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Morphological characterization of selected spiderplant (*Cleome gynandra* L.) types from western Kenya

Francis B.O. K'Opondo

Kabianga University College, Department of Agricultural Biosystems and Economics, P.O. Box 1-20201, Kabianga, Kenya

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ABSTRACT

The morphological characterization of four spiderplant types from western Kenya was carried out in a plastic greenhouse at Chepkoilel Campus of Moi University, Eldoret, Kenya. Seeds were collected from small-scale farmers in Kakamega District and from wildly growing plants within Chepkoilel Campus in Uasin Gishu District, both in western Kenya. Morphotypes were different for 3 variables out of the 7 scored for, the variables including plant structure, stem pubescence leaflet shape and leaflet apices. In the case of variable counts, morphotypes differed for 3 out of the 5 counted and the variables included days to 50% flowering, stem pubescence and number of leaflets per compound leaf. For variable measurements, morphotypes showed differences for 3 out of the 5 measured, and the variables were plant height, petiole length and fruit breadth. The morphotypes were also clustered into 3 groups by the dendrogram. In characterizing the four morphotypes, it was noted that overlaps in morphological statistics occur as expected. Despite these overlaps, significant differences were observed in analysis of variance, indicating that apart from stem and petiole colours, also other characters of importance differ.

Key words: *Cleome gynandra*, morphological characterization, morphotypes, spiderplant, western Kenya.

INTRODUCTION

Studies in Kenya indicate that there are variations in characters among spiderplant populations, for instance in plant structure (erect-semi-erect), stem pigmentation (green, pink, violet, purple), petiole pigmentation (green, pink, violet, purple), plant height (25-72 cm), number of leaflets per leaf (3-7), leaf length (3-23 cm), length of petiole (3-23 cm), leaflet length (1.7-10 cm), leaflet width (0.8-4.0 cm), leaflet shape (elliptical-oval), leaf colour (green-brownish), stem and leaf

pubescence (glabrous-abundant), days to 50% flowering (17-35), branching habit (upright-spreading), degree of flowering (low-high), fruit position on the plant (top of canopy-throughout plant), number of days to seedling emergence (4-6), seedling vigour (very strong- very weak), disease susceptibility (medium-resistant), pest susceptibility (medium-resistant), number of primary branches (2-7), fruit length (6.4-12.0 cm) and plant lodging (none-nearly 100%) [1, 2, 3, 4]. The leaf bitterness is however appreciated by many [3]. Further reports indicate the presence of a wide diversity of characters that can be seen in the field, including, more or less hairy or glandular stems; more or less branched; small or large leaves; a vertical or horizontal plant structure; early or late flowering; white or purplish flowers; short and thin or long and flabby fruits, and a range of leaf bitterness [3]. Similar differences in morphological traits have been noted for other vegetable crop species including 'Plainsman' grain amaranth (*Amaranthus caudatus*) for plant height [5]. Ethiopian mustard (*Brassica carinata*), tomato (*Lycopersicon lycopersicum*), mung bean (*Vigna radiata*) and soybean (*Glycine max*) for 50% days to flowering [6, 7] and tomato (*L. lycopersicum*) , mung bean (*V. radiata*), soybean (*G. max*) and African egg plant (*Solanum aethiopicum*) for fruit breadth and length [7].

In a study carried out elsewhere in Kenya to show whether within diversity of *C. gynandra* species had a bearing on nutritional quality, the existence of morphological types (morphotypes) of this species was reported [8]. The design which was based on the premise of an easily identifiable morphological characteristic, the stem and petiole colour, thus represented four morphological types. An earlier report, four different plant types could also be recognized, based on stem and petiole pigmentation [9]. However, it was reckoned that the intensity of pigmentation varied from light to deep [9]. Morphotypes are referred as strains of a species having established morphological differences [10]. Morphology as explained by [11] deals with the study of forms and features of different plant organs such as roots, stems, leaves, flowers, seeds and fruits, while [12] describes character or trait as a morphological, anatomical or physiological feature of an organism, which is usually a product of the action of both genotype and environment.

