high grain yields, it is important that the purity of seed be maintained at all levels, right from breeder seed up to certified seed supplied on the market.

Uganda's formal seed sector focuses on regulation in all stages of production, from breeder seed production, basic and certified seed bulking, processing, certification and seed distribution. Most of the crop hybrids on the market in Uganda have been developed and/or released by the public sector, through the National Agricultural Research Organization (NARO). Also, private sectors have commercialised a number of their own hybrids, although the process of variety testing for release, quality control in seed production and regulation of the seed market are all done by the National Seed Certification Service (NSCS) as provided for in the law (Plant and Seed Act, 2006).

As a result, the public sector has been responsible for most of the early generation seed production; while most of the small to medium scale seed companies concentrate on production of basic and certified seed. In addition, the public sector concentrates much on less commercial crops such as millet, rice, and cowpeas. Maize is the major crop commodity for private sector seed commercialisation, however, limited amounts of other crops such as sunflower, sesame, sorghum, beans, and vegetables are being commercialised. Seed production by most of the seed companies is primarily through contract farmers while

distribution and sales of seeds are effected through the agro-dealer network.

Currently, in Uganda, seed crop certification for purity and variety distinctness by the National Seed Certification Service (NSCS), is based on phenotypic/morpho-anatomical traits as provided for in their description. The phenotypic diversity is an important morpho-anatomical traits that reveals important features for verification of variety distinctness. During the quality control of seeds, most of the quality components are based on morpho-anatomical traits. These characters have been recognised to constitute universally acknowledged descriptors for varietal characterisation and inaugurating the distinctness, uniformity and stability (DUS) of crops in Plant Variety Protection (PVP) system (Begum and Kumar, 2011; Chanda et al., 2014).

The varietal traits used in DUS also accounts for the plasticity of phenotypic appearances and, thus are proficient for comparing hybrids (Law *et al.*, 2011). Despite the drawback of morpho-anatomical traits being expensive and affected by the environment, they are still very important for breeding high yielding genotypes and discriminations (Hung *et al.*, 2012; Law *et al.*, 2011; Smýkal *et al.*, 2008). Morpho-anatomical traits are, therefore, important for variety release, maintenance and seed certification.

The objective this study was to use morphoanatomical traits to determine the levels of maize hybrid seed purity on the market in Uganda.

MATERIALS AND METHODS

The study was conducted at the National Crops Resources Research Institute (NaCRRI), Namulonge (0.5297 latitude, 32.6025 longitude, 1150N elevation (masl)) in Central Uganda. In 2013/14, a total of 120 samples of four maize hybrids comprising 116 samples were collected randomly from agro-dealers in 13 districts and four samples from seed companies (reference samples) were used in the study (Table 1). Among these comprised of four popular maize hybrids maize hybrids namely; Longe 6H, Longe 7H, Longe 9H and Longe 10H from three seed companies (Seed Co A, Seed Co B, and Seed Co C. in Uganda. Entries (herein referred to as

samples) were randomised in Breeding Management System (The IBP Breeding Management System Version 3.0.8) and planted using an Alpha Lattice experimental design, which was a four by thirty simple lattice, with two replications. Each entry was planted in two-row plots of 5 metres length and 0.75 m apart. The hills were spaced 0.25 metres apart. Two seeds per hill were planted and later thinned at three weeks after emergence to one plant per hill to give a plant population of 53,333 plants per hectare. Standard agronomic and cultural practices were performed as recommended.

During the growing period, data on several morpho-anatomical traits were collected. For each source, 10×10 -m rows were planted, data on morphological traits were collected according to procedures and guidelines outlined by the International Union for the Protection of New Hybrids of Plants (UPOV, 2009) (Table 2).

The mean values for 33 morphological characters and scaling values were used to assess the dissimilarity between the reference materials from Seed Companies and samples from Agrodealers. The matrix of all quantitative traits was first standardised before calculating the Euclidean similarity distance matrix among the hybrids.

For each trait, an individual analysis of variance (ANOVA) was conducted using GenStat 12th Edition.

Principal Component Analysis (PCA) was performed on the phenotypic correlation matrix of the adjusted means, so as to estimate the relationships between the traits (Ignjatoviæ-Miciæ *et al.*, 2013), using MINITAB 14. Also, the qualitative data were subjected to PCA to identify traits that are most discriminatory among hybrids sources. A dendrogram was constructed using Ward method (Ward, 1963) to provide a general visualisation of the relationship between hybrids based on quantitative traits using GenStat 12th Edition.

