MORPHOLOGICAL AND SSR MARKER CHARACTERIZATION OF WILD AND CULTIVATED COWPEAS (*VIGNA UNGUICULATA* L. WALP)

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**Abstract:** Three hundred and ninety accessions comprising 260 cultivated and 130 wild cowpea accessions were evaluated phenotypically using 27 cowpea descriptors. Morphological evaluation of some qualitative traits revealed 11.92% and 29.23% presence of pigmentation on the stem, 1.53% and 20.76% presence of stripes on the pod, and 0% and 20% presence of hairiness on the plant of cultivated and wild cowpeas respectively. As for the molecular analysis, sixteen SSR primers were employed for genotyping 48 accessions from both wild and cultivated cowpeas. The data generated a dendrogram with three clusters, two of which consisted of wild cowpea while the third cluster comprised all the cultivated cowpeas, including the yard-long-bean (*Vigna unguiculata* subsp. *sesquipedalis*) and *Vigna unguiculata* subsp. *cylindrica* accessions. Two wild accessions of subsp. *dekindtiana*, and one each of subsp. *kgalagadensis* and *protracta* clustered with cultivated cowpea indicating their relationships with cultivated cowpea, but not with other wild cowpeas. The numbers of polymorphic SSR bands in cultivated and wild cowpeas were 38 and 54, respectively, while the PIC values were 4.47 and 6.14, respectively, showing a greater genetic diversity in wild than in cultivated cowpeas. The subsp. *dekindtiana* had the highest number (80%) of shared SSR bands with cultivated cowpea followed by subsp. *protracta* with 54% of shared bands. Five species of wild cowpea have hairs and so could be used in breeding for resistance to insects.

**Key words:** cowpea, characterization, dendrogram, genetic diversity, microsatellites.

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Introduction

Cowpea \textit{[Vigna unguiculata (L.) Walp.]} is one of the most ancient human food sources and has probably been used as a crop plant since Neolithic times. Its origin has been linked to Africa based on the extent of diversity existing among germplasm lines and the preponderance of wild relatives distributed in several parts of the continent (Xiong et al., 2016). However, it has been found distributed, adapted and grown in many countries which include many countries in tropical Africa, Asia and South America (Mahalakshmi et al., 2007).

Annually, in the developing worlds, cowpea feeds millions of peoples, provides income for smallholder farmers, is used as animal fodder, helps restore soil fertility and gives comparatively high yields in harsh environments (Amusa et al., 2019). Annual estimated world production of cowpea worldwide is about 7.64 million tonnes, 60% of which is produced in Nigeria (FAO, 2017). Despite this, several authors have raised a concern about production instability over the years as compared to what is obtainable under experimental conditions (Singh, 2005; Kamara et al., 2012; FAO, 2017; Amusa et al., 2019).

Germplasm collection is important for the maintenance of biological diversity and food security. Germplasm can range from a collection of wild species to elite, domesticated breeding lines that have undergone extensive human selection. Thousands of accessions have been collected by crop genebanks or germplasm collection stores. The conservation and characterization of these genetic resources are a necessity not only for posterity but also for utilization in different improvement programs which include yield improvement, development of pest and disease tolerant and resistant genotypes, to mention a few (Kandel and Shrestha, 2018).

DNA markers can assist in producing a more robust and acceptable characterization of this economic crop by assessing the level of genetic relationships. In particular, microsatellite (SSR) markers, which are known to have a high level of variation among taxa and with a variable number of copies that are tandemly repeated, could be a useful tool in assessing the relationships between cultivated and wild cowpea. Microsatellites are inherited as codominant and are generally dispersed throughout the genome of organisms. Microsatellites have been used extensively in studying diversity at both intra- and interspecies levels of several crops such as alfalfa (ref), eggplant (Nunome et al., 2003), wild cowpea (Ogunkanmi et al., 2008), vegetable cowpea (Ogunkanmi et al., 2007), grapevine (Fan et al., 2014), tomato (Cheng et al., 2016; Arguirre et al., 2017) and Brassica species (Thakur et al., 2018), providing a mechanism for populations to adapt to their ever-changing environment. Hence, the objective of the study was to characterize the wild and cultivated accessions of cowpea to assess the level of diversity within Nigerian cowpea accessions.
Materials and Methods

