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Genotypic Variation in Excised Leaf Culture Ability of Fodder Cowpea: A New Direction for Germplasm Evaluation

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Genotypic variability in rooting ability of excised leaves is of great importance in crop improvement programme. Rooting in excised leaves encourages leaf culture which in term helps in identification of stay green and disease resistant plants. In the present investigation we have tried to find the genotypic variation in senescence and root traits of excised forage cowpea leaves. Matured leaves were excised from 30 and 40 days old plants and immersed in water for 8-10 days. Roots were appeared in leaf petiole on 5th day of immersion. Observations were recorded on root length,

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number of roots, frequency of rooted leaves, chlorophyll content and senescence of excised leaves on 7th day. Results revealed significant variation in root length, root number, frequency of rooted leaves, chlorophyll contentand senescenceof detached leaves. The maximum root length was observed in case of UPC-2001 (8.26 cm) followed by Sweeta (7.18 cm).Length of the longest root ranged from 2.90 to 8.26 cm with a mean of 4.95 cm. Number of adventitious roots ranged from 7.8 to 36.67 with a mean of 19.46. Frequency of rooted leaf ranged from 0.10 to 1.00.The chlorophyll content in terms of SPAD units varied from 13.54 to 42.31.Senescence index ranged from 0.10 to 1.00.Three genotypes namely UPC-804, UPC-2001 and Sweetashowed the highest desirability index of 5.0. This investigation reveals variation in excised leaf culture ability of forage cowpea genotypes. The excised leaf culture is the simplest, quickest and cheapest techniquethat could be used for identification of stay green and disease resistant fodder cowpea genotypes.

Keywords: Fodder cowpea; genotypic variation; germplasm evaluation; excised leaf culture; root trait; senescence.

1. INTRODUCTION

Genetic diversity is a prerequisite for any crop improvement programme and it needs information on morphological, physiological, biochemical, cytological and molecular characters for germplasm evaluation and characterisation. Integration of more numbers of new traits may expedite the activity of germplasm evaluation and characterisation. The germplasm evaluation and characterization is essential for registration and protection of varieties. Morphological characterization is a simple approach based on visible traits and requires minimal resources to evaluate the genetic diversity in crops. Traits that are easy to measure and that shows association with plant biomass could assist plant breeders and accelerate breeding programmes in the development of cultivars with enhanced biomass production.

Fodder cowpea (*Vigna unguiculata* L. Walp) (2n=22) is an important annual herbaceous legume crop and it belongs to family fabaceae. This crop can sustain in harsh conditions like heat and drought stresses and improves soil fertility through nitrogen fixation. The morphological diversity present within fodder cowpea germplasm provides opportunities that could be exploited in crop improvement programme [1]. Kumar et al. [2] evaluated 20 forage cowpea genotypes on the basis of various morphological characters like incidence of cowpea mosaic virus, seed crowding in pod, texta structure, eye color, pod shape, pod attachment to peduncle, terminal leaflet shape, twining tendency and flower pigmentation. Sheahan [3] and National Research Council [4] reported that cowpea can either be short and bushy (as short as 20 cm) or grow like a vine through climbing supports or trailing along the ground (to a height of 2 m). Pottorff et al. [5]. observed variation in leaf size and shape of genotypes and used these traits for distinguishing cowpea varieties. Peduncle length and flower colour (purple, pink, yellow and white and blue) were used as distinguishing features for varietal identification of forage cowpea (National Research Council, 2006).

Evaluation of germplasm based on root traits of excised leaves encourages use of leaf culture technique for screening of disease resistant plants [6]. Variation in senescence of excised leaves could be used for identification of stay green genotypes [7]. To the best of authors knowledge evaluation of fodder cowpea genotypes for excised leaf culture is not available in the literature. In the present investigation an attempt has been initiated to study variation in senescence and root traits of fodder cowpea leaves deemed essential for excised leaf culture and identification of genotypes suitable for this purpose.