RESULTS

Analysis of variance. Longe 10H had the most traits with significant differences (time of anthesis, density of spikelets, time of silk emergence, length of the main axis above lowest

TABLE 1. Samples used and their origins in a study on maize seed quality control in Uganda

Region	District	Hybrids	Seed companies	Hybrids
East	Soroti	Longe 9H	Company A	Longe 7H
		Longe 7H (4) Longe 6H (3) Longe 10H (8)	Company B	Longe 6H Longe 9H Longe 7H
	Kumi	Longe 10H (2)	Company C	Longe 10H
	Bukedea	Longe 6H Longe 7H		
	Iganga	Longe 6H Longe 7H (3) Longe 9H Longe 10H		
	Bugiri	Longe 10H		
	Mbalala	Longe 10H		
North	Lira	Longe 10H (2) Longe 6H (2)		
West	Masindi	Longe 6H (8) Longe 10H (4)		
	Hoima	Longe 6H Longe 10H (2)		
	Bweyale	Longe 6H		
	Kigumba	Longe 6H		
South-west	Muhabura	Longe 6H		
Central	Masaka	Longe 10H		

Seed Companies are identified by A, B, C to maintain anonymity

side branch, length of peduncle, ear length) (Table 3). This was followed by Longe 7H (time of anthesis and anthocyanin coloration of brace roots). Lastly was Longe 6H (attitude of blade and anthocyanin colouration of base of glume were significant) (Table 3). Among the test materials, only Longe 9H had no significant difference within traits.

Principal component analysis. Principal component analysis (PCA) was used to determine

the key traits which can be used to differentiate within the same variety. The PCA of the traits resulted in the first four components for each variety, with the highest eigenvalue. The four PCs explained a total of 53.3% for Longe 6H (Table 4), 51.1% for Longe 7H (Table 5), 92.8% for Longe 9H (Table 6), and 39.1% for Longe 10H (Table 7). Longe 9H, which had the highest PCs value explaining the phenotypic variation showed that the first component accounted for 40.7% of the total variation. The second, third and four

TABLE 2. The traits that were collected using visual scoring method

Trait	Property	Scale
Anthocyanin coloration (an col)	2 Leaves unfolded	Score (1-9)
Shape of tip (shape t)	4 Leaves unfolded	Score (1-5)
Angle between blade and stem (angle bs)	Leaf just above upper ear	Score (1-9)
Attitude of blade (at of b)	Leaf just above upper ear	Score (1-9)
Degree of zig-zag	Beginning of anthesis	Score (1-3)
Anthocyanin coloration of brace roots (an col br)	Halfway anthesis	Score (1-9)
Time of anthesis (t an)	Middle third of main axis	Score (1-9)
Anthocyanin coloration of base of glumes (an col bg)	Middle third of main axis at base of glumes	Score (1-9)
Anthocyanin coloration of glumes excluding base (an col gex)	Middle third of main axis	Score (1-9)
Anthocyanin coloration of anthesis (an col anth)	Middle third of main axis on fresh anthesis	Score (1-9)
Density of spikelets (de spi)	Middle third of main axis	Score (3-7)
Angle between main axis and lateral branches	Middle third of tassel	Score (1-9)
Attitude of lateral branches (at lb)	In lower third tassel	Score (1-9)
Number of primary lateral branches (no plb)	Halfway anthesis	Score (1-9)
Time of silk emergence (t of se)	Upper ear	Score (1-9)
Anthocyanin coloration of silks (an col of s)	Upper ear	Score (1,9)
Intensity of anthocyanin coloration of silks (in of an col s)	Upper ear	Score (1-9)
Anthocyanin coloration of sheath (an col sh)	Middle of plant	Score (1-9)
Anthocyanin coloration of internodes (an col inno)	Middle of plant	Score (1-9)
Length of main axis above lowest side branch (le malb)	Watery ripe grain	Score (1-9)
Length of main axis above upper side branch (le maub)	Watery ripe grain	Score (1-9)
Width of blade (w of b)	Leaf of upper ear	Score (1-9)
Number of off types		Numbers
Length of peduncle (le of peduncle)	Upper ear	Score (1-9)
Length of husk off the tip of the ear (le hu ti e)	Upper ear	Score (1-9)
Ear length (e le)	Upper ear without husk	Score (1-9)
Ear diameter (e di)	Middle of ear	Score (1-9)
Ear shape (e sh)	Hard grain	Score (1-3)
Ear number of rows (e no rows)	Middle of ear	Score (1-9)
Type of grain (type g)	Middle third of ear	Score (1-9)
Colour of grain (col g)	Hard grain	Score (1-9)
Colouration of dorsal side of grain (col dsg)	Hard grain	Score (1-9)
Anthocyanin coloration of glumes of cob (an col gc)	Hard grain	Score (1,9)
Intensity of anthocyanin coloration of glumes of cob (in an col gc)	Hard grain	Score (1-9)

Scale adopted from UPOV (2009)

components for Longe 9H accounted for 22%, 19% and 11.1%, respectively (Table 6). The most important traits for discriminating the hybrids samples were angle between main axis and lateral branches, the attitude of lateral branches, time of silking, Anthocyanin colouration, anthocyanin colouration of anthesis, time of silking

anthocyanin colouration of silks and density of spikelets.