Although there is yet no comprehensive hypothesis and good understanding for the function and adaptative value of colour patterns of the vegetative parts of plants, polymorphism in stem and leaf colouration has been observed to occur in plants. Recent developments of molecular genetics have however, provided knowledge about the biochemical pathways of plant colouration and genes involved and their regulation [13].

Among plant pigments that are responsible for a variety of red, blue and purple colours are anthocyanins, which do accumulate in certain plant tissues at specific developmental stages. The accumulation is controlled by various environmental factors such as light, temperature, nutrients and stress [14]. These particular pigments belong to the general class of flavonoids, which have many functions attributed to them, one among them being a survival compound for flowering plants. The ability by spiderplant to grow under diverse environmental conditions could possibly be further enhanced in those morphotypes that have anthocyanin accumulation on both stems and petioles rather on either stem or petiole only, or even no accumulation on both the plant parts.

While geneticists and plant breeders are most concerned with diversity at the molecular level, such as to whether particular alleles are absent or present in a population [15, 16], farmers on the

other hand are most interested in morphological and agronomic variations, and how these can be used within the farming system to achieve sustainable livelihoods. Farmers can only easily recognize variation that can be seen by human eye. The application of morphological descriptor lists is therefore the simplest of the formal, standardized, repeatable methods of measuring crop genetic diversity [17]. Morphological characterization is a first step that should be made before more in-depth biochemical or molecular studies are attempted. However, the best way to come to good conclusion is to repeat the trials in a range of localities or alternatively by growing the plants in a controlled environment since morphological characteristics is known to be susceptible to environmental influences [18].

The objectives of this study were to establish if the spiderplant types found in western Kenya could also fit the morphological characterization pattern as found among the same plant species elsewhere in Kenya, and also to later confirm the existence of any genetic variation amongst them by carrying out further investigations using molecular technique**.**

MATERIALS AND METHODS

Plant Materials

The experiments were carried out in a plastic greenhouse at the Department of Seed, Crop and Horticultural Sciences, Chepkoilel Campus of Moi University, Eldoret, Kenya. Chepkoilel Campus is situated at longitude 0' 30'' N and latitude 35' 15'' E, at an altitude of about 2140 m asl. Seeds of spiderplant types for this study were collected from small-scale farmers in Kakamega District and from wildly growing plants within Chepkoilel Campus, Moi University in Uasin Gishu District.

The collected seeds were sown in the field at Chepkoilel Campus of Moi University, Eldoret in order to raise the material for use in this study and other subsequent studies.

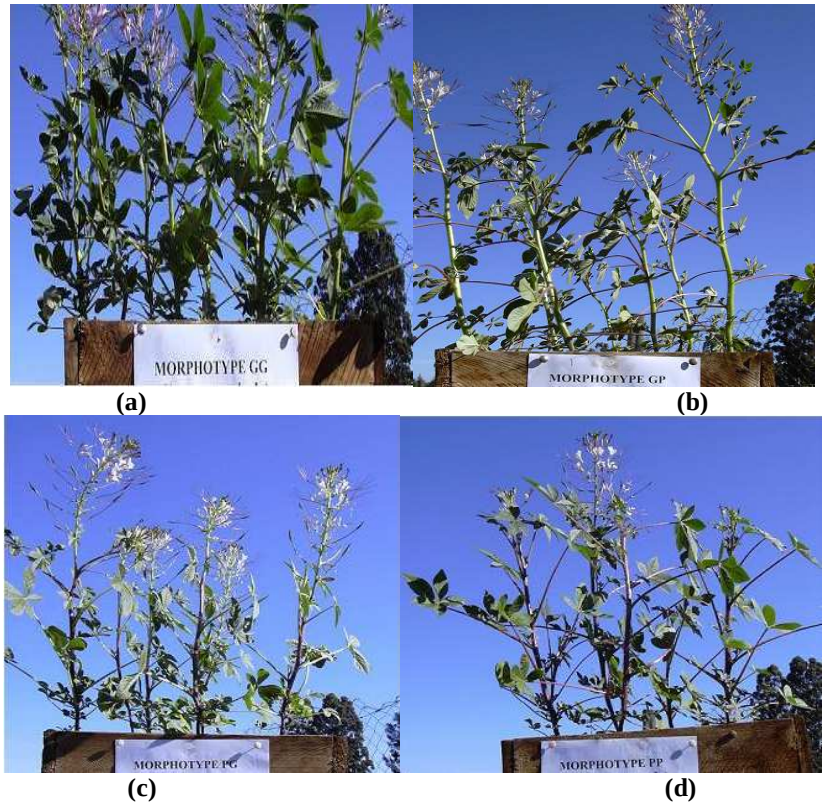
Identification and Selection of Morphotypes, and Seed Bulking