2. MATERIALS AND METHODS

The present investigation comprised of 27 forage cowpea genotypes, collected from different places of India (The list is furnished in Table 1). Eleven genotypes were collected from Mandya (genotype no. 1 to 11; Table 1), nine genotypes from Pantanagar (genotype no. 11 to 20; Table 1), four genotypes from Coimbatore (genotype no. 21 to 24; Table 1) and three from Hyderabad (genotype no. 25 to 27; Table 1) centre. The experimental trial was conducted at AICRP on FC & U, OUAT, Bhubaneswar during kharif, 2023

following RBD with three replications. The genotypes were sown in 2 rows of 4 m length with row to row distance of 60 cm and plant to plant spacing of 20cm. Matured leaves (10 numbers) from middle part of the vine were excised from 30 days old plants for the first experiment and 40 days old plants for second experiment. The excised leaves were kept in beakers filled with tap water up to a level so that the petioles were remaining immersed. The beakers were kept in the lab for 8-10 days under dark condition. The tap water in beaker was changed on each alternate day. Roots were appeared in excised leaves on 6-7 days of immersion. Observations were recorded on characters found suitable for excised leaf culture like number of roots, length of the longest root, frequency of rooted leaves, chlorophyll content and senescence on 7th day of immersion. The degree of senescence in detached leaves was indirectly measured through chlorophyll content(using Minolta SPAD meter).The SPAD reading of a genotype was taken from middle portion of three leaflets of each compound leaf and then averaged over ten compound leaves. Senescence of excised leaves was quantitatively expressed in terms of senescence index and it was calculated following the method of [8].The lab experiment was repeated twice at 10 days interval with two replications following CRD. The data were subjected to descriptive and summary statistics like mean, standard deviation, coefficient of variation and correlation estimate by SAS software version 9.3.

3. RESULTS AND DISCUSSION

The simple measures of variability like range, standard deviation and coefficient of variation for different traits are presented in Table1.Coefficient of variation was found to be 29.2, 41.8, 35.4, 25.2 and 38.0 for length of the longest root, number of lateral roots, frequency of rooted leaves, chlorophyll content and senescence index. Standard deviation was the highest for the number of lateral roots (8.13) and the lowest for senescence index (0.232). The length of the longest root ranged from 2.90 to 8.26 cm with a mean of 4.95 cm. The excised leaves of genotypes having root length more than 5.23 cm (i.e. mean + SE) were considered to have longer roots. Thegenotypes like MFC -18-2 (6.40 cm), MFC-20-3 (5.26 cm), NBC-40 (5.58 cm), NBC-43 (8.02 cm), UPC-804 (5.95 cm), UPC-2001 (8.26 cm), UPC-2002(6.48 cm), FD-1161(5.53 cm) and Sweeta (7.18 cm) in the present study found to record longer root. Number of adventitious roots in excised leaves varied from 7.80 to 36.67 with a mean of 19.46.The genotypes MFC-09-3 (36.67), MFC -18-2 (30.60), UPC-4200 (22.80), UPC-9202 (23.40), UPC-801(24.60), UPC-802(22.75), UPC-804(31.00), UPC-805 (25.30), UPC-2001(27.60), UPC-2002 (25.20) and Sweeta (34.20) recorded higher number of adventitious roots. The frequency of rooted leaves were higher (value >mean +SE)in genotypes like MFC -18-2 (1.0), MFC-18-8 (1.0), MFC-20-3 (1.0), IC-219489 (1.0), NBC-43 (1.0), UPC-4200 (1.0), UPC-9202 (1.0), UPC-801(1.0), UPC-802 (0.90),UPC-804 (1.0), UPC-805 (0.90), UPC-2001(1.0) and Sweeta (1.0) and rest genotypes had low frequency. Chlorophyll content of the excised leaves of 27 genotypes ranged from 13.09 to 42.31 SPAD units.The genotypes, MFC -18-2 (34.78 SPAD units), NBC-40 (34.60SPAD units), NBC-43 (39.25 SPAD units),UPC-9202 (30.24 SPAD units), UPC-801(33.56 SPAD units), UPC-802 (31.81 SPAD units), UPC-804 (31.67 SPAD units), UPC-805 (40.58 SPAD units), UPC-2001(38.61 SPAD units), UPC-2002 (35.60 SPAD units) and Sweeta (42.31 SPAD units) had higher chlorophyll content and rest of the genotypes had low chlorophyll content. Senescence index of detached leaves of 27 genotypes ranged from 0.14 to 0.98. Higher senescenceis marked with the yellowing of leaves due to chlorophyll degradation and low senescence is marked with the green colour of leaves. The genotypes with low senescence index (value < mean - SE) were agronomically desirable and they were IC-219489 (0.50), NBC-43 (0.33), UPC-4200 (0.42), UPC-4287 (0.57), UPC-801 (0.45), UPC-804 (0.42), UPC-2001 (0.14), UPC-2002 (0.43), IFC-9304 (0.44) andSweeta (0.15). A significant negative correlation (-0.549) was observed between SPAD value and senescence index and this indicates that with the increase in SPAD value (higher chlorophyll content) the senescence decreases. Length of the longest root in excised leaf, number of adventitious roots/excised leaf, frequency of rooted leaves showed a correlation value of -0.647, -0.400 and -0.405 with senescence index and this indicates that better rooting ability leads to decrease senescence.