The visualisation of PC scores for Longe 6H showed that different samples distributed separately in the two-dimension area, and agrodealer samples from Muhabura-45, Kigumba-44, Iganga-96, Bweyale-43, Masindi-(19, 20, 16, 102,

TABLE 3. Summary statistical of the phenotypic traits that showd significant difference

Source			Longe 10H	10Н			Longe 7H	HZ	Longe 6H	Н9
	TAnth	De Spi	T of Sel	Le MALB	Le of Ped	E Le	Anth BR	TAnth	AtofB	Anth Col BG
M.S SED LSD	2.50** 1.26 2.53	1.00 t 1.00 t	2.86*** 1.01 2.03	2.38* 1.31 2.63	4.66*** 1.67 3.34	3.01** 1.4 2.81	11.38*** 2.02 4.33	1.91** 0.98 2.1	1.69* 1.03 2.2	7.81*** 1.85 3.95

Traits: T Anth = Time of anthesis. De Spi = density of spikelets. T of S = time of silk emergence, Le MALB = length of main axis above lowest side branch. Le of Ped = length of pedunde, E Le = ear length, Anth BR = anthocyanin coloration of brace roots, T anth = Time of anthesis, At of B = attitude of blade, Anth Col BG = anthocyanin coloration of base of glume. M.S = Mean of squares, SED = Standard error of difference, LSD = Least significant difference, *= Significant at P<0.05, **= Significant at P<0.01, *** = Significant at P<0.001 and 15) and Luwero-95 were grouped with the seed company sample, which represented 40.7% of the total number of the 27 samples. This implies that 50.3% of the samples were not grouped together with seed company reference samples, indicating that they were contaminated or not similar to the references.

Score plot of Longe 7H showed that, agrodealer samples from Iganga-(106, 115, 112, 107, 114, 27 and 30), Bukedea-42, Soroti-(66, 10 and 2) and Masindi-(105 and 113) were grouped with the reference sample from Seed Company-A; whereas samples from Iganga-27 were the only samples grouped with the second Seed Sompany-B. The score plot of Longe 9H showed that only one agro-dealer's sample from Iganga-119 was grouped with the Seed Company's reference, which represented 33.3% of the total number of the samples 6. This implies that the rate of variation from the Seed Companies was 66.7%. The score plot of Longe 10H showed that the seed company sample had grouped alone as an outlier.

Cluster analysis of hybrids. Results of the cluster analysis based on Euclidean distance clustered hybrids into three to five main groups. Longe 10H samples fell into four groups, with a similarity level of 84%. The first cluster included only one agro-dealer sample, which was the seed company's sample. The second cluster contained 47 agro-dealer samples, which were sub-grouped into three different groups. The third contained one agro-dealer sample; while the fourth group included two agro-dealer samples (Fig. 1).

Longe 9H clustered into four groups, with 81% level of similarity. The first group contained two agro-dealer samples, including the seed company's sample. The second group contained two agro-dealer samples; the third one contained one agro-dealer sample as well as the fourth group (Fig. 2).

Longe 7H clustered into four groups, with 85% level of similarity. The first group contained 17 agro-dealer samples, which included the seed company-A. The second group included one agro-dealer sample as well as the fourth group. While the third group contained three agro-dealer samples and a sample from seed company-B (Fig. 3).

TABLE 4. Longe 6H eigenvectors, eigenvalues, the individual and cumulative percentage of variation explained by the first four principal components (PCs)