Three hundred and ninety accessions within the section catiang of the subgenus *Vigna* were collected from the genebank of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. This included three subspecies of cultivated cowpea and 15 wild subspecies of cowpea covering a wide geographical range of Africa and part of Asia (Table S1). The cultivated members of this selection comprised one cylindrical and seven *sesquipedalis* accessions from Asia, and 260 accessions of subspecies *unguiculata* from Africa. The remaining 130 accessions of wild cowpea were from a wide geographical range in Africa, representing countries from southwest, northeast, central, and southern Africa. An augmented complete randomized block design was used for the experimental field layout. This consisted of 15 rows, 30 m by 2 m in dimension. There were thirty accessions per row, while each accession was replicated five times with two plants per stand 50 cm apart. The 50-cm spacing was designed to give enough space to each stand and enable them to fully establish on the field without excessive interference. Among these 30 accessions, there were four diverse cultivated cowpea checks (TVu 14176, TVu 14869, Tvx 3236, and TVu 9151) planted as per the recommendations of IITA’s cowpea breeding program.

Morphological characterization was carried out using cowpea descriptors described by Padulosi et al. (1993) with slight modifications. A total of twenty-seven quantitative and qualitative characters were evaluated and analyzed using SAS software v 9.0 (2002).

Twenty-four accessions each from cultivated and wild cowpeas were selected for DNA analysis (Table S2). The selection was based on the distribution of accessions in the dendrogram constructed from the morphological data. At least one accession per cluster was selected from the dendrogram. Four seeds of each accession were sown in pots containing good loamy soil in a screen house at IITA, Ibadan, Nigeria. Two weeks after planting, newly opened fresh young leaves were picked per accession for DNA extraction. Extra leaves from the same plant were collected in polyethylene bags and stored at –80°C in the freezer as a backup. Each leaf sample was placed in a 1.5 ml Eppendorf tube, quickly frozen in liquid nitrogen, and ground with Konte pestles into a fine powder. DNA was extracted from the ground leaves of each selected accession (N = 48) according to the procedure described by Dellaporta et al. (1983). The DNA was diluted in 0.1 × TE (1mM Tris 0.1mM EDTA, pH 8.0) to 10 ng/μl concentration.

Seventy SSR primers designed for cowpea by Li et al. (2001) were screened and optimized for polymorphism and annealing temperature using two accessions each from cultivated and wild cowpeas to ensure optimal primer performance across accessions. Optimal PCR amplification across accessions was achieved with annealing temperature ranging between 54 °C and 64 °C. Primers that showed
many bands, monomorphic bands or did not amplify were not selected for use in this study. Sixteen primers that showed unambiguous and clear polymorphism with the PCR products were used for this study.

A 10 μl reaction volume containing 1.0 μl of 10 × buffer, 2.0 μl of 10 ng/μl template DNA, 1.0 μl MgCl₂, 0.8 μl mixture of 10mM dNTPs (dATP, dCTP, dGTP, and dTTP), 4.6 μl of ultra-pure water, 0.5 μl each of forward and reverse primers, and 0.1 μl red hot Taq (promega) was loaded in a Perkin Elmer MJ cycler for DNA amplification. The PCR reaction was carried out with a profile of 18 cycles at 94°C for 1 min of initial denaturing and extension at 72°C for 1 min. Annealing temperatures were progressively decreased by 0.5°C every cycle from 64°C to 54°C. The reactions continued for 30 additional cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min and ended with a 10-min extension at 72°C after about 3 hours. Microsatellite markers that showed polymorphism after screening on a 3% agarose gel were resolved for better resolution using polyacrylamide gel electrophoresis.

PCR products were separated on polyacrylamide gel containing 70 ml of freshly prepared 6% polyacrylamide solution, 350 ml of ammonium persulphite (APS), and 35ml of TEMED. The gel was run at a constant voltage of 65 volts and the current of 60 mA for 3 hours. The gel was later fixed, stained, and developed using a silver staining kit (Promega corp. Madison WI). Fragments that were resolved on gels (Figure 1) were scored as 1 or 0, i.e., the presence or absence of bands, respectively, on all the forty-eight accessions of cultivated and wild cowpeas. The bands that could not be confidently scored were regarded as missing data.