Star marks indicates value > mean + SE for all traits except senescence index where it is < mean – SE (in Table 1). The potentiality of the genotypes for excised leaf culture was judged from desirability index which was calculated by adding the number of star marks that the genotype possessed for each of the traits

Table 1. Variation in root traits and senescence index of excised fodder cowpea leaves

Genotype	Length of the longest root(cm) in excised leaf	Number of lateral roots per excised leaf	Frequency of rooted excised leaf	Chlorophyll content of excised leaf (SPAD unit)	Senescence index of excised leaf	Desirability index for leaf culture
1. MFC-08-14	3.08	11.75	0.80	25.42	0.98	0.0
2. MFC-09-3	5.06	36.67*	0.60	22.61	0.93	1.0
3. MFC -18-2	6.40*	30.60*	1.00*	34.78*	0.67	**4.0**
4. MFC-18-8	4.83	15.50	1.00*	13.54	0.85	1.0
5. MFC-18-10	3.02	12.10	0.10	25.15	0.80	0.0
6. MFC-20-3	5.26*	18.00	1.00*	27.40	0.83	2.0
7. EC-107120	4.17	19.30	0.60	24.45	1.00	0.0
8. MFC-09-1	3.76	8.30	0.70	22.39	0.59	0.0
9. NBC-40	5.58*	10.00	0.80	34.60*	0.68	2.0
10. IC-219489	4.98	13.60	1.00*	26.19	0.50*	2.0
11. NBC-43	8.02*	20.40	1.00*	39.25*	0.33*	**4.0**
12. UPC-4200	4.58	22.80*	1.00*	28.06	0.42*	3.0
13. UPC-4287	4.44	14.80	0.80	17.96	0.57*	1.0
14. UPC-9202	5.14	23.40*	1.00*	30.24*	0.72	3.0
15. UPC-801	5.12	24.60*	1.00*	33.56*	0.45*	**4.0**
16. UPC-802	4.51	22.75*	0.90*	31.81*	0.78	3.0
17. UPC-804	5.95*	31.00*	1.00*	31.67*	0.38*	**5.0**
18. UPC-805	3.98	25.30*	0.90*	40.58*	0.42*	**4.0**
19. UPC-2001	8.26*	27.60*	1.00*	38.61*	0.14*	**5.0**
20. UPC-2002	6.48*	25.20*	0.80	35.60*	0.43*	**4.0**
21. N-311	2.87	13.40	0.20	28.33	0.65	0.0
22. IFC-9304	4.80	18.00	0.50	16.95	0.44*	1.0
23. FD-1161	5.53*	16.80	0.70	27.82	0.67	1.0
24. CO-(FC)-8	4.67	13.30	0.60	22.15	0.61	0.0
25. Vijayar	3.10	8.20	0.30	25.13	0.72	0.0
26. TSFC-20-06	2.90	7.80	0.40	29.29	0.88	0.0
27. Sweeta	7.18*	34.20*	1.00*	42.31*	0.15*	**5.0**
Mean	**4.95**	**19.46**	**0.76**	**28.74**	**0.61**	
Range	**2.9-8.26**	**7.80-36.67**	**0.01-1.00**	**13.09-42.31**	**0.14-0.98**	
SE	0.28	1.56	0.051	1.394	0.045	
SD	1.45	8.13	0.269	7.249	0.232	
CV %	29.2	41.8	35.4	25.2	38.0	