Trait*	PC1	PC2	PC3	PC4
An Col	-0.05	-0.14	0.05	0.29
Shpe T	-0.25	-0.22	0.07	0.07
Angle BS	-0.18	-0.07	0.3	0.16
At of B	-0.2	-0.01	0.18	-0.11
Degree Zig	-0.16	0.08	0.22	-0.04
An Col BR	0.19	-0.09	0.21	0.13
T An	0.25	-0.29	0.04	0.06
An Col BG	0.22	-0.22	0.23	-0.1
An Col Gex	0.22	0.22	0.14	-0.14
An Col Anth	0.18	-0.15	0.2	-0.19
De Spi	0.07	-0.13	0.02	-0.06
Angle MI	0.08	0.01	0.36	0.11
At of LB	0.03	0.11	0.37	0
No of PLB	-0.21	0.23	0	0.2
T of SE	0.25	-0.29	0.02	-0.06
An Col of S	0.08	-0.11	-0.04	0.36
In of An Col S	-0.09	0	-0.03	0.43
An Col Sh	0.1	-0.21	-0.18	-0.26
An col InNO	0.28	-0.16	-0.12	0.01
Le MALB	0.17	0.13	-0.08	0.14
LeMAUB	0.18	0.03	0.05	0.23
W of B	-0.05	0.28	-0.19	0.07
Le Peduncle	0.02	0.25	0.15	-0.14
Le Hu Ti E	-0.09	-0.03	0.27	-0.07
E Le	0.14	0.24	-0.27	-0.04
E Di	0.2	0.17	0.1	0.2
Ea Sh	0.07	0.06	0.26	0.25
E No Rows	0.27	0.15	0.01	0.02
Type G	-0.1	0.21	0.2	-0.22
Col G	0.22	0.17	0.03	-0.02
Col DSG	0.2	0.26	0.01	0.06
An Col GC	0.22	0.22	0.14	-0.14
In An Col GC	0.17	0.02	-0.1	0.25
Cumulative (%)	20	34	44.9	53.3

^{*}Traits corresponding to the underlined numbers are the most significant traits that contributed much of the variation in each PC

Longe 6H clustered into five main groups, with 84% similarity level. The first group included 19 agro-dealer samples, which included the seed company's sample. The second group contained one agro-dealer sample as well as the fifth group. The third group contained 2 agro-dealer samples, while the fourth group contained 3 agro-dealer samples (Fig. 4).

The study also estimated the percentage of off-types in each variety (the number of off-types' ×100 per the total number of the samples). The

results revealed that within the samples from agro-dealers there mixture of improved seed with grain. This was done possibly to increase the seed quantity and make more profit (Table 8).

DISCUSSION

This study gives a description of a system of Quality Control and Quality Assurance of hybrids which were released with a focus on the phenotypic data. We defined quality control as

TABLE 5. Longe 7H eigenvectors, eigenvalues, the individual and cumulative percentage of variation explained by the first four principal components

Trait*	PC1	PC2	PC3	PC4
An Col	0.2	-0.28	-0.06	0.07
Shpe T	-0.04	0.11	-0.05	-0.15
Angle BS	0.01	-0.25	0.02	-0.32
At of B	0.18	0.06	0.14	-0.09
Degree Zig	-0.03	0.06	0	-0.25
An Col BR	0.1	-0.13	0.09	0.31
TAn	-0.22	-0.26	0.24	-0.07
An Col BG	0.09	0.02	0.2	0.31
An Col Gex	0.04	0.29	0.16	-0.04
An Col Anth	-0.11	-0.02	0.02	-0.17
De Spi	-0.06	-0.33	0.23	-0.16
Angle MI	0.3	-0.04	-0.2	-0.02
At of LB	0.3	-0.15	0.06	-0.17
No of PLB	0.04	0.3	-0.23	0.03
T of SE	-0.19	-0.28	0.21	-0.1
An Col of S	-0.2	0.06	-0.34	0.22
In of An Col S	0.23	-0.05	-0.06	0.11
An Col Sh	0.2	0.07	-0.08	-0.03
An col InNO	0.12	-0.2	0.02	-0.04
Le MALB	0.27	0.18	-0.03	-0.24
LeMAUB	0.28	-0.09	-0.16	-0.12
W of B	0.12	0	-0.13	-0.35
Le Peduncle	-0.06	0.08	-0.02	-0.3
Le Hu Ti E	-0.12	0.04	-0.2	-0.09
E Le	0.07	0.23	0.21	-0.3
E Di	0.21	-0.12	-0.29	-0.03
Ea Sh	-0.04	-0.2	-0.34	-0.03
E No Rows	0.25	-0.21	-0.09	0.07
Type G	-0.23	-0.02	-0.15	-0.23
Col G	0.26	0.15	0.25	0.1
Col DSG	0.24	-0.02	0.28	0
An Col GC	0.04	0.29	0.16	-0.04
In An Col GC	-0.12	-0.02	0.01	0.17
Cumulative (%)	19	32.4	42.3	51.1

^{*}Traits corresponding to the underlined numbers are the most significant traits that contributed much of the variation in each PC

steps taken to monitor and control the quality of a product as it is produced, while quality assurance is defined as a post-production review of product quality (Laurie *et al.*, 2010). In this study, 33 phenotypic traits were used to characterise a set of 120 samples of four maize hybrids (Longe 10H, 9H, 7H, and 6H) collected from agro-dealers in 13 districts and three from seed companies in Uganda.