Pairwise distance (similarity) matrices were computed using sequential, hierarchical, and nested (SAHN) clustering option of the NTSYS-pc version 2.02j software package (Rohlf, 1993). The program generated a dendrogram, which grouped the test lines on the basis of Nei genetic distance (Nei, 1972) using unweighted pair group by using mathematical average (UPGMA) cluster analysis (Sneath and Sokal, 1973). Genetic distances were calculated from the SSR data as:

\[ GD_{xy} = 1 - \frac{2N_{xy}}{N_x + N_y}, \]

where \( N_x \) and \( N_y \) are the numbers of alleles in groups X and Y, respectively, and \( N_{xy} \) is the number of alleles shared between the two groups of cultivated and wild cowpeas (Nei and Li, 1979). The correspondence between the two distance matrices (wild and cultivated cowpea lines) was assessed using simple linear correlation, cluster analysis, and principal component analysis. The polymorphism information content (PIC) provides an estimate of the discriminatory power of locus or loci by taking into account not only the number of alleles that are expressed but also the relative frequencies of those alleles. PIC values were calculated by the algorithm: \( \text{PIC} = 1 - \sum_i P_i^2 \) where \( i \) starts from 1, \( P_i^2 \) = frequency
of the \( i \)th allele (Brown et al., 1996; Ott, 1999). Only the distributions of the accessions along the first two principal components were considered in this paper.

**Results and Discussion**

In the study, twenty-seven characters were used to examine the relationships between the wild and cultivated cowpeas. It was observed that some members of the wild cowpea were found to share most of characters of cultivated cowpea and these included *dekindtiana* (TVNu 979, 305, 988, 578, 359, 1819, 315, 314, 582 669, 525, 245, 436, and 712) and *protracta* accessions (TVNu 288, 284, 390, and 267). This is in agreement with what was obtained by DNA analysis where they always aligned with the cultivated cowpea group.

For the vegetative characters, petiole length (plt) was highly correlated with rachis length (rlt) and petiolule. Results from the evaluation of some qualitative characters are presented in Table 1. The results revealed that there are more accessions (38) with purple pigmentation on their stems in wild cowpea than in cultivated cowpea (31). As for the presence of stripes on pods, cultivated cowpea had a higher percentage than wild cowpea. Accessions with the prostrate type of plant habit were more prominent than the number of accessions of erect and sub-erect type in both wild and cultivated cowpeas. Hairiness is one of the traits possessed by wild cowpea and this may correlate with their ability to resist insect pest attack. However, none of the cultivated cowpeas in this study had the trait, and hence may be the probable reason for their susceptibility to an insect pest. All the *vexillata* (14) used in this study and one accession of *rhomboidea* (TVNu 1471) showed the presence of hairs on stem and leaves. Meanwhile, some accessions of wild cowpea also possessed hairs but only on stems, and these included 5 *dekindtiana* (TVNu 305, 389, 255, 438, and 1589), two *protracta* (TVNu 505 and 273), one *trilobata* (TVNu 953), one *grandiflora* (TVNu 539), and the only two *pubescens* (TVNu 538 and 533) accessions used in this study.

Evaluation of some qualitative characters revealed that the proportion of pigmentation in wild cowpea was greater than in cultivated cowpea where the majority of the accessions had no pigment on stems. The ratio of pigment on the stem to non-pigmented stem in wild cowpea was approximately 1:3 while it was about 1:7 in cultivated cowpea (Table 1). A total of 37 accessions had an average of 20–30 leaves per plant, a large leaf area, and a copiously branched plant when examined at four weeks after planting. Thirty-four of them were cultivated cowpeas while the rest were wild cowpeas, which are all *dekindtiana* (TVNu 710, 249, and 303) accessions. This is a particular trait of agronomic importance as any of these accessions could be used as a forage crop for livestock animals. Possession of hairs in some accessions identified in this work could be a useful agronomic trait for insect-resistant improvement in cowpea. Pubescence, which is prominent in
some wild species, has been reported in several crop species and confers superior agronomic or abiotic stress adaptation. Agwaranze (1992) has observed that soybeans with dense pubescence are better adapted to high radiation, high temperature, and limited moisture conditions than those with less pubescence. He has stressed further that pod hair is a factor in *Vigna vexillata* resistance to the pod sucking bug both under the field and screenhouse conditions. IITA annual report and research highlights of 1987/88 (IITA, 1988) also recorded a high level of resistance to the *Maruca* pod borer and pod sucking bugs in hairy *V. vexillata* using fresh pods as test materials. The Pearson-correlation coefficient indicated linear relationships with strong correlation among the characters evaluated.