Seeds were sown in sets of four wooden boxes measuring 47 cm x 29 cm x 6.5 cm each. The boxes were filled with soil collected within Chepkoilel Campus, belonging to the soil order Oxisol. Soil types of this nature have oxic horizons or contain a continuous layer of planthite (*i.e.* has hydrous oxides of iron and aluminium along with silicate clay minerals) within 30 cm of the surface, and they tend to occur on the more stable, older surfaces and are therefore deeper and more highly weathered than other soils types [19]. The boxes were kept weed-free. Thinning was done 21 days after seedling emergence, to leave 10 cm between plants in a row. The crop was irrigated at least three times a week.

Thirty days after seedling emergence, plants of the desired morphotypes were identified and selected as follows: GG- green stem/green petiole type of plants, GP- green stem/purple petiole type of plants, PG- purple stem/green petiole types of plants, and PP- purple stem/purple petiole type of plants [9, 10]. The selected plants were left in the boxes while those plants that did not meet the criteria as described for the selected ones were removed. The boxes that contained similar morphotypes were grouped and placed at least 4 m away from other morphotypes to form a block and reduce the chances of cross-pollination. Plants that were observed to have shades of purple colour on the green stems and petioles or shades of green colour on the purple stems and

petioles were considered as off-types. Such off-types were continuously removed from the blocks as the plants grew to further purify the desired morphotypes. Harvesting was done in each block as fruits turned yellow, thus indicating physiological maturity. The fruits were threshed and seeds air dried under shade until the moisture content was low enough for the drying to be considered complete. Air drying under shade was taken as a better option since it allowed slow release of moisture from seed therefore avoiding the possibility of lowering viability. Air drying of seed in the sun may lead to overheating or quick drying, especially when seeds are harvested while still having high moisture content as was possibly the case with spiderplant seeds, thus affecting seed viability.

Seeds of the four selected morphotypes were sown and plants raised in ten wooden boxes of size 47 cm x 29 cm x 6.5 cm each, in order to carry out seed bulking. The boxes were filled with soil collected within Chepkoilel Campus and diammonium phosphate (DAP) fertilizer was applied to the boxes at the rate of 200 kg/ha before sowing as recommended by [20]. This was done to boost seed production. The morphotypes were isolated from each other to reduce the chances of cross pollination. All operations that included weeding, thinning and irrigation were like those described above.



(a) **(b)**

(c) **(d)**

Photography by courtesy of H.A. van Rheenen

Figure 1. Spiderplant (*Cleome gynandra* L.) morphotypes from western Kenya, which were identified and selected for this study: a) GG – green stem/green petiole type of plants (b) GP – green stem/purple petiole type of plants (c) PG – purple stem/green petiole type of plants (d) PP – purple stem/purple petiole type of plants.