Analysis of variance showed that among the test materials only Longe 9H had no significant difference within traits, but the others had some

levels of significant differences (Table 3). This meant that among the samples of Longe hybrids, only those of Longe 9H appeared to be related to each other hence the level of quality of Longe 9H compared to other hybrids was good. There were more traits which had significant differences within the same variety and this meant that the samples collected for that particular variety had low quality. These results suggest that the level of variation/contamination is higher in Longe 10H, followed by Longe 6H and Longe 7H; whereas Longe 9H appeared as a pure variety.

TABLE 6. Longe 9H eigenvectors, eigenvalues, the individual and cumulative percentage of variation explained by the first four principal components

Trait*	PC1	PC2	PC3	PC4
An Col	-0.10	-0.15	-0.33	0.08
Shpe T	-0.06	-0.17	0.19	-0.33
Angle BS	-0.15	-0.26	0.08	-0.02
At of B	-0.24	-0.09	0.01	-0.23
Degree Zig	0.25	-0.01	0.02	-0.11
An Col BR	0.16	0.17	0.17	0.17
TAn	0.24	0.11	0.05	-0.17
An Col BG	-0.15	-0.06	0.24	0.16
An Col Gex	0.24	-0.17	0.03	-0.07
An Col Anth	0.01	-0.09	0.36	-0.16
De Spi	0.18	0.18	-0.01	0.31
Angle MI	0.00	-0.30	0.11	0.25
At of LB	0.00	-0.33	0.08	0.21
No of PLB	0.22	-0.12	-0.16	-0.02
T of SE	-0.01	0.35	0.03	-0.14
An Col of S	0.05	0.16	-0.34	0.06
In of An Col S	0.11	-0.10	-0.24	-0.10
An Col Sh	-0.20	-0.14	0.12	0.14
An col InNO	-0.24	0.05	0.16	0.00
Le MALB	-0.23	0.08	0.00	-0.26
LeMAUB	-0.22	0.10	0.03	-0.25
W of B	-0.04	-0.16	-0.28	0.28
Le Peduncle	0.18	0.22	0.15	-0.05
Le Hu Ti E	-0.20	0.09	0.05	0.26
E Le	-0.10	-0.20	-0.26	-0.21
E Di	0.13	-0.30	0.05	0.00
Ea Sh	0.15	0.22	0.06	0.22
E No Rows	0.20	-0.09	0.09	-0.18
Type G	0.21	0.02	0.26	-0.01
Col G	0.24	-0.16	0.00	-0.09
Col DSG	0.25	-0.13	-0.04	-0.05
An Col GC	0.24	-0.17	0.03	-0.07
In An Col GC	-0.10	0.36	0.18	0.09
Cumulative %	40.70	62.70	81.70	92.80

^{*}Traits corresponding to the underlined numbers are the most significant traits that contributed much of the variation in each PC

Results from the present study also demonstrate the presence of high phenotypic differences between different seed sources of four hybrids.

During hybrid maize seed production, maintenance of varietal purity and confirmation of the identity of the same hybrids collected from different locations are important quality control functions in the maize seed industry. These functions have become more critical due to the reports on prevailing fake seed on the market and counterfeits. In most seed industries, the principal

causes of large variation in same samples or stocks originating from the same hybrid but sampled at a different level of value chain may be attributed to (a) issues (quality) with the parent inbred lines which were used to form the variety. Significant changes in the makeup of a germplasm may affect performance, and in the worst case result in the distribution of wrong hybrids or hybrids (Semagn *et al.*, 2012). (b) mislabeling of the seed lot; for example, in Longe 10H, the seed

TABLE 7. Longe 10H eigenvectors, eigenvalues, the individual and cumulative percentage of variation explained by the first four principal components