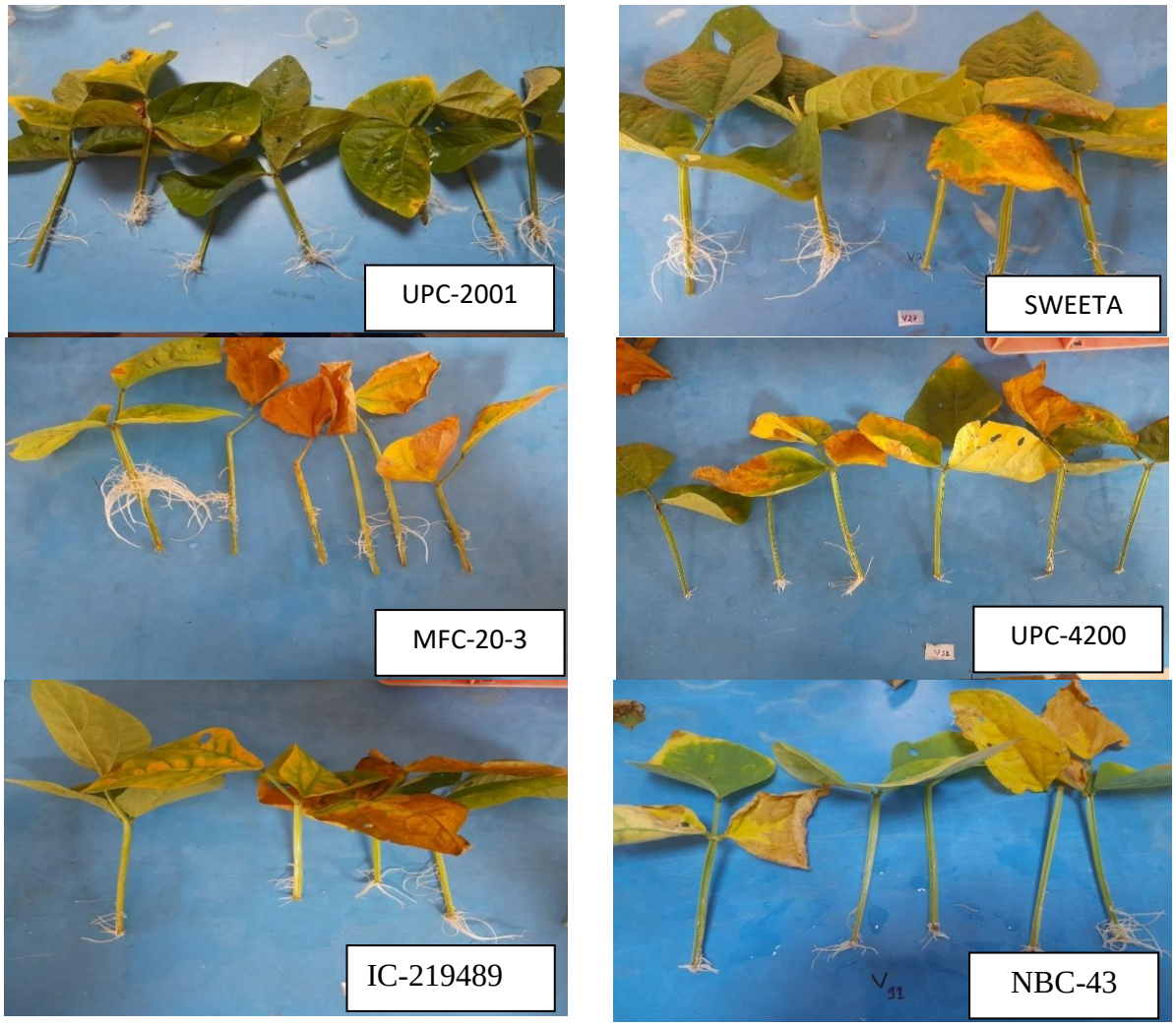

Plate 1. Rooted excised leaves of different fodder cowpea genotypes

(Table 1). The desirability index varied from 0.0 to 5.0. Three genotypes namely UPC-804, UPC-2001 and Sweeta had the desirability index of 5.0 and these weremost suitable for detached leaf culture. The genotypes,MFC -18-2, NBC-43, UPC-801, UPC-805and UPC-2002 having desirability index of 4.0 were also consideredas good for leaf culture.Two (MFC -18-2, NBC-43) out of eleven genotypes of Mandya centre, five out of nine genotypes of Pantanagar centre, one genotype (Sweeta) from Hyderabad centre showed good performance in leaf culture assays. None of the genotypes from Coimbatore centre performed better in leaf culture assays. These results revealed genetic divergence in leaf culture ability of fodder cowpea genotypes.

Development of varieties with specific traits like better nutritive value and resistance to biotic and abiotic stress along with high yield is the need of time. The sustainability of leaf culture depends on roots that provide nutrients to keep the leaves alive. When leaves are detached /excised from mother plant and kept in tap water for few days roots come out from leaf petiole to provide nutrients to the leaf and keep the leaf afresh and green. The concept of leaf culture that is used for screening of disease resistant genotypes in legumes focuses on root traits and senescence of excised leaves for proper evaluation. Detached leaf culture is now a convenient technique for many plant physiological, phytopathological and entomological studies. Earlier detached leaves were extensively used in studies of water absorption, transpiration, respiration and photosynthesis.

