

PHENOTYPIC CHARACTERIZATION FOR INDUCED MUTATION BREEDING IN RADIO SENSITIVITY STUDIES OF *Vigna unguiculata*, *Pennisetum glaucum* AND *Sorghum bicolor* VARIETIES IN NAMIBIA

LYDIA N. HORN¹, B. SOLEYMANNE² and J. VOLLMANN¹

¹Directorate of Agricultural Research and Training, Ministry of Agriculture, Water and Forestry,
Private Bag 13184, Windhoek, Namibia

²Based at the FAO/IAEA Plant Breeding Unit, Seibersdorf Laboratories, Vienna, Austria

ABSTRACT

During 2009, 95 accessions of cowpea [sourced from the Namibia Botanical Research Institute (NBRI), Windhoek, Namibia and the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria], 32 of pearl millet and 41 of Bambara groundnut, (both sourced from the NBRI, Windhoek, Namibia) were evaluated for their morphological and phenotypical characteristics at the FAO/IAEA Plant Breeding Unit's Agriculture and Biotechnology Laboratory A-2444, Seibersdorf in Austria. Accessions from the NBRI were separated according to leaves and seed colour, size and shapes before planting them for observation. The cowpea seedlings were evaluated 14 days after emergence, measuring the length of the hypocotyls and epicotyls. All accessions showed variations in growth, leaf colour and in days to maturity.

Furthermore, Namibian accessions showed a high rate of epicotyls' length compared to those of the IITA. Digital camera and image-analysis software (Sigma Scan Pro 5.0) was used to quantify the greenness, relative leaf size and cover of the cowpea leaves. Greenness was quantified as a dark green colour index (DGCI), with values from 0 to 140, from hue, saturation and brightness values as determined by the software. High value indicates low greenness content and therefore low chlorophyll content. It was noted that about 50 % Namibian cowpea accessions had low content of greenness meaning that the nodulation is also low and therefore has less nitrogen-fixing ability.

INTRODUCTION

Bambara groundnut (*Vigna subterranean* (L.) Verdic) is a very popular and indigenous crop that is commonly grown in Namibia by most subsistence farmers. Cowpea (*Vigna unguiculata*) on the other hand is also very important and grown or intercropped with pearl millet (*Pennisetum glaucum*), which is the number one staple food crop in Namibia. Due to their popularity, these crops can produce yields in soils that are regarded as poor soils and where other crops can hardly perform. Following the germplasm collection mission in Namibia soon after independence in 1991, accessions collected were taken to the NBRI for safe-keeping. The phenotypic characterization observed in seed of the same plant species was found to be different and these differences were normally in seed colour, sizes and shape. It is therefore interesting to study these accessions

and characterize them according to colour, sizes and shapes prior to genetic characterization. In addition to this exercise different seed from the crops under investigation was planted under a controlled environment in a green house. Characteristics such as the growing patterns, plant height, flowering dates and colours as well as the colour of leaves were recorded and compared among crops. This experiment was made possible through the Joint FAO/IAEA project in collaboration with the Ministry of Agriculture, Water and Forestry. It was conducted at the IAEA Plant Breeding Unit in Austria during 2009.

MATERIAL AND METHODS

The 95 accessions of cowpea, 32 of pearl millet and 41 of Bambara groundnut were planted in plastic boxes under a controlled environment in a green-house (Figure 1). The length of the hypocotyls and epicotyls was measured 14 days after emergence. Data was entered into Microsoft Excel to generate graphs.

Initially, the accessions from Namibia were mixed. A mixture of seed was observed in cowpea and Bambara groundnut accessions and therefore accessions were sorted, based on seed colour. For example the sub name with the accession number (NAM 2028) was found to consist of three different seed colours (cream, dark purple and pink). The accession numbers were reassigned as NAM 1639/2a, NAM 1639/2b, NAM 1639/2c and NAM 1639/2d respectively (Figure 2). Seeds from the same lot and with the same colour were planted in one pot and later thinned to three plants per pot. Three weeks after emergence, leaf tissues were harvested, taking one leaf from each of the three plants per pot.

The leaf tissues were used in different molecular biology assays (SSR, Page gel and AFLP). Seedlings were kept in the greenhouse up to maturity stage. Digital camera and image-analysis software (Sigma Scan Pro 5.0) was used to quantify the greenness, relative leaf size and cover of the cowpea leaves. Greenness was quantified as a dark green colour index (DGCI), with values from 0 to 140, from hue, saturation, and brightness values as determined by the software. High value indicates low greenness content and therefore low chlorophyll content. Digital images of leaves were taken against a blue background in the greenhouse. From the data graphs were created (Figure 4 to 7). The length of the epicotyls and hypocotyls in cowpea (Figure 3), as well as date to flowering, was also recorded and generated into graphs (Figure 8 to 11).