Trait*	PC1	PC2	PC3	PC4
An Col	-0.09	-0.14	0.09	-0.24
Shpe T	0.11	0.05	-0.14	0.09
Angle BS	0.19	0.17	0.03	-0.04
At of B	0.22	0.04	0.00	0.23
Degree Zig	-0.10	0.09	0.13	0.18
An Col BR	0.23	-0.06	0.08	-0.03
TAn	-0.33	0.17	-0.11	0.18
An Col BG	-0.09	-0.01	0.08	0.26
An Col Gex	-0.10	0.07	0.39	0.00
An Col Anth	0.12	-0.26	0.18	0.18
De Spi	-0.01	-0.38	0.07	0.07
Angle MI	0.34	0.07	-0.14	-0.11
At of LB	0.27	0.10	-0.20	-0.04
No of PLB	0.08	-0.19	0.08	-0.34
T of SE	-0.37	0.12	-0.10	0.20
An Col of S	0.09	0.29	0.00	-0.02
In of An Col S	0.34	-0.11	0.13	0.00
An Col Sh	-0.05	-0.09	-0.08	-0.10
An col InNO	-0.11	-0.25	0.12	-0.17
Le MALB	0.13	0.33	0.26	-0.07
Le MAUB	0.19	0.32	0.13	-0.07
W of B	0.15	-0.04	0.28	0.09
Le Peduncle	0.10	0.26	0.09	0.16
Le Hu Ti E	0.04	0.18	-0.13	0.16
E Le	0.00	0.10	0.28	0.01
E Di	0.23	-0.14	0.10	0.20
Ea Sh	0.18	-0.09	-0.23	0.01
E No Rows	0.05	-0.26	0.18	0.26
Type G	0.09	-0.06	-0.20	0.30
Col G	-0.08	0.08	-0.05	-0.48
Col DSG	0.07	0.09	0.12	-0.04
An Col GC	-0.10	0.07	0.39	0.00
In An Col GC	-0.11	0.11	0.22	0.00
Cumulative (%)	15.10	23.80	32.00	39.10

^{*}Traits corresponding to the underlined numbers are the most significant traits that contributed much of the variation in each PC

TABLE 8. Percentage off types within the field

Hybrids	Number of plants	Number of off-types	%	
Longe 10H	1285	68	5.3	
Longe 9H	54	3	5.6	
Longe 7H	391	25	6.4	
Longe 6H	487	41	8.5	

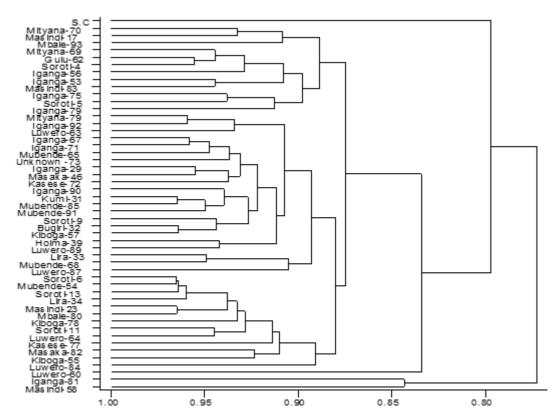


Figure 1. Longe 10H Dendrogram, based on 34 phenotypic traits using Euclidean distance matrix. *districts are indicted of the agrodealers

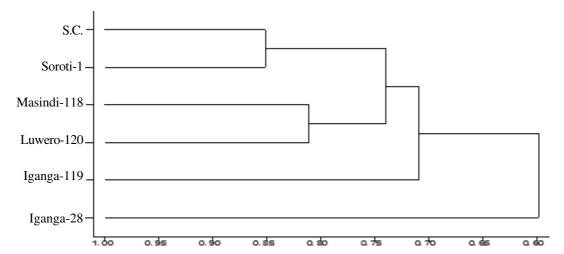


Figure 2. Longe 9H Dendrogram, based on 34 phenotypic traits using Euclidean distance matrix. *agro-dealers are represented by districts

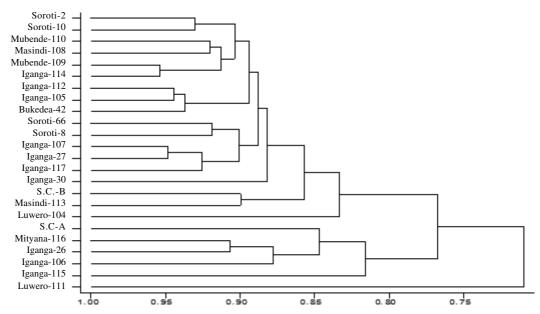


Figure 3. Longe 6H Dendrogram, based on 34 pnenotypic traits using Euclidean distance matrix. *agro-dealers are represented by districts.