The work of many scientists on excised/detached leaf culture is cited here. Subedi et al. [9] used

excised leaf culture technique for screening of cercospora leaf spot disease resistant fenugreek genotypes. Jaichopsanthia et al. [10] compared resistance/susceptibility levels of 19 mungbean genotypes to cercospora leaf spot disease following detached leaf assay. Darojat et al. [11] evaluated rubber clones for resistance to leaf fall disease pathogens using detached leaf assays. Khozestani et al. [12] provided a reliable *in-vitro* screening method to test pathogenicity of Ascochyta rabiei in chickpea by adopting detached leaf assay. Marlabeedu et al. [13] standardized a detached leaf assay technique to evaluate resistance of chickpea germplasm against beet armyworm. Hadi Ismail et al. [14] used detached leaf culture for identification and characterisation of causal pathogens of *Pestalotiopsis* leaf fall disease in rubber plants at Malaysia.

In the present investigation we observed variation in root traits of excised leaves of different genotypes. The appearance of roots from leaf petiole without exogenous auxin treatment in the present study, gives indication about the presence of endogenous auxin in leaf petiole. Variation in suchendogenous auxin level may have certain role in physiological mechanism of growth and development due to which the genotypes showed variation in their rooting ability. Das et al. [15] reported that auxin response has positive correlation with fruit yield in tomato. The genotypes collected from Mandya have high senescence index and they may be treated as devoid of stay green trait whereas most of the UPC groupof genotypes havelow senescence index and were assumed to have stay green trait.