Figure 1. Cowpea, Bambara groundnut and pearl millet accessions in the green house at Seibersdorf Plant Breeding Unit.



Figure 2. Cowpea (left) and Bambara groundnut (right) from the N BRI after being sorted into four different accessions.

RESULTS

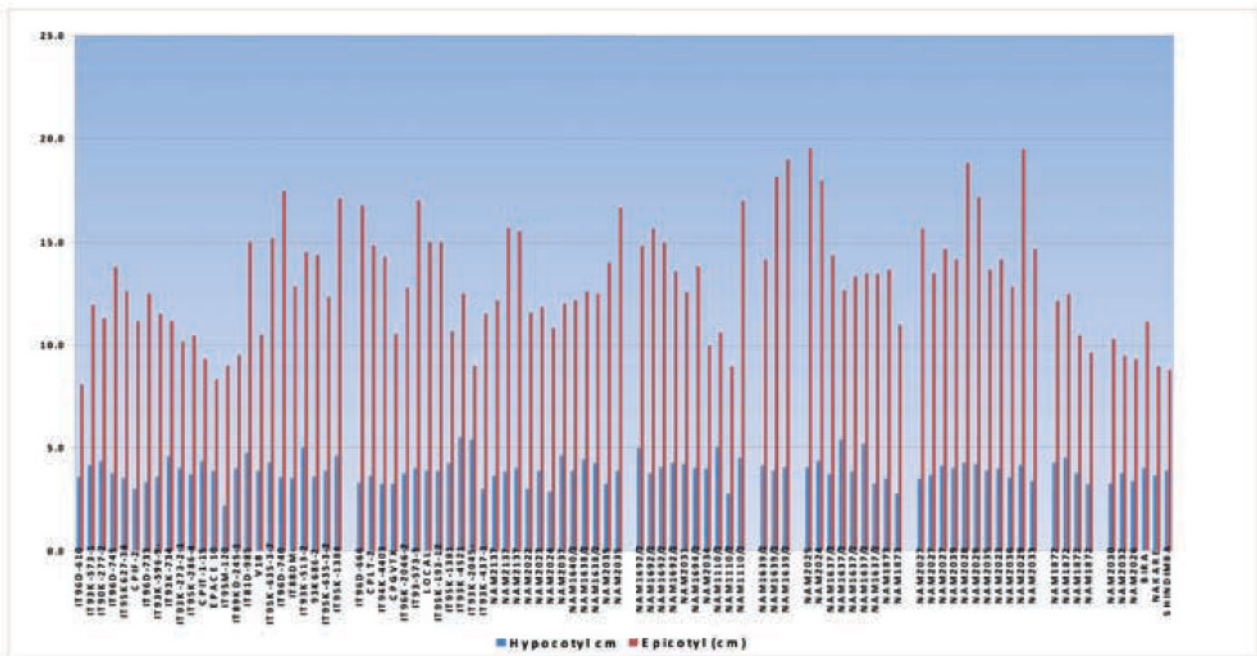


Figure 3. The differences between the length of the hypocotyls and epicotyls of cowpea plants at 14 days after emergence. Namibian accessions showed a high rate of epicotyls' length compared to those of the IITA.

Leaf analysis

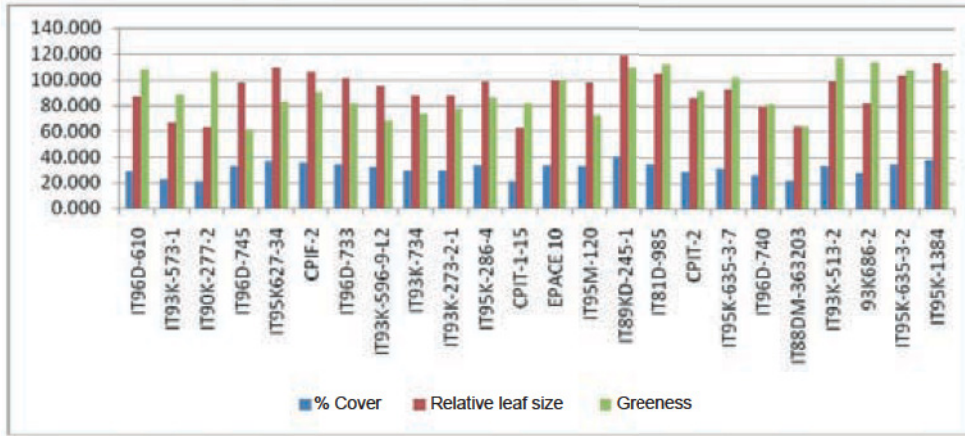


Figure 4. The percentage leaf cover, relative leaf size and greenness of 24 cowpea accessions from IITA. The IITA cowpea leaf greenness showed a high greenness content of between 100 and 110 and high relative leaf size between 80 and 100.