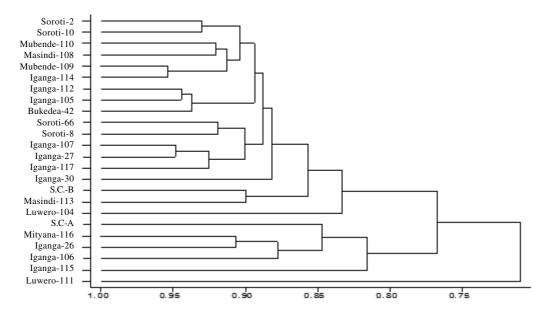


Figure 4. Longe 7H Dendrogram, based on 34 phenotypic traits using Euclidean distance matrix. *agro-dealers are represented by districts.

company sample had grouped alone as an outlier. This suggests that the seed company misspackaged the seeds (packaging seeds of another variety in Longe 10H package) during the seed processing. (c) difference in seed lots; another cause of source of variation within the same hybrid can be attributed to difference in outgrower's seed lots. Seed companies tend to contract different out-growers for bulking their certified seeds. These are backstopped but the seed companies to ensure seed quality. Different agro-dealers within the same districts may be supplied with different lots of same hybrids.

Quality control is essential for seed regulations in any seed system. Seed quality control has two components, seed certification and seed testing (Tripp and Louwaars, 1998). Seed certification verifies the quality of seed; it provides assurance that seed is of a specified variety and is of acceptable genetic purity (Tripp and Louwaars, 1998). Seed testing examines various seed quality parameters, such as germination capacity, analytical purity, and pathogen levels. The results of these tests are usually considered by the certification agency before issuing a certification label (Tripp and Louwaars, 1998). Therefore, seed certification in its more general sense, refers to both the genetic and other qualities of seed, but the phenotypic distinction is very important.

The application of PCA and cluster analysis showed that combinations of these traits, which offer effective means for modeling and classifying different samples. The interpretation of the results of PCA is usually carried out by visualisation of its PC scores (Zhang et al., 2012). The results obtained from the Score plot of Longe 7H suggest variation between the seed companies' samples, which also might contribute to the variation within agro-dealers' seeds. This might be because seed companies contract farmers to grow/multiply seed, mostly under rain-fed conditions, thereby exposing the crop to variable rainfall conditions (Langyintuo, 2004). Additionally, the grower might face difficulty in achieving isolation distances of 200 – 400 m to ensure genetic purity and seed quality (Augustine et al., 2009). For example, Longe 7H reference samples from seed companies were expected to cluster together, but was not the case, hence the purity was

compromised. This result suggests that the seed company's routine activities contributed to the different variations, by selling different versions of seed (seed with different lot numbers) to the agro-dealer. Moreover, it might be because of the agro-dealer or the seed company agent's problems such as lack of knowledge on varietal characteristics, lack of credibility on the part of some agents, adulteration of seed, and poor storage facilities.

From Cluster analysis, Longe 10H was the variety with the highest level of contamination, with almost 90.5% of agro-dealer samples contaminated. In contrast, Longe 6H had the lowest rate of contamination with almost 20.6% of the agro-dealer samples contaminated. This might be because of the high demand of Longe 10H.

Agro-dealers' samples from Iganga, Masindi, Luwero, Soroti, Bukedea and Muhabura showed high seed quality compared with the seed companies for the four hybrids under study. Samples from Lira, Hoima, Mubende, Mityana, Gulu, Kiboga, and Bugiri showed a high rate of variation. However, samples from some districts were mixed (quality seed and contaminated). For this, it is hard to distinguish districts with 100% agro-dealers' seed quality. Therefore, more analysis was needed based on the phenotypic data. All these different analyses supported the presence of variation/contamination within hybrid seeds under study at agro-dealer level. Therefore, regular monitoring and enforcement of seed regulation policies along the value chain can improve the formal seed supply system.

CONCLUSION

This study has revealed the existence of varying levels contamination of released hybrids along the seed value chain in the Uganda seed market. Variations among same hybrids collected from different agro-dealers in different districts may be attributed to seed mixing, mislabeling of hybrids and variation in seed lots. Quality control measure within different seed lots from different out-growers should be emphasized and premium price should be given to ensure quality. Also, molecular markers may be useful in discriminating among these and also confirm the phenotypic

diversity observed. There is need to strengthen quality control sector if improved productivity at farmer level is to be realised.