Thirty days after seedling emergence, one plant in each of the four bulking blocks and best conforming to the desired morphotype was selected and tagged. A single fruit was harvested from each of the tagged plants in each block, when it turned yellow. The fruits were threshed and seeds dried as described above. Each seed sample was placed into an aluminium foil packet; the packets flattened with the edge of the hand to remove air and immediately heat-sealed approximately 2 cm from the opening of the packet. Heat- or hermetic-sealing provides a simple and convenient method of controlling seed moisture content after the seeds have been dried to a level low enough for the drying to be considered complete. The seed packets were stored in a shelf at room temperature (about $20^{O}C$) until the time to plant for the characterization experiment one month later.

Characterization Experiment

The four selected morphotypes were characterized by comparing a number of variable scores, counts and measurements in order to establish whether there were differences among them. The operating hypothesis was that certain characteristics will consistently separate morphotypes. If they do, they would further support their distinctions as morphotypes. The dried seeds from the single fruits were used for the characterization experiment.

The four morphotypes were raised in an RCBD experiment with four replicates in each case by sowing seeds in wooden boxes of similar sizes as before. DAP fertilizer was applied at the rate of 200 kg/ha. Seeds were drilled in two rows per box at row-spacing of 30 cm. Number of days to seedling emergence was recorded. Scoring was done for seedling vigour using the index of scale 1 (very strong) to 4 (very weak). Thinning was done 21 days after seedling emergence, to leave 10 cm between plants. The boxes were kept weed-free throughout. The crops were irrigated at least three times a week. Thirty days after seedling emergence, any off-type plants as described before were removed to leave only those plants best conforming to the desired morphotypes selected for the study. Number of days to 50% flowering was recorded.

Two plants were tagged in each replicate immediately after the plants attained 50% flowering.

The following morphological traits were scored for: degree of flowering with index of scale 1 (low) to 3 (high); plant structure with index of score 1 (erect) or 2 (semi-erect); leaflet colour with index of score 1 (greenish) or 2 (brownish) and shape of leaflet with index of score 1 (elliptical) or 2 (ovate), all done 53 days after sowing (DAS), and branching habit with index of score 1 (horizontal) or 2 (upward) and fruit position with index of score 1 (throughout the plant) or 2 (top of canopy), both done 76 DAS.

The following counts or measurements of traits were taken: stem pubescence with index of level 1 (abundant) to 3 (glabrous); number of primary branches; number of leaflets/compound leaf; length of leaflet (cm), measured from the pulvinus of the leaflet to the tip of the leaflet, and petiole length (cm), measured from the pulvinus to the base of the leaflets, 53 DAS; plant height (cm), measured from the base of the plant to the tip of the main stem, 61 DAS; fruit length (cm), measured from the end of the fruit stalk to the tip of the fruit, and fruit breadth (cm), measured at the middle part of the fruit, which was considered as the broadest point, 76 DAS.

Data Analysis

The compiled data sheet was transferred to a spreadsheet file SPSS (Superior Package for Statistical Science) (SPSS Inc., Chicago). Analysis of variance (ANOVA) was performed on the data, differences between treatment means were tested for significance by Duncan's Multiple Range Test (DMRT).

In order to show the morphological relationships between the four morphotypes in a dendrogram, a cluster analysis was performed on the mean morphological variable data. The data for each morphological trait was assumed to represent a single locus. Data that showed significant differences were scored as presence (1) of polymorphism, while data that showed no significant differences were scored as absence (0) of polymorphism. The resulting data matrices were analysed using the POPGENE 3.2 [21] programme. A cluster analysis procedure, the Neighbour-Joining using UPGMA based on the standard genetic distance of Nei for the various loci was performed and a dendrogram was created.

RESULTS

Spiderplant morphotypes GG and PG were significantly (*P=0.05*) different from GP and PP for the degree of flowering (Table 1). While only morphotype PG was significantly different from the rest in terms of plant structure, morphotypes GG and PP were different from GP and PG for leaflet shape (Table 1).