Table 1. Morphological evaluation of some qualitative characters of 390 accessions of both cultivated and wild cowpeas.

<table>
<thead>
<tr>
<th>Traits</th>
<th>No. of cultivated accessions</th>
<th>No. of wild accessions</th>
<th>Total accessions</th>
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<tr>
<td>Presence of pigment on stem</td>
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<tr>
<td>Purple pigment</td>
<td>31</td>
<td>38</td>
<td>69</td>
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<tr>
<td>%</td>
<td>11.92</td>
<td>11.92</td>
<td>23.84</td>
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<tr>
<td>Presence of stripes on pods</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Red stripes</td>
<td>51(19.61%)</td>
<td>2</td>
<td>53</td>
</tr>
<tr>
<td>Brown stripes</td>
<td>3 (1.15%)</td>
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<tr>
<td>%</td>
<td>20.76</td>
<td>1.53</td>
<td>22.29</td>
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<td>Plant habit</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Erect</td>
<td>19(7.3%)</td>
<td>1(0.77%)</td>
<td>20</td>
</tr>
<tr>
<td>Suberect</td>
<td>18(6.92%)</td>
<td>6(4.62%)</td>
<td>24</td>
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<tr>
<td>Prostrate</td>
<td>229(88.07%)</td>
<td>117(90%)</td>
<td>346</td>
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<tr>
<td>Hairiness on:</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>stem, leaf and pod</td>
<td>0</td>
<td>14(10.77%)</td>
<td>14</td>
</tr>
<tr>
<td>Stem only</td>
<td>0</td>
<td>12(9.23%)</td>
<td>12</td>
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</table>

The Pearson-correlation coefficient was used to indicate the linear relationship between the characters (Table 2). A strong correlation was observed among the traits under study, and the number of traits for principal component analysis was reduced to only nineteen traits. This is because highly correlated traits showed no variance and can lead to an unreliable estimate of the variance in the principal components. Based on this, the first component (Prin 1) accounted for 60% of the total variation. The floral characters such as keel width (kwt), standard length (slt), adelphous stamen length, standard width (swt) and style length (sflt) were positively and highly correlated to the wing width (wwt), i.e., for a high wing width value, the other flower characters had a high value too, and contributed greatly to the diversity observed among the accessions (Table 2). A similar high and positive correlation was observed between wing length (wlt) and standard width (swt) and
wing width (wwt), keel width (kwt) with adelphous stamen length (aslt) and standard length (slt), adelphous stamen length (aslt) with single stamen length (sslt) and style length (stlt), and standard width (swt) and adelphous stamen length (aslt).

Table 2. The Pearson-correlation matrix to two decimal places of nineteen morphological characters evaluated for 390 accessions of cultivated and wild cowpeas.

<table>
<thead>
<tr>
<th></th>
<th>clt</th>
<th>cwt</th>
<th>cllt</th>
<th>wlt</th>
<th>wwt</th>
<th>klt</th>
<th>kwt</th>
<th>slt</th>
<th>swt</th>
<th>aslt</th>
<th>sslt</th>
<th>stlt</th>
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<td>0.12</td>
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<td>0.26</td>
<td>0.49</td>
<td>0.56</td>
<td>0.61</td>
<td>0.5</td>
<td>0.44</td>
<td>0.34</td>
<td>0.37</td>
<td>0.41</td>
<td>0.42</td>
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The characters are abbreviated as: calyx length (clt); calyx width (cwt); calyx lobe lt (cclt); total calyx lt (tclt); wing lt (wlt); wing width (wwt); keel lt (klt); keel width (kwt); standard lt (slt); standard width (swt); adelphous stamen lt (aslt); single stamen lt (sslt); style lt (stlt); terminal leaflet width (tlwt); terminal leaflet lt (tltt); petiolule lt (pllt); petiole lt (plt); rachis lt (rlt) and pulvin lt (pult).