4. CONCLUSION

The present investigation reveals presence of substantial variation in senescence and root traits of excised leaves of fodder cowpea genotypes which further could be employed for crop improvement programmes.This excised leaf culture is the simplest, quickest and cheapest technique for identification of biotic and abiotic stress tolerantfodder cowpea genotypes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Egbadzor KF, Ofori K, Yeboah M, Aboagye LM, Opoku-Agyema MO, Danquah EY,Samuel K. Diversity in 113 cowpea (*Vigna unguiculata*(L) Walp) accessions assessed with 458 SNP markers.Springer Plus.2014;3:541-546.
2. Kumar S, Phogat D and Bhusal N. Characterization of elite forage cowpea genotypes for various DUS traits. Forageres. 2015;40(4):232-236
3. Sheahan CM. Plant guide for cowpea (*Vigna unguiculata*), USDA-natural resources conservation service, Cape May Plant Materials Center, Cape May;2012.
4. National Research Council, "Cowpea". Lost Crops of Africa: Vegetables. Washington DC: The National Academies Press. 2006;2:104–117.
5. Pottorff M, Ehlers Jeffrey D, Fatokun C, Roberts Philip A, Timothy J. Leaf morphology in Cowpea (*Vigna unguiculata*(L.) Walp) QTL analysis, physical mapping and identifying a candidate gene using synteny with model legume species, BMC Genomics. 2012;13:234-238.
6. Foolad MR, Sullenberger MT and Ashrafi H. Detached leaf-let evaluation of tomato germplasm for late blight resistance and its correspondence to field and green house screening. Americ. Phyto-pathol. Society.2015;9:718–722.
7. Kobata T, Shinonaga M, Yoshida H, Tomisaka K, Akai K. Stay green trait assessment using the leaf incubation method to examine the maintenance of assimilation rates under high temperature conditions during the grain filling period in rice. Plant Prod. Sci. 2015;18:254–266.
8. Das S, Mishra RC, Sinha SK, Pattanaik MC. Variation in streptomycin-induced bleaching and dark induced senescence of rice (*Oryza sativa*) genotypes and their relationship with yield and adaptability. J. Plant Breed. Crop Sci. 2010;2:139–147.
9. Subedi U, Acharya S, Chatterton S, Thomas J, and Friebel D. Techniques for screening Cercospora leaf spot resistant fenugreek genotypes. Canadian Journal of Plant Science. 2019; 99(3):324-337.
10. Jaichopsanthia T, Siwapithakpong K, Pookhamsak P, Khairum A, Chueakhunthod W, Tharapreuksapong A, Tantasawat PA. Correlation between mycelium-inoculated detached leaf and field assessments of resistance to *Cercospora canescens* in mungbean.

Journal of Food Science and Agricultural Technology. 2023;7:42-48.

11. Darojat MR, Ardie SW, Oktavia F, Sudarsono. New leaf fall disease in rubber-pathogen characterization and rubber clone resistance evaluation using detached leaf assay. Biodiversitas. 2023;24:1935-1945.
12. Khozestani FR, Tavallaie ZF, Shokouhifar F, Mamarabadi M. Introduction of an *In vitro* pathogenicity test of *Ascochyta rabiei* on chickpea leaves detached from resistant and susceptible cultivars. Iranian Journal of Pulses Research. 2023;14(2):211-222.
13. Marlabeedu S, Mishra SP, Sharma HC, Jaba J, Tatineni RB, Dasari S. Standardization of detached leaf assay to screen chickpeas for resistance to beet armyworm, Spodoptera exigua (Hübner, 1808). Acta Agriculturae Slovenica. 2023;119(1):1–8.
14. Hadi Ismail MZ, Mahyudin MM, Noran AS, Ahmad Zambri AM, Maiden NA, Atan Safiah. Identification and characterisation of causal pathogens of Pestalotiopsis leaf fall disease in Hevea brasiliensisusing a detached leaf technique. J Rubber Res. 2024;17:243-249.
15. Das S, Satpathy P, Dash SK, Mohanty A, Sarkar S, Pradhan B, Sahu GS,Tripathy. Genotypic variation in auxin-induced rooting response of detached tomato leaves: An innovative approach for indirect evaluation of yielding ability in tomato (*Solanum lycopersicum*L.). Current Science. 2022;123(4):568-573.

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