Table 1. Morphological trait scores and their standard errors of four spiderplant (Cleome gynandra) morphotypes from Western Kenya:
GG – green stem/green petiole type of plants; GP – green stem/purple petiole type of plants; PG – purple stem/green petiole type of plants; PP – purple stem/purple petiole type of plants.

Trait index	GG (Mean ± S.E.)	GP (Mean ± S.E.)	PG (Mean ± S.E.)	PP (Mean ± S.E.)
Seedling vigour	1.8 ± 0.3a	2.0 ± 0.0a	2.0 ± 0.4a	1.5 ± 0.3a
Degree of flowering	2.0 ± 0.0a	2.8 ± 0.3b	2.0 ± 0.0a	2.5 ± 0.3b
Plant structure	1.0 ± 0.0a	1.3 ± 0.3ab	1.8 ± 0.3b	1.0 ± 0.0a
Branching habit	2.0 ± 0.0a	2.0 ± 0.0a	2.0 ± 0.0a	2.0 ± 0.0a
Leaf colour	1.0 ± 0.0a	1.0 ± 0.0a	1.0 ± 0.0a	1.0 ± 0.0a
Leaflet shape	1.8 ± 0.3a	1.0 ± 0.0b	1.0 ± 0.0b	2.0 ± 0.0a
Fruit position	1.0 ± 0.0a	1.0 ± 0.0a	1.0 ± 0.0a	1.0 ± 0.0a

*Figures followed by different letters within the same row are significantly (P=0.05) different according toDuncan's multiple range test. * = Significant at the 5% level, NS= Not significant. Data are means ±S.E. of four replications of two plants each for the four spiderplant morphotypes.*

For the number of days to 50% flowering and stem pubescence, it is only spiderplant morphotype GG that was significantly (*P=0.05*) different from the rest (Table 2). However, morphotypes GP was different from GG and PP considering the number of leaflets per compound leaf (Table 2).

Table 2. Morphological trait counts and their standard errors of four spiderplant (*Cleome gynandra*) morphotypes from Western Kenya:
GG – green stem/green petiole type of plants; GP – green stem/purple petiole type of plants; PG – purple stem/green petiole type of plants; PP – purple stem/purple petiole type of plants.

Trait index	GG (Mean ± S.E.)	GP (Mean ± S.E.)	PG (Mean ± S.E.)	PP (Mean ± S.E.)
Number of days to seedling emergence	5.0 ± 0.0a	5.0 ± 0.0a	5.0 ± 0.0a	5.0 ± 0.0a
Number of days to 50% flowering	36.5 ± 2.1a	42.3 ± 0.3b	40.8 ± 1.3b	40.3 ± 0.8b
Stem pubescence	2.0 ± 0.0a	1.3 ± 0.3b	1.0 ± 0.0b	1.0 ± 0.0b
Primary branches (no)	10.8 ± 0.5a	13.5 ± 1.2ab	12.5 ± 1.3ab	14.0 ± 0.7b
Leaflets/ compound leaf (no)	6.0 ± 0.0a	5.0 ± 0.0b	5.5 ± 0.3ab	5.8 ± 0.3a

*Figures followed by different letters within the same row are significantly (P=0.05) different according to Duncan's multiple range test. * = Significant at the 5% level, NS= Not significant.*
Data are means ±S.E. of four replications of two plants each for the four spiderplant morphotypes.

While spiderplant morphotypes GG and GP were significantly (*P=0.05*) different from PG and PP for plant height, it is only morphotype PP that was different from the rest, taking into account petiole length (Table 3). And for fruit breadth, morphotype GG was different from the rest (Table 3).

Table 3. Mean morphological trait measurements and their standard errors of four spiderplant (*Cleome gynandra*) morphotypes from Western Kenya:
GG – green stem/green petiole type of plants; GP – green stem/purple petiole type of plants; PG – purple stem/green petiole type of plants; PP – purple stem/purple petiole type of plants.