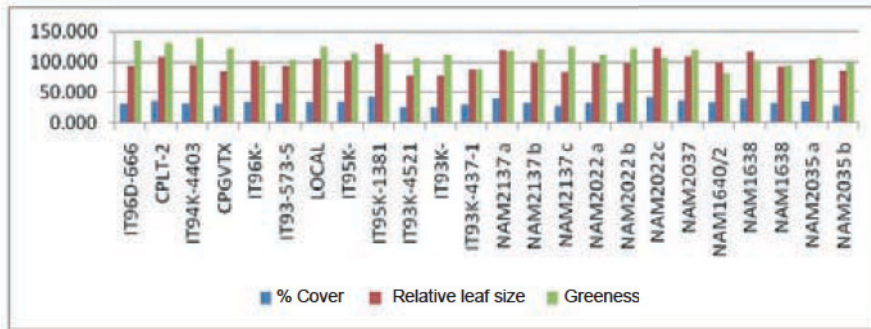


Figure 5. The percentage leaf cover, relative leaf size and greenness of 24 cowpea accessions from Namibia. The Namibian cowpea leaf greenness showed lower greenness content of up to 180 in comparison to IITA accessions.

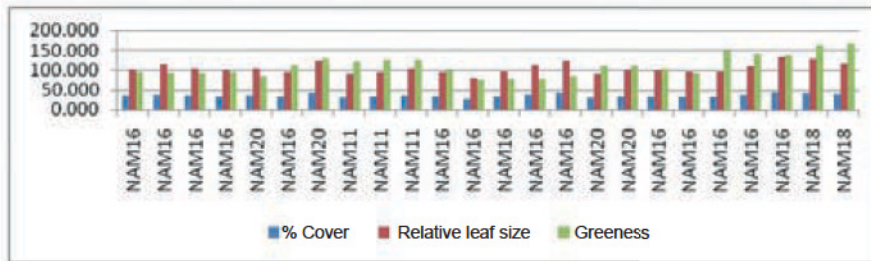


Figure 6. The percentage leaf cover, relative leaf size and greenness of 24 cowpea accessions from Namibia. The Namibian cowpea leaf greenness showed lower greenness content of up to 180 in comparison to IITA accessions.

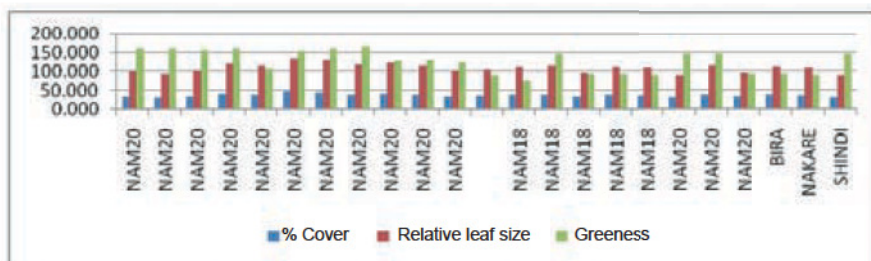


Figure 7. The percentage leaf cover, relative leaf size and greenness of 24 cowpea accessions from Namibia. The Namibian cowpea leaf greenness showed a lower greenness content of up to 180 in comparison to IITA accessions. Note that the lower the value (percentage cover, relative leaf size and greenness) the higher the content.