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REFERENCES

- Barnett, C., Chisvo, M., and Pinto, Y. 2011. Country case studies on the pass value chain strategy /Approach And Its Impact / Effect On Smallholder Farmer Prepared by/ EAST AFRICA SYNTHESIS REPORT, (January).
- Begum, T. and Kumar, D. 2011. Usefulness of morphological characteristics for DUS testing of jute (*Corchorus olitorius* L. and *C. capsularis* L.). 9: 11.
- Beshir, B. 2011. Open pollinated maize seed systems linkages through farmer research group in Central Rift Valley of Ethiopia. *Farmers' Access to Seed.* pp. 43.
- Chanda, R., Mukanga, M., Mwala, M., Osiru, D. S., and Macrobert, J. 2014. A comparative analysis of distinctness, uniformity and stability (DUS) data in discriminating selected Southern African maize (*Zea mays* L.) inbred lines. *African Journal of Agricultural Research* 9(41): 3056-3076. http://doi.org/10.5897/AJAR2014.8755
- FNSU-UDS. 2011. Three country (Burkina Faso, Ghana and Mali) case studies on the PASS Value Chain Strategy / Approach and its Effect on Smallholder Farmer Yields in Africa. Draft report Submitted to Alliance for a Green Revolution in Africa. Food and Nutrition

- (April). Retrieved from http://www.mendeley.com/research/three-country-burkina-faso-ghana-mali-case-studies-pass-value-chain-strategy-approach-effect-smallho/
- Hung, H.Y., Browne, C., Guill, K., Coles, N., Eller, M., Garcia, A., Lepak, N., Melia-Hancock, S., Oropeza-Rosas, M., Salvo, S., Upadyayula, N., Buckler, E. S., Flint-Garcia, S., Mcmullen, M. D., Rocheford, T. R. and Holland, J. B. 2012. The relationship between parental genetic or phenotypic divergence and progeny variation in the maize nested association mapping population. *Heredity* 108: 490-499.
- Ignjatoviæ-Miciæ, D., Ristiæ, D., Babiæ, V., Andjelkoviæ, V., Markoviæ, K. and Vanèetoviæ, J. 2013. Genetic assessment of maize landraces from former Yugoslavia. *Genetika* 45(2): 405-417. http://doi.org/10.2298/GENSR1302405I
- Langyintuo, A.S. 2004. Challenges of the Seed Sector of Southern Africa. International Maize and Wheat Improvement Center (CIMMYT) Report, Harare, Zimbabwe, 39. Retrieved from www.cimmyt.org/gis/rfseedsafrica/
- Laurie, C.C., Doheny, K.F., Mirel, D.B., Pugh, E.W., Bierut, L.J., Bhangale, T., Boehm, F., Caporaso, N. E., Cornelis, M. C., Edenberg, H. J., Gabriel, S. B., Harris, E. L., Hu, F. B., Jacobs, K. B., Kraft, P., Landi, M. T., Lumley, T., Manolio, T. A., Mchugh, C., Painter, I., Paschall, J., Rice, J. P., Rice, K. M., Zheng, X., Weir, B. S. and For The, G.I. 2010. Quality control and quality assurance in genotypic data for genome-wide association studies. *Genetic Epidemiology* 34: 591-602.
- Law, J., Anderson, S., Jones, E., Nelson, B., Mulaosmanovic, E., Hall, B. and Smith, J. 2011. Approaches to improve the determination of eligibility for plant variety protection: I evaluation of morphological characteristics. *Maydica* 56: 113-131.
- Mubangizi, E., Ntamu, D.N., Mwesigwa T.W. and Thijssen, M. 2012. ISSD Briefing Note -September 2012 Uganda Seed Sector Assessment. ISSD Africa, (September) 1-7.
- Mrutu, B.A. 2015. Assessment of seed genetic purity of hybrid maize variety UH6303 in southern highlands of Tanzania by random amplified polymorphic DNA (RAPD) markers.

- African Journal of Agricultural Research 10: 2911-2918.
- Semagn, K., Beyene, Y., Makumbi, D., Mugo, S., Prasanna, B.M., Magorokosho, C. and Atlin, G. 2012. Quality control genotyping for assessment of genetic identity and purity in diverse tropical maize inbred lines. *Theoretical and Applied Genetics* 125: 1487-501.
- Smýkal, P., Horáèek, J., Dostálová, R. and Hýbl, M. 2008. Variety discrimination in pea (*Pisum sativum* L.) by molecular, biochemical and morphological markers. *Journal of Applied Genetics* 49: 155-166.
- Tripp, R. and Louwaars, N. 1998. Seed regulation: choices on the road to reform. *Food Policy*, 22: 433-446.
- UPOV. 2009. Guidelines for the conduct of tests for distinctness, uniformity, and stability; maize. TG/2/7. UPOV, Geneva, Switzerland.
- Ward, J.H. 1963. Hierarchical grouping to optimize an objective function, *Journals American Statistical Association* 58:236-244.
- Zhang, X., Liu, F., He, Y. and Li, X. 2012. Application of hyperspectral imaging and chemometric calibrations for variety discrimination of maize seeds. *Sensors* 12(12): 17234-17246. http://doi.org/10.3390/s121217234