Trait index	GG (Mean ± S.E.)	GP (Mean ± S.E.)	PG (Mean ± S.E.)	PP (Mean ± S.E.)
Plant height (cm)	78.6 ± 2.8a	70.8 ± 5.0a	92.3 ± 3.4b	102.1 ± 3.5b
Leaflet length (cm)	5.3 ± 0.3a	5.1 ± 0.2a	5.9 ± 0.3a	5.8 ± 0.4a
Petiole length (cm)	14.4 ± 0.6a	14.4 ± 0.5a	15.8 ± 0.5a	17.7 ± 0.6b
Fruit length (cm)	14.0 ± 0.5a	13.4 ± 0.4a	13.5 ± 0.4a	13.8 ± 0.5a
Fruit breadth (cm)	0.8 ± 0.0a	1.0 ± 0.1b	1.0 ± 0.1b	1.4 ± 0.3b

*Figures followed by different letters within the same row are significantly (P=0.05) different according to Duncan's multiple range test. * = Significant at the 5% level, NS= Not significant.*
Data are means ±S.E. of four replications of two plants each for the four spiderplant morphotypes.

The dendrogram showing the relationships between the morphotypes, obtained from a matrix calculated by means of distance coefficients of the morphological trait data is presented in Figure 2. The separation of the morphotypes by the dendrogram is indicated, with conformation of three clusters. This shows the existence of variations among the morphotypes with regard to morphological traits studied.

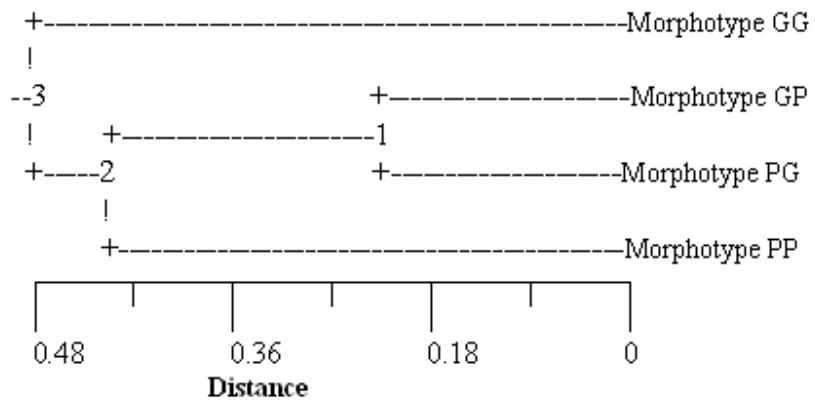


Figure 2. Neighbour-joining dendrogram based on Nei's genetic distances [22] generated by mean morphological trait data for four spiderplant morphotypes from Western Kenya.

DISCUSSION

The analysis of variance for the 17 morphological characters exhibited significant (at 5%) difference for 9 of the characters because of the treatment, indicating the existence of variability for these traits, amongst the morphotypes studied. The existence of variation in the 9 characters was in close agreement with the findings of by other investigators [1, 2, 3, 4]. Results reported for other vegetable crop species also lend further support to this observation [5, 6, 7]. The mean performances of individual morphotypes are presented in Tables 1, 2 and 3.

Morphotype PP (102.1 cm) was the tallest overall. GG (36.6 days) flowered earliest. Morphotypes GP, PG and PP, which took over 40 days to flower, also produced broader fruits compared to GG. Longest petioles were produced by PP (17.7 cm). The plant height reported for GG and GP were within the range reported elsewhere [1, 2], although morphotypes PG and PP were way above this range. For the number of days to flowering, it is only morphotype GG that had almost similar findings made by others [1, 2]; GP, PG and PP all had days above 40.