Primers used varied in allele identification per accession in the study (Figure 1). A total of 92 resolved DNA bands were amplified by sixteen SSR primers in the 48 accessions evaluated. The distance matrices generated from genotyping data were used to construct the dendrogram (Figure 2). Three clusters were identified at a similarity coefficient of 82% that separated cultivated lines from their wild relatives. Clusters ‘A’ and ‘C’ consisted mainly of wild cowpea, while cluster ‘B’ comprised cultivated cowpea including accessions of subsp. Sesquipedalis and...
subsp. cylindrical. Two dekindtiana (3 and 6), one kgalagadensis (10) and one protracta (20) wild accessions clustered with the cultivated cowpea. Accessions 13 and 14 were widely separated, and this may be attributed to leaf characteristics of these accessions. Their morphological evaluation revealed that accession Tvnu 1471 (14) (belonging to rhomboidea) had simple leaf characteristics as against the trifoliate leaf in rhomboidea accession Tvnu 1473 (13). Two accessions, Tvnu 533 and 538 (23 and 24) belonging to the ssp pubescens were also grouped separately from each other. Accession Tvnu 533 (23) did not cluster in either of the two clusters (A and B) while accession Tvnu 538 (24) was grouped in cluster ‘A’. Mensensis (19) was observed as an outgroup to all the evaluated accessions in the study. Mensensis is a variety of subspecies dekindtiana with the long calyx lobe as against the short calyx lobe found in other wild cowpeas. This is a characteristic of V. unguiculata subsp. dekindtiana var. mensensis.

![Figure 1. The electrophoregram of the combined accessions of cultivated and wild cowpeas with SSR primer VM 27. (Code: 1–24 = wild, 25–48 = cultivated with lanes 1–24 showing more segregation than lanes 25–48).](image)

The information generated from the dendrogram showed that the cultivated cowpea was closely related to the wild species. This is important for understanding the taxonomy of the genus Vigna, the origin of cultivated cowpea and future cowpea breeding. Several hypotheses based on morphological attributes have been proposed about the origin of cultivated cowpea (Rawal, 1975; Lush, 1979; Steele and Mehra, 1980; Ng and Marechal, 1985). The wild annual subsp. dekindtiana has been suggested as the probable progenitor of the cultivated cowpea. Lush and Evans (1981) have also speculated that the subsp.subsp. dekindtiana is a more probable progenitor type than subsp.subsp. mensensis because it is morphologically closer to the cultivated cowpea. He stressed further that subsp. dekindtiana could have originated from hybrids of subsp. mensensis with subspecies unguiculata.
This corroborates with the work of Pasquet (1999), who reported subsp. *spontanea* as the closest variety to cultivated cowpea. It is also quite possible that what was classified as subsp. *spontanea* is also referred to as *dekindtiana*, i.e., the same subsp. Fatokun et al. (1993) used RFLP markers to demonstrate that *Vigna unguiculata* subsp. *unguiculata* var. *sesquipedalis* showed high phyletic relationships with cultivated cowpea and its wild relatives of subsp. *dekindtiana*. However, results from this work did not show a contrary opinion from past findings.

![Figure 2](image_url)


The principal component analyses further clarified the relationships between cultivated and wild cowpeas. For molecular data, the first and the second principal
components (PCs) accounted for 67.45% and 3.75%, respectively, of the total variations in the correlation matrix, which was considered a good summary of the data. The two-dimensional plot (Figure 3) based on these two axes showed a clear pattern of differentiation although there was the overlapping of some wild accessions in the group of cultivated ones. For instance, two wild subspecies *dekindtiana* (3) and *protracta* (20) were grouped together with the cultivated accessions. Wild lines were distinctly separated from the cultivated group, especially *rhomboidea* and *mensensis* with Tvu1471 (14) and Tvu 1561 (19), respectively, indicating their distinctiveness as revealed by the UPGMA dendrogram. After examining the gels developed from the sixteen polymorphic primers for shared bands between wild and cultivated cowpeas, the results showed that *dekindtiana* had the highest percentage (80%) of shared bands with cultivated cowpea, followed by *protracta* at 54%, while others were *kgalagadensis* at 48%, *pubescens* at 40%, *congolensis* at 39%, *mensensis* at 36%, *ovata* at 28%, and *rhomboidea* at 27%. The high percentages obtained for both *dekindtiana* and *protracta* may be a result of their closeness to the cultivated cowpea, as close relatives are likely to have matching bands. The study also revealed a higher PIC value in wild cowpea (6.14) than in cultivated cowpea (4.47).