Number of days to flowering

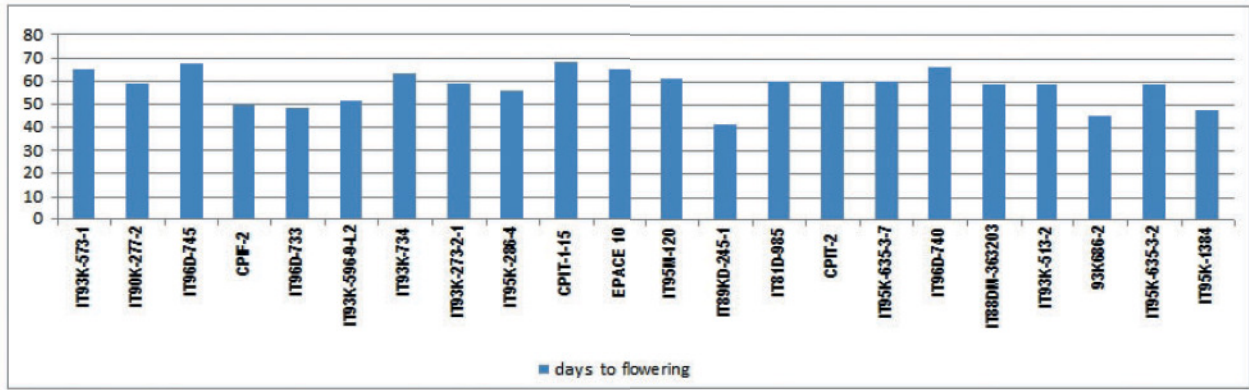


Figure 8. The number of days to flowering. The IITA accessions flowered within 100 days after emergence. IT89KD-245-1 flowered at 40 days.

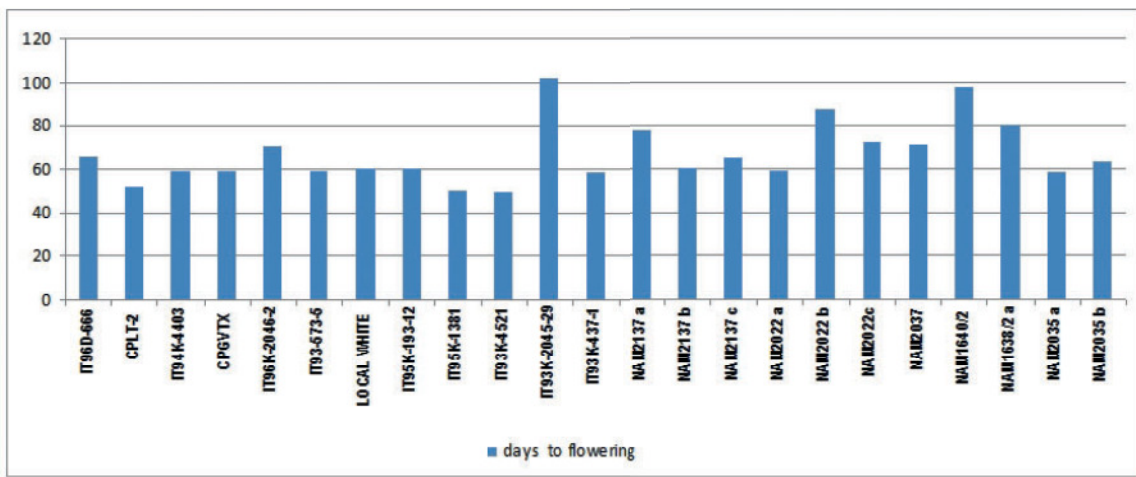


Figure 9. The number of days to flowering. Most of the IITA accessions flowered within 40-60 days after emergence while Namibian accessions NAM21317b, NAM2022a and local White flowered within 59-60 days after emergence. The rest of the Namibian accessions flowered from 60 days to 100 days after emergence.

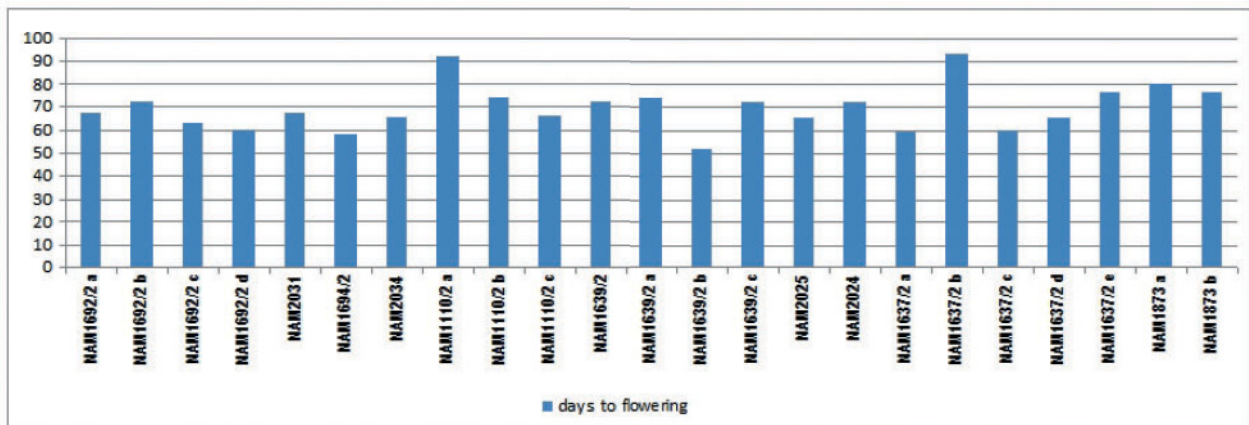


Figure 10. The number of days to flowering of Namibian accessions flowers from 50 days to 90 days after emergence. Separated accessions have shown a distinctive difference with flowering dates.