Morphotypes GP and PP showed high degree of flowering, and this was moderate for GG and PG. Moderate to high flowering degree as exhibited by the four morphotypes should be an important character when considering seed production since more flowering is likely to lead to high fruit and seed production. GG, GP and PP were all erect while it was only morphotype PG that was semi-erect. Morphotypes GP and PG had elliptically-shaped leaflets while for GG and PP, the shape of the leaflets was ovate.

In general, the more than half traits considered showed variation, and especially the agronomically important traits such as plant height, days to flowering, plant structure, number of leaflets per compound leaf, petiole length and fruit breadth. Some of the variations shown among the spiderplant morphotypes studied, particularly the agronomic traits, could partly be attributed to selection pressure being effected by farmers for those characters they consider to be of importance to them, as they continue to putting spiderplant under more domestication through cultivation. Spiderplant belongs to the genus *Cleome* with over 200 species [23] that are reported to consist of highly polymorphic herbaceous plants. This could point to why while using only

four morphotypes in this study, more than half of the morphological traits considered showed significant differences among the morphotypes.

Morphotypes however did not vary for traits like number of days to seedling emergence, seedling vigour, leaf colour, branching habit, fruit position, number of primary branches, length of leaflet and fruit length. The values for the following characters were within the range of those reported by other authors [1,2]: number of days to seedling emergence (4-8), seedling vigour (strong), leaf colour (green), branching habit (upward), fruit position (throughout plant), leaflet length (5.1-5.9 cm). This may point to the fact that some of the germplasm collected and characterized in the study by the other investigators [1,2] from elsewhere in Kenya shared some some genes with the morphotypes collected from western Kenya and used in this study. Only two characters were however in contrast: number of primary branches (11-14) and fruit length (13.4-14 cm). Further, it is probable that farmers do not apply any selection pressure for these characters that were found not to be significantly different among the morphotypes, as they consider them of less agronomic importance to them.

The morphotypes were clustered into three groups by the dendrogram. In this study, morphotypes GP and PG that formed the first group were closely clustered, followed by morphotypes GP and PG, and PP that were in the second group. While morphotype GG formed the third group with the rest of the morphotypes, it was distantly placed. Comparing the morphological dendrogram with the molecular one in a later study, the clusters are shown to differ. There should be no surprise for this observed difference since the grouping is based on different criteria. Different evolutionary path of development could be responsible for these observed differences among the clusters. It has further been intimated that while genes interact with other genes, the way they are expressed is influenced by the environment [24], and for a number of morphological traits this generally appears to be so.

The distinguishing features for the four respective morphotypes collected from western Kenya and selected to be used in this study can thus be summarized as follows: morphotype GG can be described as a green-stemmed and green-petioled plant with moderate degree of flowering, reaching to a height of less than 80 cm and growth being erect type, moderately pubescent stem, is early flowering and with rather narrower fruits; GP is a plant with green stem and purple petiole and having a high degree of flowering, less than 80 cm tall with erect type of growth, whose stem is abundantly pubescent, is late flowering and whose fruits are broader; PG is a type of plant whose stem and petiole are respectively, purple- and green-coloured, has a moderate degree of flowering and with a height of above 80 cm, type of growth being semi-erect, stem that is abundantly pubescent, flowers late while producing broadly appearing fruits, and PP is a purple-stemmed and purple- petioled plant, with high degree of flowering that reaches more than 80 cm tall, and whose growth type is erect with abundantly pubescent stem, is late flowering and has broader fruits.

CONCLUSION

In characterizing the four morphotypes, it was noted that overlaps in morphological statistics occur as expected. Despite these overlaps, significant differences were observed in analysis of

variance, indicating that apart from stem and petiole colours, also other characters of importance differ.

Given that morphological characteristics are affected by environmental influences, the use of molecular markers such as random amplified polymorphic DNA, restriction fragment length polymorphism and amplified fragment length polymorphism would complement this study by identifying polymorphism that is not affected by the environment**.**

It is concluded, the four morphotypes may be useful for further testing in view of variety release; and for use in breeding programmes.

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