Figure 3. The principal component analysis of forty-eight accessions of cowpea (cultivated and wild) using SSR primers, (1–24 are wild, and 25–48 are cultivated cowpeas).
From the study, both dekindtiana and protracta consistently showed good relationships with the cultivated cowpea and clustered together. This is an indication of genetic relatedness with cultivated cowpea and probable domestication of these wild relatives. This suggests that breeders could use these two wild relatives to improve the cultivated cowpea with the intent of introgressing useful traits into cultivated cowpea. Studies have confirmed the cross-compatibility between cultivated and wild cowpeas, especially the dekindtiana.

The polymorphic information content (PIC) value for cultivated cowpea was 4.467 from a total of thirty-eight alleles detected from sixteen SSR primers. This high value was in agreement with the results obtained in past works (Ali et al., 2015) although this value was lower than those reported in other legumes, like P. vulgaris (Singh et al., 1991) and some other non-legumes like rice (Second, 1985). This could be because cowpea was domesticated at only one place. However, wild cowpea had a higher PIC value of 6.136 from fifty-four alleles with the same number of SSR primers. The estimates of PIC and allele frequency from this study indicated that wild cowpea was much more genetically diverse than cultivated cowpea. We also observed lower genetic variation within cultivated cowpea compared to the wild accessions, probably due to wider geographic distribution of the wild species, with rhomboidea (14) and mensensis (19) far apart from remaining accessions.

Conclusion

The study revealed a diverse morphological trait between cultivated and wild cowpeas evaluated in the study, with hairiness only found on wild cowpea pods and stems. The molecular evaluation showed wild cowpea lines were distinctly separated from the cultivated group, especially rhomboidea and mensensis. However, both morphological trait evaluation and molecular data showed lower genetic variability between both cultivated and wild cowpea species evaluated in the study although widely distributed geographically.

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MORFOLOŠKA KARAKTERIZACIJA I KARAKTERIZACIJA MARKEROM SSR DIVLJIH I UZGAJANIH VIGNI (VIGNA UNGUICULATA L. WALP)

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Rezime

Tristadevedeset genotipova koji su obuhvatali 260 gajenih i 130 genotipova divljih vigne fenotipski su ocenjivani korišćenjem 27 deskriptora vigne. Morfološka evaluacija nekih kvalitativnih osobina otkrila je od 11,92% do 29,23% prisustvo pigmentacije na stabljiki, 1,53% do 20,76% prisustvo pruga na mahuni i 0% do 20% prisustvo dlakavosti na biljci gajene odnosno divlje vigne. U pogledu molekularne analize, šesnaest SSR prajmera su korišćeni za genotipizaciju 48 genotipova koji pripadaju kako divljoj tako i gajenoj vigni. Podaci su dali dendogram sa tri klastera, od kojih su se dva sastojala od divlje vigne dok je treći obuhvatio sve gajene vigne, uključujući i genotipove sa dugačkom mahunom (Vigna unguiculata subsp. sesquipedalis) i Vigna unguiculata podvrste cylindrica. Dva divlja genotipa podvrste dekindtiana, i po jedan iz podvrsta kgalagadensis i protracta grupisali su se sa genotipovima gajenih vogni ukazujući na njihove veze, ali ne i sa drugim divljim vgnama. Broj polimorfnih traka SSR kod gajenih i divljih vogni bio je 54 i 38 odnosno 54, dok su vrednosti PIC bile 6,14 odnosno 4,47, što pokazuje veću genetsku raznovrsnost kod divljih nego kod gajenih vogni. Podvrsta dekindtiana imala je najveći broj (80%) prikazanih traka primenom SSR markera sa gajenom vognom, a zatim je sledila podvrsta protracta sa 54% prikazanih traka. Pet vrsta divljih vigne ima dlake, te bi se tako mogle koristiti u oplemenivanju za otpornost prema insektima.

Ključne reči: vigna, karakterizacija, dendrogram, genetska raznovrsnost, mikrosateliti.

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