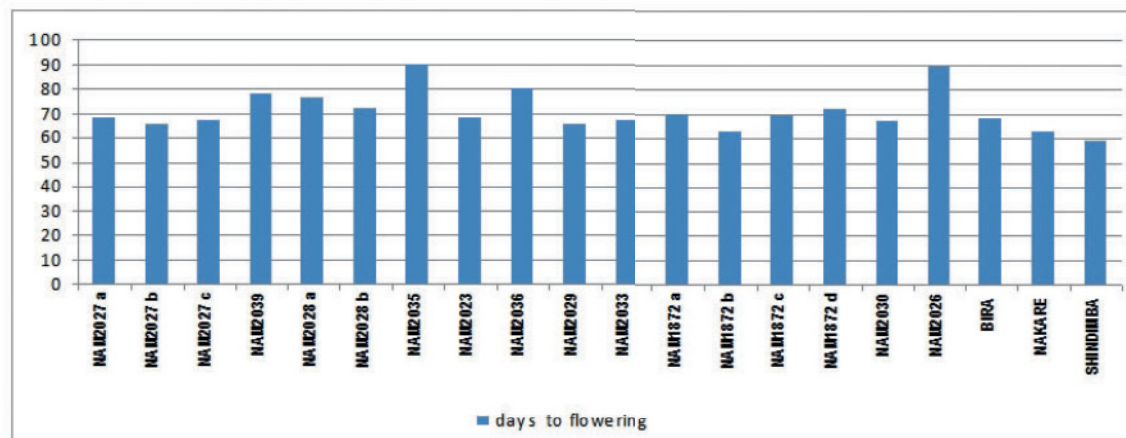


Figure 11. The number of days to flowering of Namibian accessions which flowers from 60 days to 90 days after emergence. Among the released varieties Shindimba flowered at 59 days while Bira flowered at 68 days after emergence. The late maturing species were the NAM 2035 and NAM 2026 with 90 days after emergence.

DISCUSSION AND SUMMARY

The epicotyls and hypocotyls analysis in cowpea accessions showed the variation in plant heights between Namibian and IITA accessions. However the length of epicotyls and hypocotyls showed correlations between accessions (Figure 3). Namibian accessions showed a higher rate of epicotyls' length in seedlings of 14 days with the ranges between 8 cm and 18 cm. This information correlates with the observed fact that most of the Namibian cowpea showed a trailing/creeping growing habit thus taking longer to flower and to reach maturity unlike the IITA accessions with short epicotyls, which grew as an erect bush and matured early. Furthermore, it was observed that separated accessions such as NAM 2137a and NAM 2137c did not flower at the same time but at different times, 59 and 78 days after emergence respectively. This supports the idea that separating the two accessions based on the fact that their colours were different was relevant. It could however be agreed, based on the morphological characteristics, that the accessions taken from the same lot but with different colour, shape and sizes are really different from one another.

The pigment determination using digital images to quantify in-season N status of crops gives an idea of how much N a plant is able to absorb. It was also observed that plants with dark green leaves had many more root nodules compared to those with lighter green leaves. This was observed in IITA accessions where dark green leaves were observed and a high content of nodules on the roots. Furthermore, about 50 % of cowpea accessions from Namibia had lighter green leaves when compared to those obtained from IITA and therefore low content of greenness was recorded. However, Namibian cowpeas were found to possess a very large leaf surface (Figure 4 to 7) and most of them had a creeping/climbing habit. They took between two to three months to set pods which is not the case with the IITA accessions of which the majority flowered within 40 days after emergence. Namibian accessions in general have large pods and grains and the germplasm was found to be rich in diversity as many different seed colours (black, white, purple, red, brown and

grey, caramel, cream, pink and spotted) were observed. Multiple flower colours were also observed in Namibian accessions. The most dominating flower colour observed was purple and yellow followed by a white colour.

CONCLUSION

Cowpeas in Namibia lack green pigments and this is said to influence the nitrogen fixation ability. The IITA cowpea has more green pigments and therefore better nitrogen fixation abilities. Erect and bushy type cowpeas showed early flowering and thus early maturity in relation to creeping types which took longer to flower and mature. With the induced mutation breeding, it is expected that improved varieties could be developed for Namibia to suit its requirements.

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